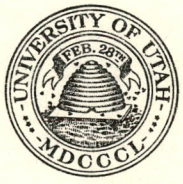
COO-119-242

Research in Radiobiology

Annual Report of Work in Progress
in the Internal Irradiation Program

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



Respectfully Submitted by:
THOMAS F. DOUGHERTY, Director

MARCH 31, 1970

CONTRACT NO. AT (11-1)-119

DISCLAIMER

COO-119-242

RESEARCH IN RADIOBIOLOGY

Annual Report of Work in Progress in the Internal Irradiation Program

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

Respectfully Submitted by:
Thomas F. Dougherty, Director

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Contract No. AT(11-1)-119

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GROUP LEADERS AND SECTIONS

Division of Radiobiology, Department of Anatomy,

University of Utah College of Medicine

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

AVAILABILITY OF PREVIOUS REPORTS

Copies of our reports may be obtained from the Clearinghouse for Federal Scientific and Technical Information, Springfield, Virginia. Paper copies $ 3.00 each; microfiche copies $ 0.65 each.

Report	Date	Title
TID-7639	Jun 1954	Consultants Meeting
AECU-3418	Mar 1955	Annual Report
AECU-3109	Sep 1955	Semi-Annual Report
TID-16458	Mar 1956	Annual Report
TID-16459	Sep 1956	Semi-Annual Report
AECU-3522	Mar 1957	Annual Report
AECU-3583	Sep 1957	Semi-Annual Report
COO-215	Mar 1958	Annual Report
COO-216*	Mar 1958	Escape of Radon & Thoron
COO-217	Sep 1958	Semi-Annual Report
AECU-4112	Feb 1959	Radioactive Fallout
COO-218	Mar 1959	Annual Report
COO-219*	Sep 1959	Semi-Annual Report
COO-220	Mar 1960	Research in Radiobiology
COO-221	Aug 1960	Interim Report of ^{90}Sr
COO-222	Sep 1960	Research in Radiobiology
COO-223	Mar 1961	Research in Radiobiology
COO-224*	Sep 1961	Research in Radiobiology
COO-225	Mar 1962	Research in Radiobiology
COO-226	Sep 1962	Research in Radiobiology

AVAILABILITY OF PREVIOUS REPORTS (Con't.)

Report	Date	Title
COO-227*	Mar 1963	Research in Radiobiology
COO-228*	Sep 1963	Research in Radiobiology
COO-119-229	Mar 1964	Research in Radiobiology
COO-119-230*	Jul 1964	Safety Manual
COO-119-231*	Sep 1964	Research in Radiobiology
COO-119-232*	Mar 1965	Research in Radiobiology
COO-119-233*	Sep 1965	Research in Radiobiology
COO-119-234*	Mar 1966	Research in Radiobiology
COO-119-235	Sep 1966	Research in Radiobiology
COO-119-236*	Mar 1967	Research in Radiobiology
COO-119-237*	Mar 1968	Research in Radiobiology
COO-119-238*	Aug 1968	Rb in RBC, Plasma & Urine
COO-119-239*	Dec 1968	Cs, Rb and K Metabolism
COO-119-240*	Mar 1969	Research in Radiobiology
COO-119-241	Mar 1970	Retention & Dosimetry
COO-119-242	Mar 1970	Research in Radiobiology

* Also available on request from this laboratory.

CURRENT CENSUS OF THE BEAGLE COLONY

March 31, 1970

^{241}Am (americium)	Test dogs	60
^{239}Pu (plutonium)	Toxicity dogs	61
	Test dogs	77
^{228}Th (radiothorium)	Toxicity dogs	31
	Test dogs	
^{228}Ra (mesothorium)	Toxicity dogs	31
	Test dogs	0
^{226}Ra (radium)	Toxicity dogs	43
	Test dogs	35
^{224}Ra (quickradium)	Test dogs	12
^{210}Pb (lead)	Test dogs	2
^{90}Sr (strontium)	Toxicity dogs	48
	Test dogs	2
X-Ray	Test dogs	4
Ancillary (breeding and others)		38
Unassigned dogs		100
	TOTAL	544

INJECTION TABLES

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

Dogs are put into the toxicity study at graded injection levels. At each level, about half the dogs are male and half female. Litter mates are used whenever possible. Each animal receives the designated dose of one radionuclide in a single I. V. injection. 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, ^{226}Ra, ^{239}Pu, ^{228}Ra, ^{228}Th and ^{90}Sr. The current injection program involves ^{226}Ra and ^{239}Pu at lower dose levels and test animals receiving various radionuclides of current interest.

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

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*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 ^{226}Ra retention = 0.25 after 271 days
average ^{239}Pu retention = 0.90 after 6 days
average ^{228}Ra retention = 0.25 after 214 days
average ^{228}Th retention = 0.90 after 6 days
average ^{90}Sr retention = 0.25 after 134 days

"retained" activities are the same for all the radionuclides except ^{90}Sr, in which case they are greater by a factor of 10. Injection level 1 is the basis of the scheme, and is 10 times the maximum permissible concentration of ^{226}Ra in man. Level 1 = 10 x $\frac{0.1\ \mu Ci\ ^{226}Ra}{70\ kg\ man}$ = 0.0143 "retained" μCi/kg. All other injection levels are simple multiples of level 1 as shown below.

Level 0.1	is	1/27	of level 1
Level 0.2	is	1/9	of level 1
Level 0.5	is	1/3	of level 1
Level 1.5	is	2	times level 1
Level 1.7	is	3	times level 1
Level 2	is	6	times level 1
Level 3	is	18	times level 1
Level 4	is	54	times level 1
Level 4.5	is	94	times level 1
Level 5	is	162	times level 1

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

Next comes a code letter for the radionuclide: R = ^{226}Ra, P = ^{239}Pu, M = ^{228}Ra, T = ^{228}Th, S = ^{90}Sr, Q = ^{224}Ra, J = ^{85}Sr, W = ^{241}Am, L = ^{210}Pb, and A = Ancillary.

"A" following the regular dog number means that the dog is a replacement. "H" following the regular dog number means that the dog received its dose in more than one injection. "B", "C", or "D" denotes assignment to serial sacrifice schedule. "E" in the final position is used to denote that the dog listed is not a Beagle from our colony.

Any of the above letters denoting a radionuclide may follow the final number, in which case the letter indicates that two radionuclides were given. The injection level refers to the radionuclide appearing first in the identifying code.

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

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

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

DOSIMETRY

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

$$^{226}Ra = 0.412e^{-0.558t} + 0.105e^{-0.0730t} + 0.196e^{-0.00488t} + 0.287e^{-0.000299t}$$
(5-level only)

$$^{226}Ra = 0.251e^{-0.982t} + 0.211e^{-0.269t} + 0.210e^{-0.0155t} + 0.177e^{-0.00204t} + 0.151e^{-0.000150t}$$
(lower levels)

$$^{222}Rn/^{226}Ra = 0.075\left(1-e^{-0.181t}\right)^{0.158} t$$
(all levels)

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

$$^{228}Ra = 0.251e^{-0.982t} + 0.211e^{-0.269t} + 0.210e^{-0.0158t} + 0.177e^{-0.00237t} + 0.151e^{-0.000479t}$$
(pure at t = 0)

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

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

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

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

$$^{90}Sr = 0.36e^{-0.95t} + 0.29e^{-0.12t} + 0.10e^{-0.0091t} + 0.12e^{-0.0019t} + 0.13e^{-0.00015t}$$

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

Detailed retention data and dosimetric analyses have been presented in a special report COO-119-241.

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

TABLE I. TOXICITY ANIMALS (MAR. 31 1970)

A. RADIUM-226

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
M001R0.0	558	8.03		20 4 53		3116	
M002R0.0	487	14.60		16 11 53		3675	
F003R0.0	601	11.40		10 3 54		2139	
M004R0.0	461	11.00		7 4 54		5145	
M005R0.0	460	6.57		22 6 54		4018	
F006R0.0	483	8.43		27 7 54		3182	
M007R0.0	511	11.00		24 8 54		3360	
F008R0.0	638	8.21		21 12 54		3361	
F009R0.0	700	11.70		11 4 55		1550	
M010R0.0	522	10.90		27 7 55		4698	
F011R0.0	544	10.20		20 12 55		4575	
F012R0.0	501	8.68		17 1 56		4283	
M013R0.0	515	12.30		4 3 64	2218		
F014R0.0	536	10.80		23 10 64	1985		
M015R0.0	564	12.80		4 2 65	1881		
F016R0.0	469	10.00		7 4 65	1819		
M017R0.0	469	12.50		27 4 66	1434		
F018R0.0	497	12.00		25 5 66	1406		
F019R0.0	526	8.42		13 10 66	1265		
M020R0.0	536	9.70		29 12 66	1188		
F021R0.0	549	9.90		26 1 67	1160		
M022R0.0	533	12.10		22 3 67	1105		
F031R0.0B	536	10.60		23 10 64	1985		
F031R0.0C	536	9.88		23 10 64	1985		
F031R0.0D	542	9.90		21 9 65	1652		
F032R0.0B	542	7.80		21 9 65	1652		
F032R0.0C	532	11.70		21 9 65	1652		
F032R0.0D	532	9.70		21 9 65	1652		
F033R0.0B	532	9.80		21 9 65	1652		
F033R0.0C	496	9.50		25 5 66	1406		
F033R0.0D	496	11.80		25 5 66	1406		
F034R0.0B	525	8.20		26 1 67	1160		
F034R0.0C	520	8.90		22 3 67	1105		
F034R0.0D	484	9.90		22 3 67	1105		
F035R0.0B	502	9.41		1 2 68	789		
F035R0.0C	502	9.38		1 2 68	789		
F035R0.0D	552	8.86		9 1 69	446		
F036R0.0B	467	10.10		2 7 68	637		
F036R0.0C	467	9.17		2 7 68	637		
F036R0.0D	467	9.08		2 7 68	637		
F037R0.0B	801	11.10		20 5 69	315		
F042R0.0B	338	8.00		25 4 69		33	

DOG NUMBER	COMMENTS ON DEAD DOGS
M001R0.0	SEMINOMA, LYMPHOSARCOMA
M002R0.0	TRANSITIONAL CELL CARCINOMA
F003R0.0	STATUS EPILEPTICUS
M004R0.0	CHRONIC INTERSTITIAL NEPHRITIS; THROMBOSIS
M005R0.0	OBTURATING PULMONARY EMBOLISM
F006R0.0	STATUS EPILEPTICUS
M007R0.0	STATUS EPILEPTICUS, NEPHRITIS
F008R0.0	PANCREATIC ADENOCARCINOMA
F009R0.0	AORTIC BODY TUMOR
M010R0.0	NEPHRITIS
F011R0.0	VAGINAL FIBROMA
F012R0.0	UNDETERMINED
M013R0.0	
F014R0.0	
M015R0.0	
F016R0.0	
M017R0.0	
F018R0.0	
F019R0.0	
M020R0.0	
F021R0.0	
M022R0.0	
F031R0.0B	
F031R0.0C	
F031R0.0D	
F032R0.0B	
F032R0.0C	
F032R0.0D	
F033R0.0B	
F033R0.0C	
F033R0.0D	
F034R0.0B	
F034R0.0C	
F034R0.0D	
F035R0.0B	
F035R0.0C	
F035R0.0D	
F036R0.0B	
F036R0.0C	
F036R0.0D	
F037R0.0B	
F042R0.0B	SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
M013R0.2	529	9.77	0.00577	4	3	64	2218		
F014R0.2	460	8.10	0.00836	23	10	64	1985		
M015R0.2	504	10.80	0.00873	4	2	65	1881		
F016R0.2	485	8.90	0.00665	7	4	65	1819		
M017R0.2	494	11.80	0.00711	27	4	66	1434		
F018R0.2	497	9.30	0.00652	25	5	66	1406		
F019R0.2	526	10.60	0.00785	13	10	66	1265		
M020R0.2	546	11.40	0.00676	29	12	66	1188		
F021R0.2	549	11.50	0.00687	26	1	67	1160		
M022R0.2	533	12.90	0.00961	22	3	67	1105		
M013R0.5	529	11.00	0.0171	4	3	64	2218		
F014R0.5	510	9.75	0.022	23	10	64	1985		
M015R0.5	490	10.40	0.0263	4	2	65	1881		
F016R0.5	500	11.40	0.0205	7	4	65	1819		
M017R0.5	494	9.20	0.0215	27	4	66	1434		
F018R0.5	496	9.10	0.0197	25	5	66	1406		
F019R0.5	526	10.00	0.023	13	10	66	1265		
M020R0.5	536	13.20	0.0206	29	12	66	1188		
F021R0.5	538	8.80	0.0208	26	1	67	1160		
M022R0.5	520	12.30	0.029	22	3	67	1105		
M031R0.5B	508	11.40	0.021	27	4	66	1434		
F031R0.5C	537	9.40	0.0235	22	12	65	1560		
F031R0.5D	537	11.70	0.0238	22	12	65	1560		
M032R0.5B	496	13.40	0.0196	25	5	66	1406		
F032R0.5C	519	10.10	0.0239	22	12	65	1560		
F032R0.5D	509	10.10	0.024	22	12	65	1560		
M033R0.5B	497	12.90	0.0194	25	5	66	1406		
F033R0.5C	527	10.60	0.0212	27	4	66	1434		
F033R0.5D	527	8.70	0.0217	27	4	66	1434		
M034R0.5B	496	10.50	0.0196	25	5	66	1406		
F034R0.5C	524	9.90	0.0215	27	4	66	1434		
F034R0.5D	508	9.70	0.0212	27	4	66	1434		
M035R0.5B	536	10.40	0.0205	29	12	66	1188		
F035R0.5C	532	9.00	0.0201	29	12	66	1188		
F035R0.5D	532	10.20	0.0202	29	12	66	1188		
M001R1.0	471	8.48	0.0618	20	4	53		5727	170
M002R1.0	627	10.00	0.0876	16	11	53		4054	237
F003R1.0	706	8.68	0.0576	10	3	54		3850	151

DOG NUMBER	COMMENTS ON DEAD DOGS
M013R0.2	
F014R0.2	
M015R0.2	
F016R0.2	
M017R0.2	
F018R0.2	
F019R0.2	
M020R0.2	
F021R0.2	
M022R0.2	
M013R0.5	
F014R0.5	
M015R0.5	
F016R0.5	
M017R0.5	
F018R0.5	
F019R0.5	
M020R0.5	
F021R0.5	
M022R0.5	
M031R0.5B	
F031R0.5C	
F031R0.5D	
M032R0.5B	
F032R0.5C	
F032R0.5D	
M033R0.5B	
F033R0.5C	
F033R0.5D	
M034R0.5B	
F034R0.5C	
F034R0.5D	
M035R0.5B	
F035R0.5C	
F035R0.5D	
M001R1.0	MELANOMA ORAL CAVITY
M002R1.0	SEMINOMA
F003R1.0	MAMMARY GLAND CARCINOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
M004R1.0	414	8.60	0.0642	7	4	54		2038	108
M005R1.0	490	11.70	0.0436	22	6	54		3780	112
F006R1.0	483	7.23	0.0584	27	7	54		5260	187
M007R1.0	511	11.40	0.0651	24	8	54		3544	167
F008R1.0	861	8.98	0.0559	21	12	54		2988	93
F009R1.0	781	9.88	0.0521	11	4	55		4399	99
M010R1.0	525	11.50	0.0573	27	7	55		4003	157
F011R1.0	511	11.20	0.0522	20	12	55	5215		
F012R1.0	501	9.71	0.0444	17	1	56		3978	124
M013R1.0	529	11.70	0.0527	4	3	64	2218		
F014R1.0	510	10.50	0.0701	23	10	64		1729	148
M015R1.0	490	8.88	0.0797	4	2	65		893	82
F016R1.0	501	8.99	0.0611	7	4	65	1819		
M017R1.0	494	11.40	0.0639	27	4	66	1434		
F018R1.0	496	10.00	0.0589	25	5	66	1406		
F019R1.0	526	11.60	0.0682	13	10	66	1265		
M020R1.0	536	10.00	0.061	29	12	66	1188		
F021R1.0	525	8.10	0.0633	26	1	67	1160		
M022R1.0	484	10.90	0.0861	22	3	67	1105		
F031R1.0B	509	10.40	0.0712	22	12	65	1560		
M001R1.7	523	9.98	0.137	17	1	56		4438	341
M002R1.7	598	7.85	0.163	30	11	56		1273	116
M002R1.7A	493	12.00	0.222	6	3	63	2582		
F003R1.7	473	13.10	0.165	20	12	55		3267	385
M004R1.7	514	6.20	0.163	20	12	55	5215		
M005R1.7	511	10.10	0.151	20	12	55		4108	488
F006R1.7	491	7.90	0.152	20	12	55		3432	417
M007R1.7	598	7.17	0.163	30	11	56		3142	309
F008R1.7	491	9.50	0.154	20	12	55		2577	378
F009R1.7	598	7.55	0.168	30	11	56		3914	262
M010R1.7	590	9.57	0.167	30	11	56		557	109
M010R1.7A	545	10.60	0.183	7	1	59	4101		
F011R1.7	598	8.17	0.165	30	11	56	4869		
F012R1.7	590	8.95	0.167	30	11	56		2399	210
M001R2.0	471	8.74	0.382	20	4	53		3440	860
M002R2.0	592	8.21	0.387	16	11	53		3775	625
F003R2.0	541	8.53	0.347	10	3	54		4459	872
M004R2.0	414	10.50	0.361	7	4	54		325	189

DOG NUMBER	COMMENTS ON DEAD DOGS
M004R1.0	TRAUMA
M005R1.0	TRANSITIONAL CELL CARCINOMA, HYDRONEPHROSIS
F006R1.0	NEPHRITIS
M007R1.0	STATUS EPILEPTICUS
F008R1.0	LYMPHOSARCOMA
F009R1.0	PNEUMONIA
M010R1.0	FIBROSARCOMA (GINGIVA)
F011R1.0	
F012R1.0	MELANOMA, GINGIVA
M013R1.0	
F014R1.0	UNDETERMINED (NO NEOPLASIA)
M015R1.0	UNDETERMINED (NO NEOPLASIA)
F016R1.0	
M017R1.0	
F018R1.0	
F019R1.0	
M020R1.0	
F021R1.0	
M022R1.0	
F031R1.0B	
M001R1.7	OBTURATING ABDOMINAL AORTA AND PULMONARY EMBOLISM
M002R1.7	LYMPHOSARCOMA
M002R1.7A	
F003R1.7	MAMMARY GLAND CARCINOMA
M004R1.7	
M005R1.7	OSTEOSARCOMA
F006R1.7	
M007R1.7	BACTERIAL TOXEMIA, INTERSTITIAL CELL ADENOMA
F008R1.7	DRUG ALLERGY
F009R1.7	PYOMETRA
M010R1.7	TRAUMA
M010R1.7A	
F011R1.7	
F012R1.7	UNDETERMINED (NO BONE TUMOR)
M001R2.0	HEMANGIOSARCOMA (SPLEEN)
M002R2.0	OSTEOSARCOMA
F003R2.0	RETICULUM CELL SARCOMA (NON-SKELETAL)
M004R2.0	PERFORATED ILEUM

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
M004R2.0A	420	10.60	0.306	11 4 55	4368	1142
M005R2.0	461	11.50	0.267	22 6 54	4703	994
F006R2.0	486	10.60	0.36	27 7 54	4615	1264
M007R2.0	514	11.10	0.413	24 8 54	3425	922
F008R2.0	572	6.95	0.331	21 12 54	4781	996
F009R2.0	592	9.38	0.317	11 4 55	3998	1016
M010R2.0	523	9.95	0.345	27 7 55	3569	1220
F011R2.0	495	9.30	0.31	20 12 55	3297	728
F012R2.0	497	10.30	0.281	17 1 56	2948	742
M001R3.0	473	8.91	1.2	20 4 53	2850	2395
M002R3.0	470	9.02	1.21	16 11 53	2226	1727
F003R3.0	386	7.74	1.11	10 3 54	2497	2323
M004R3.0	412	11.70	1.16	7 4 54	1917	2361
M005R3.0	461	13.00	0.846	22 6 54	2955	2317
F006R3.0	486	9.75	1.14	27 7 54	1932	2246
M007R3.0	514	12.30	1.29	24 8 54	2099	3029
F008R3.0	542	7.76	1.03	21 12 54	2612	1916
F009R3.0	551	8.02	0.987	11 4 55	2487	1839
M010R3.0	525	10.10	1.06	27 7 55	1737	2336
F011R3.0	495	12.90	0.938	20 12 55	1610	1333
F012R3.0	497	11.40	0.883	17 1 56	1897	1639
M001R4.0	471	9.08	3.51	20 4 53	1606	6575
M002R4.0	470	9.53	3.55	16 11 53	1884	6150
F003R4.0	384	8.65	3.33	10 3 54	490	2208
F003R4.0A	598	7.20	3.1	30 11 56	1614	3855
M004R4.0	408	8.83	3.47	7 4 54	1518	6063
M005R4.0	461	13.20	2.42	22 6 54	1659	4505
F006R4.0	486	8.55	3.44	27 7 54	1939	7133
M007R4.0	453	9.55	3.88	24 8 54	1647	5844
F008R4.0	474	8.94	3.14	21 12 54	1324	4615
F009R4.0	542	8.53	3.02	11 4 55	1471	4095
M010R4.0	527	10.80	3.28	27 7 55	1553	7582
F011R4.0	491	10.40	2.84	20 12 55	1469	5273
F012R4.0	496	9.61	2.81	17 1 56	1435	3877
M001R5.0	473	9.87	10.5	20 4 53	908	14943

DOG NUMBER	COMMENTS ON DEAD DOGS
M004R2.0A	VALVULAR ENDOCARDITIS
M005R2.0	OSTEOSARCOMA, ADRENAL CORTICAL CARCINOMA
F006R2.0	EPIDERMOID CARCINOMA (TYMPANIC BULLA)
M007R2.0	OSTEOSARCOMA, CUSHING SYNDROME
F008R2.0	
F009R2.0	MAMMARY CARCINOMA
M010R2.0	OSTEOSARCOMA
F011R2.0	OSTEOSARCOMA
F012R2.0	MAMMARY ADENOCARCINOMA
M001R3.0	OSTEOSARCOMA
M002R3.0	OSTEOSARCOMA
F003R3.0	OSTEOSARCOMA
M004R3.0	OSTEOSARCOMA
M005R3.0	OSTEOSARCOMA
F006R3.0	OSTEOSARCOMA
M007R3.0	OSTEOSARCOMA
F008R3.0	OSTEOSARCOMA
F009R3.0	OSTEOSARCOMA
M010R3.0	OSTEOSARCOMA
F011R3.0	PYOMETRITIS + SECONDARY PERITONITIS
F012R3.0	OSTEOSARCOMA
M001R4.0	OSTEOSARCOMA
M002R4.0	OSTEOSARCOMA
F003R4.0	CANINE DISTEMPER
F003R4.0A	OSTEOSARCOMA
M004R4.0	OSTEOSARCOMA
M005R4.0	OSTEOSARCOMA
F006R4.0	OSTEOSARCOMA
M007R4.0	OSTEOSARCOMA
F008R4.0	OSTEOSARCOMA
F009R4.0	OSTEOSARCOMA
M010R4.0	OSTEOSARCOMA
F011R4.0	OSTEOSARCOMA
F012R4.0	OSTEOSARCOMA
M001R5.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
M002R5.0	470	8.85	10.8	16	11	53	1380	18071
F003R5.0	380	7.82	10.1	10	3	54	481	7147
M004R5.0	408	8.90	10.6	7	4	54	1091	16417
M005R5.0	458	10.90	10.1	22	6	54	1220	15433
F006R5.0	466	9.66	10.2	27	7	54	1015	15414
M007R5.0	453	8.85	11.9	24	8	54	1288	16708
F008R5.0	474	7.76	9.68	21	12	54	968	11564
F009R5.0	420	9.16	9.48	11	4	55	1288	15941
M010R5.0	527	10.70	10.2	27	7	55	825	11179

DOG NUMBER	COMMENTS ON DEAD DOGS
M002R5.0	OSTEOSARCOMA
F003R5.0	CANINE DISTEMPER
M004R5.0	OSTEOSARCOMA
M005R5.0	OSTEOSARCOMA
F006R5.0	OSTEOSARCOMA
M007R5.0	OSTEOSARCOMA
F008R5.0	OSTEOSARCOMA
F009R5.0	OSTEOSARCOMA + ANEMIA
M010R5.0	OSTEOSARCOMA + FRACTURED MANDIBLE

B. PLUTONIUM-239

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
M001P0.0	443	9.70		1	12	52		4003	
F002P0.0	424	6.36		2	3	53		2755	
M003P0.0	515	10.80		1	6	53		5362	
M004P0.0	426	10.70		16	9	53		5138	
F005P0.0	620	9.75		14	10	53		4088	
F006P0.0	410	5.59		12	5	54		4499	
F007P0.0	515	6.90		25	10	54		5344	
M008P0.0	585	10.90		15	3	55		4072	
F009P0.0	658	11.00		22	11	55		3032	
F010P0.0	658	11.00		22	11	55		3971	
M011P0.0	602	10.30		24	4	56		3821	
M012P0.0	630	10.90		29	5	56		4143	
F013P0.0	517	9.47		4	3	64	2218		
F014P0.0	452	9.89		12	5	64	2149		
M015P0.0	527	12.10		23	10	64	1985		
M016P0.0	485	13.90		7	4	65	1819		
M017P0.0	551	12.20		18	11	66	1239		
F018P0.0	536	11.40		29	11	66	1218		
M019P0.0	536	13.10		29	11	66	1218		
F020P0.0	546	8.50		29	12	66	1188		
M021P0.0	549	13.30		26	1	67	1160		
F022P0.0	489	10.60		25	5	67	1041		
M031P0.0B	452	11.80		12	5	64		1763	
M031P0.0C	452	12.60		12	5	64	2149		
M032P0.0B	452	11.20		12	5	64	2149		
M032P0.0C	542	10.30		21	9	65	1652		
M033P0.0B	517	12.10		21	9	65	1652		
M033P0.0C	503	11.70		18	11	65	1594		
M034P0.0B	525	13.50		26	1	67	1160		
M034P0.0C	484	12.70		22	3	67	1105		
M035P0.0B	484	12.50		22	3	67	1105		
M035P0.0C	484	13.10		22	3	67	1105		
M036P0.0B	489	11.00		25	5	67	1041		
M036P0.0C	489	12.20		25	5	67	1041		
M037P0.0B	507	11.70		22	6	67	1013		
M037P0.0C	493	10.40		22	6	67	1013		
M038P0.0B	529	10.70		16	11	67	866		
M038P0.0C	529	12.20		16	11	67	866		
M039P0.0B	503	10.70		21	12	67	831		
M039P0.0C	503	10.10		21	12	67	831		
M040P0.0B	484	10.30		30	7	68	609		
M040P0.0C	552	11.40		9	1	69	446		
M041P0.0B	560	9.49		17	1	69	438		
M042P0.0B	338	11.60		25	4	69		32	

DOG NUMBER	COMMENTS ON DEAD DOGS
M001P0.0	SPLENIC RUPTURE, METASTATIC SEMINOMA
F002P0.0	ANESTHETIC ACCIDENT
M003P0.0	PANCREATIC ADENOCARCINOMA
M004P0.0	THYROID CARCINOMA, NEPHRITIS
F005P0.0	ADRENAL CORTICAL CARCINOMA
F006P0.0	OBTURATING PULMONARY EMBOLISM
F007P0.0	RHABDOMYOSARCOMA
M008P0.0	CIRCULATORY FAILURE
F009P0.0	PULMONARY EMBOLISM, NEPHRITIS
F010P0.0	LEUKEMIA
M011P0.0	FIBROSARCOMA (SPLEEN)
M012P0.0	TESTICULAR CARCINOMA
F013P0.0	
F014P0.0	
M015P0.0	
M016P0.0	
M017P0.0	
F018P0.0	
M019P0.0	
F020P0.0	
M021P0.0	
F022P0.0	
M031P0.0B	STATUS EPILEPTICUS; BILE DUCT OBSTRUCTION
M031P0.0C	
M032P0.0B	
M032P0.0C	
M033P0.0B	
M033P0.0C	
M034P0.0B	
M034P0.0C	
M035P0.0B	
M035P0.0C	
M036P0.0B	
M036P0.0C	
M037P0.0B	
M037P0.0C	
M038P0.0B	
M038P0.0C	
M039P0.0B	
M039P0.0C	
M040P0.0B	
M040P0.0C	
M041P0.0B	
M042P0.0B	SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (µCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
F013P0.1	515	9.46	0.00068	4	3	64	2218	
F014P0.1	452	10.30	0.00055	12	5	64	2149	
M015P0.1	536	9.67	0.00071	23	10	64	1985	
M016P0.1	501	12.00	0.00059	7	4	65	1819	
M017P0.1	551	12.26	0.00057	18	11	66	1239	
F018P0.1	536	9.26	0.0007	29	11	66	1218	
M019P0.1	536	11.60	0.00065	29	11	66	1219	
F020P0.1	536	9.80	0.00075	29	12	66	1188	
M021P0.1	536	11.30	0.00059	26	1	67	1160	
F022P0.1	489	9.80	0.00059	25	5	67	1041	
M031P0.1B	517	12.20	0.00068	4	3	64	2218	
F032P0.1B	549	10.40	0.00059	18	11	65	1594	
M033P0.1B	549	10.80	0.00079	18	11	65	1594	
F034P0.1B	535	11.10	0.00056	18	11	66	1239	
M035P0.1B	489	10.30	0.00059	25	5	67	1041	
F036P0.1B	495	9.79	0.0006	22	6	67	1013	
M037P0.1B	495	11.30	0.00059	22	6	67	1013	
F038P0.1B	515	9.52	0.00057	21	12	67	831	
M039P0.1B	490	10.50	0.00056	21	12	67	831	
F013P0.2	517	9.44	0.00206	4	3	64	2218	
F014P0.2	516	7.44	0.00175	12	5	64	2149	
M015P0.2	505	10.90	0.00201	23	10	64	1985	
M016P0.2	500	11.40	0.00165	7	4	65	1819	
M017P0.2	535	11.80	0.00171	18	11	66	1239	
F018P0.2	530	9.46	0.002	29	11	66	1218	
M019P0.2	530	12.10	0.00198	29	11	66	1218	
F020P0.2	532	8.30	0.00224	29	12	66	1188	
M021P0.2	538	12.10	0.00181	26	1	67	1160	
F022P0.2	485	8.30	0.00176	25	5	67	1041	
M031P0.2B	515	10.70	0.00185	4	3	64	2218	
F031P0.2C	452	11.90	0.00169	12	5	64	2149	
F031P0.2D	429	9.35	0.00186	12	5	64	2149	
M032P0.2B	549	13.60	0.00178	18	11	65	1594	
F032P0.2C	494	10.10	0.00183	4	2	65	1881	
F032P0.2D	490	8.04	0.00195	4	2	65	1881	
M033P0.2B	515	14.50	0.00176	18	11	65	1594	
F033P0.2C	549	12.50	0.00176	18	11	65	1594	
F033P0.2D	515	12.70	0.00176	18	11	65	1594	
M034P0.2B	535	12.70	0.0017	18	11	66	1239	
F034P0.2C	535	11.50	0.00172	18	11	66	1239	
F034P0.2D	519	9.92	0.00167	18	11	66	1239	

DOG NUMBER	COMMENTS ON DEAD DOGS
F013P0.1	
F014P0.1	
M015P0.1	
M016P0.1	
M017P0.1	
F018P0.1	
M019P0.1	
F020P0.1	
M021P0.1	
F022P0.1	
M031P0.1B	
F032P0.1B	
M033P0.1B	
F034P0.1B	
M035P0.1B	
F036P0.1B	
M037P0.1B	
F038P0.1B	
M039P0.1B	
F013P0.2	
F014P0.2	
M015P0.2	
M016P0.2	
M017P0.2	
F018P0.2	
M019P0.2	
F020P0.2	
M021P0.2	
F022P0.2	
M031P0.2B	
F031P0.2C	
F031P0.2D	
M032P0.2B	
F032P0.2C	
F032P0.2D	
M033P0.2B	
F033P0.2C	
F033P0.2D	
M034P0.2B	
F034P0.2C	
F034P0.2D	

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
M035P0.2B	489	11.20	0.00173	25	5	67	1041		
F035P0.2C	507	10.50	0.00175	22	6	67	1013		
F035P0.2D	507	9.10	0.00175	22	6	67	1013		
M036P0.2B	479	12.90	0.00177	25	5	67	1041		
F036P0.2C	493	10.40	0.00177	22	6	67	1013		
F036P0.2D	569	8.74	0.00146	16	11	67	866		
M037P0.2B	529	10.60	0.00149	16	11	67	866		
F037P0.2C	529	10.10	0.0015	16	11	67	866		
F037P0.2D	529	7.14	0.00153	16	11	67	866		
M038P0.2B	517	10.00	0.00152	16	11	67	866		
F038P0.2C	503	7.95	0.00211	21	12	67	831		
F038P0.2D	499	9.08	0.00176	21	12	67	831		
F039P0.2C	499	9.46	0.00173	21	12	67	831		
F039P0.2D	499	9.34	0.00176	21	12	67	831		
F042P0.2C	589	9.55	0.00176	4	9	69	208		
F013P0.5	517	9.93	0.0054	4	3	64	2218		
F014P0.5	516	9.96	0.00493	12	5	64	2149		
M015P0.5	505	8.41	0.00627	23	10	64	1985		
M016P0.5	501	12.60	0.00521	7	4	65	1819		
M017P0.5	535	13.40	0.00506	18	11	66	1239		
F018P0.5	530	8.98	0.00594	29	11	66	1218		
M019P0.5	530	11.90	0.00645	29	11	66	1218		
F020P0.5	532	9.30	0.00553	29	12	66	1188		
M021P0.5	536	9.80	0.00526	26	1	67	1160		
F022P0.5	485	8.10	0.00525	25	5	67	1041		
M031P0.5B	515	10.50	0.00549	4	3	64		1648	14
M032P0.5B	549	13.60	0.00546	18	11	65	1594		
F032P0.5C	494	8.44	0.00571	4	2	65	1861		
F033P0.5B	503	10.10	0.00559	18	11	65	1594		
M034P0.5B	530	12.50	0.00642	29	11	66	1218		
F035P0.5B	501	9.54	0.0052	22	6	67	1013		
M036P0.5B	479	11.50	0.00527	25	5	67	1041		
F037P0.5B	517	8.39	0.00454	16	11	67	866		
M038P0.5B	517	10.50	0.00448	16	11	67	866		
F039P0.5B	490	10.90	0.00526	21	12	67	831		
F043P0.5B	545	11.60	0.00484	3	10	69	179		
F043P0.5C	535	10.70	0.0048	3	10	69	179		
M044P0.5B	445	11.50	0.0036	3	6	69		99	1
M045P0.5B	472	10.30	0.0035	3	6	69		42	1
M046P0.5B	484	11.80	0.00336	3	6	69		7	1

DOG NUMBER	COMMENTS ON DEAD DOGS
M035P0.2B	
F035P0.2C	
F035P0.2D	
M036P0.2B	
F036P0.2C	
F036P0.2D	
M037P0.2B	
F037P0.2C	
F037P0.2D	
M038P0.2B	
F038P0.2C	
F038P0.2D	
F039P0.2C	
F039P0.2D	
F042P0.2C	
F013P0.5	
F014P0.5	
M015P0.5	
M016P0.5	
M017P0.5	
F018P0.5	
M019P0.5	
F020P0.5	
M021P0.5	
F022P0.5	
M031P0.5B	STATUS EPILEPTICUS
M032P0.5B	
F032P0.5C	
F033P0.5B	
M034P0.5B	
F035P0.5B	
M036P0.5B	
F037P0.5B	
M038P0.5B	
F039P0.5B	
F043P0.5B	
F043P0.5C	
M044P0.5B	SPECIAL STUDY
M045P0.5B	SPECIAL STUDY
M046P0.5B	SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
F014P0.7	535	8.98	0.00947	22 7 69	252		
M015P0.7	533	10.30	0.00941	22 7 69	252		
M016P0.7	516	11.90	0.0102	4 9 69	208		
M017P0.7	540	8.04	0.0103	3 10 69	179		
F018P0.7	521	9.66	0.00942	22 7 69	252		
F020P0.7	521	9.18	0.00926	22 7 69	252		
F022P0.7	538	9.69	0.0108	4 9 69	208		
F023P0.7	538	9.56	0.0108	4 9 69	208		
F024P0.7	516	8.90	0.011	4 9 69	208		
M001P1.0	442	9.41	0.015	1 12 52		4572	98
F002P1.0	422	6.85	0.0163	2 3 53		4810	112
M003P1.0	515	8.00	0.0165	1 6 53		4292	102
M004P1.0	608	9.97	0.0139	16 9 53		4569	91
F005P1.0	620	8.80	0.0142	14 10 53		1509	30
F005P1.0A	472	11.00	0.0168	3 9 58		3764	91
F006P1.0	410	7.38	0.014	12 5 54		4292	86
F007P1.0	510	6.36	0.0167	25 10 54		3981	96
M008P1.0	453	10.60	0.0172	15 3 55		3367	83
F009P1.0	556	7.87	0.0168	9 9 55		2257	60
F010P1.0	641	12.00	0.0152	22 11 55		3649	80
M011P1.0	602	8.90	0.0157	24 4 56	5089		
M012P1.0	629	9.67	0.0167	29 5 56		2374	60
M013P1.0	504	12.70	0.0153	3 9 58	4227		
F014P1.0	533	10.40	0.0141	22 7 69	252		
M015P1.0	516	12.80	0.0159	4 9 69	208		
M016P1.0	516	10.60	0.0165	4 9 69	208		
M017P1.0	537	10.90	0.0151	3 10 69	179		
F018P1.0	531	9.89	0.014	22 7 69	252		
F020P1.0	521	10.40	0.0141	22 7 69	252		
F022P1.0	531	9.04	0.0139	22 7 69	252		
F023P1.0	538	11.20	0.0163	4 9 69	208		
F024P1.0	516	10.40	0.0163	4 9 69	208		
M001P1.7	657	8.72	0.0475	26 6 56		3025	210
F002P1.7	527	8.62	0.0431	22 11 55		3430	215
M003P1.7	642	8.63	0.0495	26 6 56		3430	246
M004P1.7	673	8.37	0.0484	10 10 56		3312	233
F005P1.7	642	11.60	0.0493	26 6 56		2659	190
F006P1.7	642	10.30	0.0459	26 6 56		2221	170

DOG NUMBER	COMMENTS ON DEAD DOGS
F014P0.7	
M015P0.7	
M016P0.7	
M017P0.7	
F018P0.7	
F020P0.7	
F022P0.7	
F023P0.7	
F024P0.7	
M001P1.0	OSTEOSARCOMA
F002P1.0	CIRCULATORY FAILURE
M003P1.0	OSTEOSARCOMA
M004P1.0	BILE DUCT CARCINOMA
F005P1.0	COLITIS. ENTERITIS + SECONDARY HEPATIC NECROSIS
F005P1.0A	THYROID CARCINOMA
F006P1.0	CARCINOMA OF COLON
F007P1.0	TRAUMA LYMPHADENOPHATHY
M008P1.0	OSTEOSARCOMA
F009P1.0	OSTEOSARCOMA
F010P1.0	MAMMARY CARCINOMA
M011P1.0	
M012P1.0	CHRONIC PANCREATITIS
M013P1.0	
F014P1.0	
M015P1.0	
M016P1.0	
M017P1.0	
F018P1.0	
F020P1.0	
F022P1.0	
F023P1.0	
F024P1.0	
M001P1.7	OSTEOSARCOMA
F002P1.7	OSTEOSARCOMA
M003P1.7	CHROMOPHOBE ADENOMA OF PITUITARY, PROSTATE CARCINOMA
M004P1.7	OSTEOSARCOMA
F005P1.7	OSTEOSARCOMA
F006P1.7	OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/78	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
F007P1.7	756	9.73	0.0481	10	10	56		3353	234
M008P1.7	673	13.60	0.0479	10	10	56		3282	229
F009P1.7	756	9.72	0.0485	10	10	56		2500	180
F010P1.7	739	10.60	0.0495	10	10	56		467	40
F010P1.7A	472	8.07	0.0457	3	9	56		4214	277
M011P1.7	599	11.60	0.0486	24	4	56		2777	200
M012P1.7	673	9.41	0.0491	10	10	56		2973	213
M013P1.7	504	10.60	0.0473	3	9	58	4227		
M001P2.0	442	7.61	0.0853	1	12	52		2985	370
F002P2.0	422	7.73	0.112	2	3	53		2780	460
M003P2.0	485	10.50	0.094	1	6	53		3185	420
M004P2.0	608	9.84	0.0862	16	9	53		2948	380
F005P2.0	594	8.12	0.0846	14	10	53		2423	310
F006P2.0	417	7.54	0.0902	12	5	54		2947	370
F007P2.0	485	8.40	0.0996	25	10	54		2093	310
M008P2.0	406	9.73	0.0957	15	3	55		1761	250
F009P2.0	552	9.72	0.101	9	9	55		2014	300
F010P2.0	551	7.94	0.0968	22	11	55		2912	410
M011P2.0	599	10.30	0.0961	24	4	56		1617	230
M012P2.0	622	9.98	0.1	29	5	56		2264	370
M001P3.0	417	8.00	0.261	1	12	52		1476	580
F002P3.0	422	6.85	0.312	2	3	53		1947	900
M003P3.0	485	8.74	0.291	1	6	53		1604	700
M004P3.0	608	8.51	0.292	16	9	53		1950	840
F005P3.0	650	8.22	0.288	14	10	53		1504	630
F006P3.0	415	8.38	0.282	12	5	54		1617	670
F007P3.0	485	9.00	0.314	25	10	54		1627	750
M008P3.0	406	9.73	0.3	15	3	55		1771	780
F009P3.0	552	7.67	0.3	9	9	55		1894	840
F010P3.0	533	8.94	0.298	22	11	55		1547	700
M011P3.0	599	10.50	0.309	24	4	56		1198	550
M012P3.0	622	10.20	0.308	29	5	56		1659	760
M001P4.0	442	7.61	0.823	1	12	52		1724	2100
F002P4.0	567	8.65	1.03	2	3	53		1556	2380
M003P4.0	485	9.36	0.929	1	6	53		1198	1680

DOG NUMBER	COMMENTS ON DEAD DOGS
F007P1.7	OSTEOSARCOMA
M008P1.7	OSTEOSARCOMA
F009P1.7	OSTEOSARCOMA
F010P1.7	ACUTE ENTERITIS
F010P1.7A	OSTEOSARCOMA
M011P1.7	BILE DUCT CARCINOMA
M012P1.7	LEUKEMIA
M013P1.7	
M001P2.0	OSTEOSARCOMA
F002P2.0	OSTEOSARCOMA
M003P2.0	OSTEOSARCOMA
M004P2.0	OSTEOSARCOMA
F005P2.0	OSTEOSARCOMA
F006P2.0	OSTEOSARCOMA
F007P2.0	SQUAMOUS CELL CARCINOMA (FRONTAL SINUS)
M008P2.0	ASPIRATION PNEUMONIA
F009P2.0	OSTEOSARCOMA
F010P2.0	OSTEOSARCOMA
M011P2.0	OSTEOSARCOMA
M012P2.0	OSTEOSARCOMA
M001P3.0	OSTEOSARCOMA
F002P3.0	OSTEOSARCOMA
M003P3.0	OSTEOSARCOMA
M004P3.0	OSTEOSARCOMA
F005P3.0	OSTEOSARCOMA
F006P3.0	OSTEOSARCOMA
F007P3.0	OSTEOSARCOMA
M008P3.0	OSTEOSARCOMA
F009P3.0	OSTEOSARCOMA
F010P3.0	OSTEOSARCOMA
M011P3.0	OSTEOSARCOMA
M012P3.0	OSTEOSARCOMA
M001P4.0	OSTEOSARCOMA
F002P4.0	OSTEOSARCOMA
M003P4.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
M004P4.0	566	8.74	0.974	16 9 53	1066	1560
F005P4.0	650	7.05	0.872	14 10 53	1245	1650
F006P4.0	420	9.26	0.811	12 5 54	1357	1660
F007P4.0	485	8.45	0.963	25 10 54	1198	1730
M008P4.0	651	9.22	0.887	15 3 55	1157	1560
F009P4.0	552	8.58	0.96	9 9 55	1343	1920
F010P4.0	527	6.48	0.868	22 11 55	1241	1660
M011P4.0	596	9.56	0.927	24 4 56	1288	1790
M012P4.0	598	11.40	0.838	29 5 56	1463	1840
M001P5.0	417	8.86	2.67	1 12 52	1324	5370
F002P5.0	1150	8.75	3.3	2 3 53	1576	7830
M003P5.0	515	8.10	3.0	1 6 53	499	2340
M004P5.0	566	9.18	3.17	16 9 53	1562	7380
F005P5.0	691	8.77	2.77	14 10 53	2059	8690
F006P5.0	407	7.90	2.57	12 5 54	1194	4620
F007P5.0	482	8.33	2.99	25 10 54	1491	6630
M008P5.0	497	9.55	2.69	15 3 55	1192	4840
F009P5.0	552	9.45	2.73	9 9 55	1145	4750

DOG NUMBER	COMMENTS ON DEAD DOGS
M004P4.0	OSTEOSARCOMA
F005P4.0	OSTEOSARCOMA
F006P4.0	OSTEOSARCOMA
F007P4.0	OSTEOSARCOMA
M008P4.0	OSTEOSARCOMA
F009P4.0	OSTEOSARCOMA
F010P4.0	OSTEOSARCOMA
M011P4.0	OSTEOSARCOMA
M012P4.0	OSTEOSARCOMA
M001P5.0	OSTEOSARCOMA
F002P5.0	OSTEOSARCOMA + FRACTURED MANDIBLE
M003P5.0	LIVER DEGENERATION + ASCITES
M004P5.0	OSTEOSARCOMA
F005P5.0	OSTEOSARCOMA, LIVER DEGENERATION + HEPATIC HEMORRHAGE
F006P5.0	OSTEOSARCOMA
F007P5.0	OSTEOSARCOMA + CRIPPLING FRACTURE
M008P5.0	GINGIVITIS
F009P5.0	OSTEOSARCOMA, EPISTAXIS + CIRCULATORY COLLAPSE

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
F001M0.0	732	7.33		4 1 54		3451	
F002M0.0	545	6.94		29 11 54	5601		
M003M0.0	579	13.00		13 3 56		5056	
M004M0.0	601	10.30		15 1 57		4816	
F005M0.0	671	11.20		5 3 57		4581	
M006M0.0	492	7.56		23 4 57	4725		
F007M0.0	595	8.71		4 6 57		1414	
F007M0.0A	594	10.90		15 1 63	2632		
F008M0.0	654	11.60		9 3 60	3674		
M009M0.0	575	12.40		13 4 60	3639		
M010M0.0	581	13.30		17 7 62	2814		
F011M0.0	475	9.31		18 9 62	2751		
M012M0.0	695	10.00		22 12 60	3386		
F001M0.5	492	9.47	0.0173	17 7 62	2814		
F002M0.5	492	9.15	0.0173	17 7 62	2814		
M003M0.5	493	10.80	0.0199	18 9 62	2751		
M004M0.5	475	12.80	0.0199	18 9 62	2751		
F005M0.5	534	7.83	0.0172	23 10 62	2716		
M006M0.5	510	10.30	0.0171	23 10 62	2716		
F007M0.5	492	8.87	0.0172	17 7 62	2814		
F008M0.5	654	12.60	0.0159	9 3 60	3674		
M009M0.5	485	11.90	0.017	13 4 60	3639		
M010M0.5	492	10.60	0.0174	17 7 62	2814		
F011M0.5	505	7.82	0.0202	18 9 62	2751		
M012M0.5	510	10.60	0.0165	23 10 62	2716		
F001M1.0	718	7.75	0.0463	4 1 54		2952	196
F001M1.0A	590	8.07	0.0512	23 10 62	2716		
F002M1.0	459	8.25	0.0324	29 11 54		5267	305
M003M1.0	575	13.80	0.0589	13 3 56		3157	306
M004M1.0	601	9.90	0.0481	15 1 57		4260	131
F005M1.0	658	8.80	0.049	5 3 57		4565	192
M006M1.0	521	10.60	0.0468	23 4 57		3402	272
F007M1.0	534	9.89	0.0489	4 6 57		2159	149
F008M1.0	654	12.40	0.0491	9 3 60	3674		
M009M1.0	485	10.10	0.0504	13 4 60	3639		
M010M1.0	492	9.43	0.0501	17 7 62	2814		
F011M1.0	505	8.91	0.0613	18 9 62	2751		
M012M1.0	528	9.27	0.0498	23 10 62	2716		

DOG NUMBER	COMMENTS ON DEAD DOGS
F001M0.0	PURULENT MENINGOENCEPHALITIS
F002M0.0	
M003M0.0	BRAIN INFARCTION
M004M0.0	VALVULAR ENDOCARDITIS; MYOCARDIAL INFARCTION
F005M0.0	MAMMARY CARCINOMA
M006M0.0	
F007M0.0	STATUS EPILEPTICUS
F007M0.0A	
F008M0.0	
M009M0.0	
M010M0.0	
F011M0.0	
M012M0.0	
F001M0.5	
F002M0.5	
M003M0.5	
M004M0.5	
F005M0.5	
M006M0.5	
F007M0.5	
F008M0.5	
M009M0.5	
M010M0.5	
F011M0.5	
M012M0.5	
F001M1.0	SARCOMA (SPLEEN)
F001M1.0A	
F002M1.0	OSTEOSARCOMA
M003M1.0	OSTEOSARCOMA
M004M1.0	PNEUMONIA; PANCREATITIS
F005M1.0	MALIGNANT MELANOMA (EYE)
M006M1.0	EPIDERMOID CARCINOMA (PENIS)
F007M1.0	SARCOMA (HEART)
F008M1.0	
M009M1.0	
M010M1.0	
F011M1.0	
M012M1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
F001M1.7	510	7.52	0.151	23 10 62	2716		
F002M1.7	560	9.90	0.183	13 3 56		2383	733
M003M1.7	576	11.00	0.18	13 3 56		2709	723
M004M1.7	601	8.94	0.143	15 1 57		2864	424
F005M1.7	658	12.80	0.141	5 3 57		3234	625
M006M1.7	521	10.00	0.144	23 4 57		3424	393
F007M1.7	534	10.20	0.146	4 6 57		2646	600
F008M1.7	654	10.80	0.148	9 3 60		2486	386
M009M1.7	485	12.60	0.149	13 4 60		2799	672
M010M1.7	492	10.10	0.124	17 7 62	2814		
F011M1.7	505	10.70	0.179	18 9 62	2751		
M012M1.7	524	9.28	0.153	23 10 62	2716		
F001M2.0	676	7.60	0.276	4 1 54		1780	870
F002M2.0	517	8.25	0.194	29 11 54		965	198
M003M2.0	576	11.00	0.358	13 3 56		619	355
M004M2.0	601	9.88	0.282	15 1 57		2282	1033
F005M2.0	509	8.30	0.295	5 3 57		2688	928
M006M2.0	502	12.40	0.306	23 4 57		2674	1382
F007M2.0	534	10.10	0.298	4 6 57		2239	1064
F008M2.0	654	12.40	0.3	9 3 60		2386	928
M009M2.0	630	9.99	0.302	13 4 60		1254	561
M010M2.0	430	11.20	0.311	17 7 62		2373	1448
F011M2.0	505	7.03	0.381	18 9 62	2751		
M012M2.0	524	9.47	0.306	23 10 62		2471	1494
F001M3.0	519	10.40	0.858	4 1 54		918	1833
F002M3.0	460	6.70	0.612	29 11 54		1856	2075
M003M3.0	579	10.40	0.965	13 3 56		1185	2464
M004M3.0	601	10.20	0.916	15 1 57		1176	1592
F005M3.0	531	8.51	0.94	5 3 57		1869	2148
M006M3.0	502	9.09	0.953	23 4 57		1421	1906
F007M3.0	534	9.94	0.907	4 6 57		1463	3145
F008M3.0	633	11.80	0.95	9 3 60		1447	2158
M009M3.0	630	9.83	0.918	13 4 60		1570	2277
M010M3.0	581	10.40	1.0	17 7 62		1575	2318
F011M3.0	499	11.00	1.19	18 9 62		1395	2467
M012M3.0	510	12.90	0.987	23 10 62		1638	2365

DOG NUMBER	COMMENTS ON DEAD DOGS
F001M1.7	
F002M1.7	OSTEOSARCOMA
M003M1.7	OSTEOSARCOMA
M004M1.7	CARCINOMA SMALL INTESTINE
F005M1.7	OSTEOSARCOMA
M006M1.7	OSTEOSARCOMA
F007M1.7	OSTEOSARCOMA
F008M1.7	OSTEOSARCOMA
M009M1.7	OSTEOSARCOMA
M010M1.7	
F011M1.7	
M012M1.7	
F001M2.0	OSTEOSARCOMA
F002M2.0	INTESTINAL HEMORRHAGE
M003M2.0	PNEUMONIA
M004M2.0	OSTEOSARCOMA
F005M2.0	OSTEOSARCOMA
M006M2.0	OSTEOSARCOMA
F007M2.0	CHRONIC PANCREATITIS
F008M2.0	OSTEOSARCOMA
M009M2.0	OSTEOSARCOMA
M010M2.0	OSTEOSARCOMA
F011M2.0	
M012M2.0	OSTEOSARCOMA
F001M3.0	OSTEOSARCOMA
F002M3.0	OSTEOSARCOMA
M003M3.0	OSTEOSARCOMA
M004M3.0	OSTEOSARCOMA
F005M3.0	OSTEOSARCOMA
M006M3.0	OSTEOSARCOMA
F007M3.0	OSTEOSARCOMA
F008M3.0	OSTEOSARCOMA
M009M3.0	OSTEOSARCOMA
M010M3.0	OSTEOSARCOMA
F011M3.0	OSTEOSARCOMA
M012M3.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
F001M4.0	510	7.56	2.6	4 1 54	841	5614
F002M4.0	460	6.95	1.86	29 11 54	778	2272
M003M4.0	579	9.65	3.37	13 3 56	418	1795
M003M4.0A	494	7.34	2.64	4 6 57	1063	5604
M004M4.0	609	7.84	2.47	15 1 57	896	2680
F005M4.0	509	9.63	2.67	5 3 57	1064	4358
M006M4.0	502	9.49	2.66	23 4 57	1121	4784
F007M4.0	544	8.40	2.67	4 6 57	1253	4636
F001M5.0	494	7.77	8.11	4 1 54	232	3854
F002M5.0	460	7.35	5.46	29 11 54	780	7666
M003M5.0	579	8.87	10.4	13 3 56	688	14151
M004M5.0	482	7.29	7.89	15 1 57	561	5662
F005M5.0	658	11.10	8.48	5 3 57	770	9559
M006M5.0	580	7.53	8.67	23 4 57	792	6761
F007M5.0	494	7.35	8.92	4 6 57	966	18094

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*(μCi ^{228}Th/μCi ^{228}Ra) injected = 0.15 for F1M1.0, 2.0, 3.0, 4.0, 5.0.

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

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

DOG NUMBER	COMMENTS ON DEAD DOGS
F001M4.0	OSTEOSARCOMA + CRIPPLING FRACTURE
F002M4.0	OSTEOSARCOMA
M003M4.0	STRANGULATED INGUINAL HERNIA
M003M4.0A	OSTEOSARCOMA, NEPHRITIS, ULCERATIVE GINGIVITIS + PNEUMONIA
M004M4.0	FRACTURED MANDIBLE + ULCERATIVE GINGIVITIS
F005M4.0	OSTEOSARCOMA
M006M4.0	OSTEOSARCOMA
F007M4.0	OSTEOSARCOMA
F001M5.0	NEPHRITIS + SEVERE ANEMIA
F002M5.0	CRIPPLING FRACTURES
M003M5.0	ULCERATIVE GINGIVITIS
M004M5.0	CRIPPLING FRACTURE
F005M5.0	ULCERATIVE GINGIVITIS
M006M5.0	OSTEOSARCOMA +CRIPPLING FRACTURE
F007M5.0	ULCERATIVE GINGIVITIS, MYOCARDIAL INFARCTION + GLAUCOMA

D. THORIUM-228 (RADIOTHORIUM)

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
M001T0.0	493	8.24		8	2	54		4895	
M002T0.0	488	7.28		28	9	54		5510	
F003T0.0	797	11.60		6	6	55		2592	
M004T0.0	591	8.10		18	10	55		3072	
M005T0.0	458	10.40		14	10	58	4186		
F006T0.0	484	9.64		10	1	61		171	
F006T0.0A	686	8.61		15	12	60	3393		
M007T0.0	517	10.50		7	2	61		1412	
M008T0.0	535	10.80		24	5	61	3233		
F009T0.0	569	8.28		29	6	61	3197		
F010T0.0	536	10.40		28	7	61	3168		
F011T0.0	530	9.45		4	6	63	2492		
F012T0.0	492	9.09		9	7	63	2457		
M001T0.2	682	11.40	0.00164	27	3	62	2926		
M002T0.2	682	10.40	0.00160	27	3	62	2926		
F003T0.2	478	9.86	0.00163	27	3	62	2926		
M004T0.2	478	10.00	0.00160	27	3	62	2926		
M005T0.2	625	13.80	0.00162	9	2	60		869	10
M005T0.2A	530	13.40	0.00173	4	6	63	2492		
F006T0.2	489	8.85	0.00176	10	1	61	3367		
M007T0.2	532	10.50	0.00159	7	2	61	3339		
M008T0.2	494	13.90	0.00189	24	5	61	3233		
F009T0.2	569	7.82	0.00171	29	6	61	3197		
F010T0.2	508	10.50	0.0017	28	7	61	3168		
F011T0.2	530	9.76	0.00171	4	6	63	2492		
F012T0.2	492	7.37	0.0019	9	7	63	2457		
M001T0.5	699	14.30	0.00496	7	9	56		3471	45
M002T0.5	455	10.50	0.0049	28	9	54		1976	41
F003T0.5	659	8.59	0.00485	6	6	55		3032	44
M004T0.5	516	8.58	0.0054	18	10	55		2159	46
M005T0.5	513	8.46	0.00522	14	10	58	4186		
F006T0.5	489	9.66	0.0051	10	1	61	3367		
M007T0.5	532	9.11	0.00491	7	2	61	3339		
M008T0.5	533	9.53	0.00562	24	5	61	3233		
F009T0.5	569	8.62	0.00529	29	6	61	3197		
F010T0.5	508	10.20	0.0051	28	7	61	3168		
F011T0.5	530	7.78	0.00518	4	6	63	2492		
F012T0.5	492	9.94	0.00567	9	7	63		1682	45

DOG NUMBER	COMMENTS ON DEAD DOGS
M001T0.0	RETICULUM CELL SARCOMA (SOFT TISSUE)
M002T0.0	NEPHRITIS
F003T0.0	BRAIN HEMORRHAGE
M004T0.0	LYMPHOSARCOMA
M005T0.0	
F006T0.0	TRAUMA
F006T0.0A	
M007T0.0	BRAIN HEMORRHAGE
M008T0.0	
F009T0.0	
F010T0.0	
F011T0.0	
F012T0.0	
M001T0.2	
M002T0.2	
F003T0.2	
M004T0.2	
M005T0.2	STRANGULATION ON VOMITUS + GRAND MAL
M005T0.2A	
F006T0.2	
M007T0.2	
M008T0.2	
F009T0.2	
F010T0.2	
F011T0.2	
F012T0.2	
M001T0.5	CEREBRAL INFARCTION HEMORRHAGE
M002T0.5	STRANGULATION ON VOMITUS + GRAND MAL
F003T0.5	PYOMETRITIS + SECONDARY PERITONITIS
M004T0.5	STATUS EPILEPTICUS + PNEUMONIA
M005T0.5	
F006T0.5	
M007T0.5	
M008T0.5	
F009T0.5	
F010T0.5	
F011T0.5	
F012T0.5	LIVER DEGENERATION, ANESTHETIC REACTION

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
M001T1.0	493	9.36	0.0146	8	2	54		3172	132
M002T1.0	699	9.27	0.0146	7	9	56		4570	135
F003T1.0	723	8.84	0.0145	7	9	56		4142	133
M004T1.0	699	8.27	0.0146	7	9	56		3217	132
M005T1.0	513	11.90	0.0146	14	10	58		2886	130
F006T1.0	469	8.81	0.015	10	1	61		3273	136
M007T1.0	532	9.18	0.0147	7	2	61	3339		
M008T1.0	533	8.69	0.0166	24	5	61	3233		
F009T1.0	527	10.00	0.016	29	6	61		2546	140
F010T1.0	508	10.20	0.015	28	7	61	3168		
F011T1.0	521	7.55	0.0154	4	6	63	2492		
F012T1.0	472	9.96	0.0167	9	7	63		1263	118
M001T1.5	699	7.95	0.0289	7	9	56		2894	258
M002T1.5	458	10.00	0.0293	28	9	54		2576	257
F003T1.5	609	10.30	0.0303	6	6	55		1921	249
M004T1.5	591	8.59	0.0299	18	10	55		2309	257
M005T1.5	598	9.65	0.0286	9	2	60		1624	223
F006T1.5	489	8.14	0.0292	10	1	61		2373	252
M007T1.5	517	8.83	0.0292	7	2	61		364	95
M007T1.5A	521	9.08	0.0311	4	6	63	2492		
M008T1.5	494	11.60	0.0324	24	5	61		2665	286
F009T1.5	527	8.80	0.0306	29	6	61		2983	274
F010T1.5	508	11.60	0.0296	28	7	61		1859	241
F011T1.5	518	11.40	0.0305	4	6	63		2408	281
F012T1.5	465	7.56	0.0329	9	7	63		2120	278
M001T2.0	491	10.20	0.0976	8	2	54		1282	693
M002T2.0	483	9.16	0.0875	28	9	54		1234	611
F003T2.0	474	7.87	0.0908	6	6	55		1541	695
M004T2.0	553	13.00	0.09	18	10	55		78	70
M004T2.0A	650	10.60	0.0899	7	9	56		1222	625
M005T2.0	598	9.12	0.0848	9	2	60		1085	556
F006T2.0	451	8.65	0.0879	10	1	61		1108	583
M007T2.0	517	8.85	0.0881	7	2	61		1015	558
M008T2.0	533	10.70	0.0981	24	5	61		1078	641
F009T2.0	527	8.09	0.0979	29	6	61		1209	677
F010T2.0	508	10.70	0.0919	28	7	61		1022	584
F011T2.0	518	10.80	0.0904	4	6	63		1038	579
F012T2.0	464	8.92	0.1	9	7	63		1449	748

DOG NUMBER	COMMENTS ON DEAD DOGS
M001T1.0	OSTEOSARCOMA
M002T1.0	PERIANAL GLAND ADENOMA
F003T1.0	UNDIFFERENTIATED MALIGNANCY (SOFT TISSUE)
M004T1.0	OSTEOSARCOMA THYROID CARCINOMA PERIANAL ADENOCARCINOMA
M005T1.0	STATUS EPILEPTICUS
F006T1.0	STOMACH PERFORATION
M007T1.0	
M008T1.0	
F009T1.0	LEIOMYOSARCOMA
F010T1.0	
F011T1.0	
F012T1.0	PNEUMONIA
M001T1.5	OSTEOSARCOMA
M002T1.5	OSTEOSARCOMA
F003T1.5	COMA OF UNKNOWN ETIOLOGY (NO BONE TUMOR)
M004T1.5	OSTEOSARCOMA
M005T1.5	OSTEOSARCOMA
F006T1.5	OSTEOSARCOMA
M007T1.5	LEPTOSPYROSIS
M007T1.5A	
M008T1.5	OSTEOSARCOMA
F009T1.5	OSTEOSARCOMA
F010T1.5	OSTEOSARCOMA
F011T1.5	OSTEOSARCOMA
F012T1.5	OSTEOSARCOMA
M001T2.0	OSTEOSARCOMA
M002T2.0	OSTEOSARCOMA
F003T2.0	OSTEOSARCOMA
M004T2.0	TRAUMA
M004T2.0A	OSTEOSARCOMA
M005T2.0	OSTEOSARCOMA
F006T2.0	OSTEOSARCOMA
M007T2.0	OSTEOSARCOMA
M008T2.0	OSTEOSARCOMA
F009T2.0	OSTEOSARCOMA
F010T2.0	OSTEOSARCOMA
F011T2.0	OSTEOSARCOMA
F012T2.0	OSTEOSARCOMA

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
M001T3.0	314	9.15	0.301	8	2	54	988	1879
M002T3.0	456	11.90	0.301	28	9	54	859	1736
F003T3.0	471	12.00	0.272	6	6	55	547	1164
M004T3.0	606	9.69	0.285	18	10	55	801	1575
M005T3.0	571	10.70	0.269	9	2	60	890	1584
F006T3.0	451	8.83	0.282	10	1	61	1156	1909
M007T3.0	427	9.90	0.266	7	2	61	861	1536
M008T3.0	494	10.10	0.313	24	5	61	685	1566
F009T3.0	511	11.50	0.298	29	6	61	1062	1933
F010T3.0	508	9.26	0.28	28	7	61	971	1732
F011T3.0	518	10.30	0.29	4	6	63	791	1591
F012T3.0	459	11.50	0.32	9	7	63	804	1773
M001T4.0	480	8.32	0.882	8	2	54	645	4237
M002T4.0	458	8.32	0.916	28	9	54	833	5185
F003T4.0	461	7.25	0.8	6	6	55	763	4290
M004T4.0	606	8.81	0.835	18	10	55	793	4587
M001T5.0	480	9.48	2.76	8	2	54	212	5457
M002T5.0	483	8.22	2.63	28	9	54	97	2535

DOG NUMBER	COMMENTS ON DEAD DOGS
M001T3.0	OSTEOSARCOMA + SEVERE ANEMIA
M002T3.0	OSTEOSARCOMA + TRAUMA
F003T3.0	OSTEOSARCOMA
M004T3.0	OSTEOSARCOMA
M005T3.0	OSTEOSARCOMA
F006T3.0	OSTEOSARCOMA
M007T3.0	OSTEOSARCOMA
M008T3.0	OSTEOSARCOMA
F009T3.0	OSTEOSARCOMA
F010T3.0	OSTEOSARCOMA
F011T3.0	OSTEOSARCOMA
F012T3.0	HEMANGIOSARCOMA (HUMERUS)
M001T4.0	OSTEOSARCOMA + CRIPPLING FRACTURE
M002T4.0	OSTEOSARCOMA, CRIPPLING FRACTURE + NEPHRITIS
F003T4.0	ULCERATIVE GINGIVITIS + NEPHRITIS
M004T4.0	ULCERATIVE GINGIVITIS
M001T5.0	NEPHRITIS
M002T5.0	PANCYTOPENIA

F. STRONTIUM-90

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
F001S0.0	502	8.48		18 1 55		5484	
M002S0.0	600	11.10		14 2 56		3838	
M003S0.0	493	9.03		11 9 57		3516	
F004S0.0	520	8.19		15 10 57	4550		
M005S0.0	542	10.60		19 11 57		4158	
M006S0.0	466	9.68		27 5 58	4326		
F007S0.0A	462	9.46		7 1 59		3303	
F008S0.0	483	9.29		19 5 59	3969		
F009S0.0	549	12.40		11 8 59		708	
F009S0.0A	535	11.20		4 6 63	2492		
M010S0.0	522	13.90		29 9 59	3836		
F011S0.0	541	9.60		3 11 59	3801		
M012S0.0	605	8.99		6 1 60	3737		
F001S1.0	1524	6.84	0.573	18 1 55		308	21
F001S1.0A	521	9.38	0.588	14 2 56	5159		
M002S1.0	567	8.81	0.606	14 2 56		5077	90
M003S1.0	493	10.90	0.572	11 9 57	4584		
F004S1.0	525	8.96	0.56	15 10 57	4550		
M005S1.0	555	10.20	0.532	19 11 57		2705	71
M006S1.0	466	9.56	0.581	27 5 58	4326		
F007S1.0	524	9.94	0.517	11 11 58	4158		
F008S1.0	483	10.80	0.697	19 5 59		2784	79
F009S1.0	549	11.60	0.534	11 8 59		3601	87
M010S1.0	522	11.50	0.558	29 9 59	3836		
F011S1.0	543	10.30	0.55	3 11 59	3801		
M012S1.0	607	13.70	0.559	6 1 60	3737		
F001S1.7	526	7.41	1.78	14 2 56	5159		
M002S1.7	567	11.60	1.84	14 2 56		4297	405
M003S1.7	493	9.19	1.69	11 9 57	4584		
F004S1.7	522	9.60	1.68	15 10 57	4550		
M005S1.7	560	9.85	1.6	19 11 57		1715	165
M005S1.7A	493	11.40	1.78	6 3 63	2582		
M006S1.7	466	10.60	1.72	27 5 58	4326		
F007S1.7	488	10.20	1.6	11 11 58		3990	248
M008S1.7	472	8.47	2.03	19 5 59		1973	214
F009S1.7	549	10.00	1.62	11 8 59	3885		
M010S1.7	519	13.60	1.66	29 9 59		2947	306
F011S1.7	543	11.00	1.68	3 11 59		3180	239

DOG NUMBER	COMMENTS ON DEAD DOGS
F001S0.0	ADRENAL CORTICAL CARCINOMA; MAMMARY CARCINOMA
M002S0.0	BRONCHOGENIC CARCINOMA
M003S0.0	OBTURATING AORTIC EMBOLISM, NEPHRITIS
F004S0.0	
M005S0.0	TRANSITIONAL CELL CARCINOMA
M006S0.0	
F007S0.0A	DIABETES MELLITUS
F008S0.0	
F009S0.0	TRAUMA
F009S0.0A	
M010S0.0	
F011S0.0	
M012S0.0	
F001S1.0	SACRIFICED -IMPROPER INJECTION AGE-
F001S1.0A	
M002S1.0	AORTIC BODY TUMOR
M003S1.0	
F004S1.0	
M005S1.0	STATUS EPILEPTICUS
M006S1.0	
F007S1.0	
F008S1.0	PANCREATIC ISLET CELL CARCINOMA
F009S1.0	FOREIGN BODY PNEUMONIA; ENTERITIS
M010S1.0	
F011S1.0	
M012S1.0	
F001S1.7	
M002S1.7	HEMANGIOSARCOMA (SOFT TISSUE ORIGIN)
M003S1.7	
F004S1.7	
M005S1.7	COMA OF UNKNOWN ETIOLOGY (NO BONE TUMOR)
M005S1.7A	
M006S1.7	
F007S1.7	ARTHRITIS; MAMMARY CARCINOMA
F008S1.7	STATUS EPILEPTICUS, CHRONIC PANCREATITIS
F009S1.7	
M010S1.7	OBTURATING PULMONARY EMBOLISM, NEPHRITIS
F011S1.7	AORTIC THROMBUS; METASTATIC CALCIFICATION OF LUNGS

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
M012S1.7	607	11.90	1.68	6	1	60	3737		
F001S2.0	502	5.59	3.7	18	1	55		3269	477
M002S2.0	567	8.97	3.42	14	2	56		3768	631
M003S2.0	494	7.82	3.39	11	9	57		4295	670
F004S2.0	522	9.68	3.41	15	10	57	4550		
M005S2.0	560	8.72	3.24	19	11	57		3253	531
M006S2.0	466	9.19	3.5	27	5	58	4326		
F007S2.0	488	11.20	3.19	11	11	58		3421	457
F008S2.0	465	9.49	4.14	19	5	59		3955	685
F009S2.0	473	14.10	3.28	11	8	59		2467	566
M010S2.0	508	10.70	3.34	29	9	59		3436	593
F011S2.0	543	10.40	3.41	3	11	59	3801		
M012S2.0	607	11.60	3.49	6	1	60	3737		
F001S3.0	468	7.36	11.6	18	1	55		5126	3289
M002S3.0	565	9.62	11.6	14	2	56		4263	2605
M003S3.0	494	11.40	10.8	11	9	57	4584		
F004S3.0	527	9.17	10.6	15	10	57		3101	1490
M005S3.0	557	8.90	10.1	19	11	57	4515		
M006S3.0	466	9.44	10.9	27	5	58	4326		
F007S3.0	486	9.80	10.1	11	11	58		4018	1816
F008S3.0	465	12.50	12.9	19	5	59	3969		
F009S3.0	468	10.00	10.1	11	8	59	3885		
M010S3.0	519	12.50	10.3	29	9	59		2898	2099
F011S3.0	541	9.00	10.8	3	11	59	3801		
M012S3.0	605	8.43	10.2	6	1	60	3737		
F001S4.0	468	8.74	33.3	18	1	55		3682	6613
M002S4.0	567	11.20	32.6	14	2	56		2093	5688
M003S4.0	593	9.83	32.1	11	9	57		2781	4139
F004S4.0	528	8.24	32.1	15	10	57	4550		
M005S4.0	562	9.65	30.6	19	11	57		4427	6277
M006S4.0	504	16.00	32.7	3	9	58		3530	6899
F007S4.0	478	10.90	30.9	11	11	58	4158		
F008S4.0	465	10.90	40.6	19	5	59		2206	7766
F009S4.0	468	9.56	30.6	11	8	59	3885		
M010S4.0	517	8.20	31.3	29	9	59	3836		

DOG NUMBER	COMMENTS ON DEAD DOGS
M012S1.7	
F001S2.0	BACTERIAL PNEUMONIA
M002S2.0	UNDETERMINED SOFT TISSUE SARCOMA +BRONCHIOGENIC CARCINOMA
M003S2.0	STATUS EPILEPTICUS; THYROID CARCINOMA
F004S2.0	
M005S2.0	ULCERATIVE STOMATITIS
M006S2.0	
F007S2.0	PANCREATIC ISLET CELL ADENOMA
F008S2.0	PNEUMONIA
F009S2.0	UNDETERMINED (NO BONE TUMOR)
M010S2.0	BACTERIAL VALVULAR ENDOCARDITIS
F011S2.0	
M012S2.0	
F001S3.0	
M002S3.0	NEPHRITIS
M003S3.0	
F004S3.0	MAMMARY CARCINOMA
M005S3.0	
M006S3.0	
F007S3.0	MAMMARY CARCINOMA; THYROID CARCINOMA
F008S3.0	
F009S3.0	
M010S3.0	FIBROSARCOMA (GINGIVA)
F011S3.0	
M012S3.0	
F001S4.0	NOT DETERMINED (NO OSTEOSARCOMA)
M002S4.0	SQUAMOUS CELL CARCINOMA -GINGIVA-
M003S4.0	OBTURATING PULMONARY EMBOLISM
F004S4.0	
M005S4.0	HEMANGIOSARCOMA (SPLEEN)
M006S4.0	SEMINOMA
F007S4.0	
F008S4.0	UNDETERMINED (NO BONE TUMOR)
F009S4.0	
M010S4.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
F011S4.0	542	8.86	32.7	3 11 59		2114	3324
M012S4.0	605	10.90	32.3	6 1 60	3737		
F001S4.5	530	9.00	64.2	16 3 66	1476		
M002S4.5	530	12.20	63.6	16 3 66	1476		
M003S4.5	530	11.90	63.8	16 3 66	1476		
F004S4.5	530	9.80	64.5	16 3 66	1476		
M005S4.5	496	13.30	61.3	16 3 66		993	5424
M006S4.5	496	12.00	63.8	16 3 66	1476		
F007S4.5	511	9.90	64.5	16 3 66	1476		
F008S4.5	511	9.90	64.5	16 3 66	1476		
F009S4.5	511	10.30	64.0	16 3 66		1028	5201
M010S4.5	496	14.00	60.9	16 3 66	1476		
F011S4.5	496	11.90	63.8	16 3 66	1476		
M012S4.5	485	11.40	63.7	16 3 66	1476		
F001S5.0	434	9.38	103.0	18 1 55		960	8384
M002S5.0	551	12.20	102.0	14 2 56		255	3213
M002S5.0A	545	11.40	96.6	7 1 59		1740	12295
M003S5.0	507	10.30	102.0	15 10 57		2256	16559
F004S5.0	528	11.40	105.0	15 10 57		1448	9527
M005S5.0	621	8.53	95.2	19 11 57		1285	10243
M006S5.0	504	9.33	98.8	3 9 58		35	652
M006S5.0A	462	11.20	94.2	7 1 59		1021	11538
F007S5.0	478	10.20	92.7	11 11 58		1129	10899
F008S5.0	535	11.20	90.5	7 1 59		1469	11132
F009S5.0	459	8.82	93.5	11 8 59		1982	13607
M010S5.0	517	8.55	95.9	29 9 59		990	7657
F011S5.0	542	8.97	102.0	3 11 59		1667	10016
M012S5.0	606	12.50	99.2	6 1 60		1165	8128

DOG NUMBER	COMMENTS ON DEAD DOGS
F011S4.0	BLOOD DYSCRASIA, PYOMETRA
M012S4.0	
F001S4.5	
M002S4.5	
M003S4.5	
F004S4.5	
M005S4.5	OSTEOSARCOMA
M006S4.5	
F007S4.5	
F008S4.5	
F009S4.5	OSTEOSARCOMA
M010S4.5	
F011S4.5	
M012S4.5	
F001S5.0	OSTEOSARCOMA
M002S5.0	STRANGULATED INGUINAL HERNIA
M002S5.0A	OSTEOSARCOMA
M003S5.0	OSTEOSARCOMA
F004S5.0	OSTEOSARCOMA
M005S5.0	SEVERE ANEMIA, AUTOAGGLUTINATION, INFARCTION, SPLENOMEGALY
M006S5.0	INTESTINAL HEMORRHAGE
M006S5.0A	OSTEOSARCOMA, INFARCTION + THROMBOCYTOPENIA
F007S5.0	STATUS EPILEPTICUS
F008S5.0	OSTEOSARCOMA
F009S5.0	SQUAMOUS CELL CARCINOMA ARISING FROM FRONTAL SINUS
M010S5.0	SEVERE ANEMIA + THROMBOCYTOPENIA
F011S5.0	HEMANGIOSARCOMA (LEFT MANDIBLE)
M012S5.0	HEMANGIOSARCOMA (RIB)

TABLE II. TEST ANIMALS (MAR. 31 1970)

A. RADIUM-226*

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DEATH	DOSE TO SKELETON (RADS)
T001R5.0	995	11.10	10.3	1	12	52	1074		9469
T002R5.0	919	8.40	4.39	12	1	53	1368		4724
T003R5.0	1467	8.29	4.76	12	1	53	428		1316
T004R5.0	459	10.00	10.6	6	7	53	1		24
T005R5.0	126	6.14	11.7	6	10	53	1		25
T006R5.0	126	6.14	11.4	6	10	53	1		24
T007R5.0	126	6.14	11.8	6	10	53	1		25
T008R5.0	290	5.52	1.92	10	5	55	58		488
T009R5.0	2276	10.40	1.94	10	5	55	58		585
T010R5.0	43	1.02	1.96	10	5	55	49		293
T011R5.0	43	1.58	1.91	10	5	55	49		363
T012R5.0	397	12.30	9.72	9	5	56	225		2707
T013R5.0	397	7.59	9.76	9	5	56	188		2319
T014R4.0	674	8.12	3.17	11	7	56	72		380
T015R4.0	672	9.03	3.11	11	7	56	2127		5117
T016R5.0	604	12.40	9.68	11	7	57	12		183
T017R5.0H	383	12.20	9.87	28	10	58	1147		13655
T018R5.0H	383	11.10	10.8	28	10	58	1226		12628
T019R5.0H	383	11.30	10.7	28	10	58	1219		11580
T020R5.0H	383	11.40	10.6	28	10	58	1330		12937
T021R5.0H	381	11.80	10.1	28	10	58	386		3710
T022R5.0H	381	11.90	10.1	28	10	58	587		5911
T023R4.0H	384	9.50	4.05	25	11	58	1471		4340
T024R4.0H	384	11.90	3.24	25	11	58	1505		5593
T025R4.0H	379	11.30	3.42	25	11	58	1309		4672
T026R4.0H	379	11.00	3.48	25	11	58	1780		4719
T027R4.0H	372	11.50	3.34	25	11	58	1414		3382
T028R3.0H	372	11.70	1.11	25	11	58	387		357
T029R5.0	474	13.50	10.4	3	3	59	216		3404

DOG NUMBER	COMMENTS ON DEAD DOGS
T001R5.0	OSTEOSARCOMA
T002R5.0	OSTEOSARCOMA
T003R5.0	SPECIAL STUDY
T004R5.0	SPECIAL STUDY
T005R5.0	SPECIAL STUDY
T006R5.0	SPECIAL STUDY
T007R5.0	SPECIAL STUDY
T008R5.0	SPECIAL STUDY
T009R5.0	SPECIAL STUDY
T010R5.0	SPECIAL STUDY
T011R5.0	SPECIAL STUDY
T012R5.0	SPECIAL STUDY
T013R5.0	SPECIAL STUDY
T014R4.0	SPECIAL STUDY
T015R4.0	OSTEOSARCOMA
T016R5.0	SPECIAL STUDY
T017R5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T018R5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T019R5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T020R5.0H	OSTEOSARCOMA + ULCERATIVE GINGIVITIS
T021R5.0H	NEPHRITIS
T022R5.0H	CRIPPLING FRACTURES
T023R4.0H	OSTEOSARCOMA
T024R4.0H	OSTEOSARCOMA
T025R4.0H	OSTEOSARCOMA
T026R4.0H	OSTEOSARCOMA
T027R4.0H	OSTEOSARCOMA
T028R3.0H	SPECIAL STUDY
T029R5.0	NEPHRITIS

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T030R5.0	474	11.50	10.4	3 3 59	178	2851
T031R5.0	471	10.50	10.4	3 3 59	303	4758
T032R3.0	471	11.40	1.13	3 3 59	2294	1864
T033R3.0	471	10.60	1.15	3 3 59	1822	1921
T034R3.0	470	15.70	1.12	3 3 59	1737	1605
T035R3.0J	670	9.44	0.951	5 5 59	8	14
T036R4.0	695	10.20	2.99	22 12 60	1154	3647
T037R4.0	695	9.53	3.0	22 12 60	1627	3234
T038R4.0	695	10.10	3.02	22 12 60	1503	3657
T040R1.0	899	13.00	0.0483	3 4 62	7	1
T041R1.0	899	12.70	0.0487	3 4 62	63	3
T042R1.7	967	14.00	0.146	4 4 62	7	2
T043R1.7	963	13.20	0.145	4 4 62	64	11
T044R3.0	938	11.10	0.937	4 4 62	68	71
T045R3.0	939	13.60	0.941	5 4 62	7	12
T046R3.0	810	12.50	0.928	5 4 62	69	105
T047R6.0	99	5.27	29.4	11 6 62	4	352
T048R6.0	2842	11.20	25.1	27 12 62	49	1597
T049R5.0	485	10.60	7.54	2 5 63	5	111
T050R5.0	485	13.70	7.46	2 5 63	15	274
T051R5.0	418	13.30	8.48	8 5 63	92	1689
T052R5.0	418	10.70	8.57	8 5 63	15	258
T053R5.0	418	12.00	8.5	8 5 63	33	611

DOG NUMBER	COMMENTS ON DEAD DOGS
T030R5.0	NEPHRITIS
T031R5.0	NEPHRITIS
T032R3.0	OSTEOSARCOMA
T033R3.0	OSTEOSARCOMA, NEPHRITIS
T034R3.0	OSTEOSARCOMA
T035R3.0J	SPECIAL STUDY
T036R4.0	OSTEOSARCOMA
T037R4.0	OSTEOSARCOMA
T038R4.0	OSTEOSARCOMA
T040R1.0	SPECIAL STUDY
T041R1.0	SPECIAL STUDY
T042R1.7	SPECIAL STUDY
T043R1.7	SPECIAL STUDY
T044R3.0	SPECIAL STUDY
T045R3.0	SPECIAL STUDY
T046R3.0	SPECIAL STUDY
T047R6.0	SPECIAL STUDY
T048R6.0	LEUKOPENIA, PNEUMONIA + SPECIAL MELANOMA STUDY
T049R5.0	SPECIAL STUDY
T050R5.0	SPECIAL STUDY
T051R5.0	SPECIAL STUDY
T052R5.0	SPECIAL STUDY
T053R5.0	SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T054R5.0	417	11.40	8.76	22 5 63	5	88
T055R5.0	417	11.60	8.61	22 5 63	33	534
T056R5.0	417	11.60	8.61	22 5 63	90	1461
T057R4.0	501	12.10	2.72	15 8 63	14	54
T058R4.0	496	11.70	2.41	15 8 63	61	286
T059R4.0	496	9.64	2.57	15 8 63	63	272
T060R4.0	490	12.10	2.33	15 8 63	117	415
T061R4.0	490	9.48	2.7	15 8 63	371	1567
T062R4.0	490	8.63	2.68	15 8 63	460	1586
T063R3.0	559	8.72	0.899	29 1 64	36	51
T064R3.0	551	8.42	0.919	29 1 64	63	73
T065R3.0	551	11.60	0.922	29 1 64	70	99
T066R3.0	549	10.10	0.904	29 1 64	132	140
T067R3.0	549	12.70	0.898	29 1 64	134	168
T068R3.0	549	12.10	0.917	29 1 64	1667	1033
T069R3.0	499	8.84	0.919	29 1 64	622	651
T070R3.0	499	14.20	0.922	29 1 64	1996	2114
T071R5.0	4025	13.80	9.23	28 1 69	42	698

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

T35R3J also received 99 μCi ^{85}Sr.

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

DOG NUMBER	COMMENTS ON DEAD DOGS
T054R5.0	SPECIAL STUDY
T055R5.0	SPECIAL STUDY
T056R5.0	SPECIAL STUDY
T057R4.0	SPECIAL STUDY
T058R4.0	SPECIAL STUDY
T059R4.0	SPECIAL STUDY
T060R4.0	SPECIAL STUDY
T061R4.0	SPECIAL STUDY
T062R4.0	SPECIAL STUDY
T063R3.0	SPECIAL STUDY
T064R3.0	SPECIAL STUDY
T065R3.0	SPECIAL STUDY
T066R3.0	SPECIAL STUDY
T067R3.0	SPECIAL STUDY
T068R3.0	OSTEOSARCOMA
T069R3.0	SPECIAL STUDY
T070R3.0	OSTEOSARCOMA
T071R5.0	MELANOMA ORAL CAVITY

B. PLUTONIUM-239*

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi /KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T000P5.0	647	11.40	3.05	24	6	52	1	10
T001P5.0	581	12.70	3.04	13	10	52	29	160
T002P5.0	914	11.90	6.85	15	9	52	44	520
T003P5.0	942	9.65	3.22	13	10	52	610	3040
T004P5.0	1016	8.78	3.02	13	10	52	365	1750
T005P5.0	474	10.40	2.69	14	12	54	400	1720
T006P5.0	527	6.16	2.73	14	12	54	406	1750
T007P5.0	475	7.40	2.68	14	12	54	777	3230
T008P5.0	527	8.32	2.67	14	12	54	863	3540
T009P5.0	551	10.30	2.8	22	11	55	15	80
T010P5.0	534	11.90	2.74	23	11	55	15	70
T011P5.0	516	12.10	2.76	22	11	55	28	140
T012P5.0	487	9.23	2.74	23	11	55	28	140
T013P5.0	587	8.27	3.16	24	4	56	3	20
T014P5.0	587	9.38	2.43	24	4	56	7	30
T015P5.0	737	8.32	2.79	15	10	56	1	10
T016P5.0	673	10.70	2.85	10	10	56	92	440
T017P5.0	739	11.10	3.01	12	2	57	210	990
T018P5.0	739	8.16	2.83	12	2	57	217	960
T019P5.0	688	8.86	2.91	15	12	60	1400	6129
T020P5.0	688	13.00	2.68	15	12	60	474	1970
T021P5.0	688	10.30	2.72	15	12	60	939	3940
T023P1.0	1485	13.10	0.0172	28	7	61	96	3
T024P1.0	559	13.10	0.0172	28	7	61	97	3
T025P1.0	559	13.80	0.0167	28	7	61	467	10
T026P1.0	556	12.00	0.016	28	7	61	647	20
T027P3.0	556	11.50	0.332	28	7	61	755	390
T028P1.0	552	10.50	0.015	9	8	61	559	10
T029P3.0	552	12.10	0.296	9	8	61	560	260

DOG NUMBER	COMMENTS ON DEAD DOGS
T000P5.0	SPECIAL STUDY
T001P5.0	SPECIAL STUDY
T002P5.0	SPECIAL STUDY
T003P5.0	SPECIAL STUDY
T004P5.0	SPECIAL STUDY
T005P5.0	SPECIAL STUDY
T006P5.0	SPECIAL STUDY
T007P5.0	SPECIAL STUDY
T008P5.0	SPECIAL STUDY
T009P5.0	SPECIAL STUDY
T010P5.0	SPECIAL STUDY
T011P5.0	SPECIAL STUDY
T012P5.0	SPECIAL STUDY
T013P5.0	SPECIAL STUDY
T014P5.0	SPECIAL STUDY
T015P5.0	SPECIAL STUDY
T016P5.0	SPECIAL STUDY
T017P5.0	SPECIAL STUDY
T018P5.0	SPECIAL STUDY
T019P5.0	OSTEOSARCOMA,BLOOD DYSCRASIA,LIVER DEGENERATION
T020P5.0	LIVER DEGENERATION, ASCITES + THROMBOCYTOPENIA
T021P5.0	TOXIC NEPHRITIS + LIVER DEGENERATION
T023P1.0	SPECIAL STUDY
T024P1.0	SPECIAL STUDY
T025P1.0	SPECIAL STUDY
T026P1.0	SPECIAL STUDY
T027P3.0	SPECIAL STUDY
T028P1.0	SPECIAL STUDY
T029P3.0	SPECIAL STUDY

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T030P1.0	548	12.40	0.0148	9 8 61	35	1
T031P3.0	519	13.00	0.305	9 8 61	40	20
T032P1.0	520	8.47	0.0162	9 8 61	274	10
T033P1.0	550	10.70	0.0153	15 9 61	375	10
T034P1.0	550	9.68	0.0154	15 9 61	746	20
T035P3.0	550	11.90	0.303	15 9 61	362	180
T036P1.0	544	10.40	0.0158	15 9 61	5	
T037P1.0	542	8.59	0.0148	15 9 61	186	4
T038P3.0	489	7.96	0.304	15 9 61	187	90
T039P1.0	1534	10.70	0.0151	15 9 61	376	10
T040P1.0	1534	9.92	0.0177	15 9 61	769	20
T041P5.0	543	8.50	3.01	30 11 64	1227	5586
T042P5.0	510	11.40	2.4	10 2 65	13	57
T043P5.0H	600	14.00	2.86	15 7 65	40	200
T044P5.0H	517	12.00	2.72	21 9 65	35	168
T045P5.0H	420	12.30	2.98	28 10 65	5/24	1
T046P5.0	420	11.90	3.01	28 10 65	732	3402
T047P5.0	803	12.40	3.02	30 11 65	69	353
T048P5.0	554	8.50	2.61	11 3 66	1327	5222
T049P1.0	103	5.00	0.0162	5 7 66	1365	

DOG NUMBER	COMMENTS ON DEAD DOGS
T030P1.0	SPECIAL STUDY
T031P3.0	SPECIAL STUDY
T032P1.0	SPECIAL STUDY
T033P1.0	SPECIAL STUDY
T034P1.0	SPECIAL STUDY
T035P5.0	SPECIAL STUDY
T036P1.0	SPECIAL STUDY
T037P1.0	SPECIAL STUDY
T038P3.0	SPECIAL STUDY
T039P1.0	SPECIAL STUDY
T040P1.0	SPECIAL STUDY
T041P5.0	PURPURA HEMORRHAGICA;AUTOHEMAGGLUTINATION;LIVER DEGENERATN
T042P5.0	SPECIAL STUDY
T043P5.0H	SPECIAL STUDY
T044P5.0H	SPECIAL STUDY
T045P5.0H	SPECIAL STUDY
T046P5.0	LIVER DEGENERATION
T047P5.0	SPECIAL STUDY
T048P5.0	UNDIFFERETIATED SARCOMA (BONE)
T049P1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μCi /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DEATH	DOSE TO SKELETON (RADS)
T050P3.0	103	5.30	0.296	5 7 66	1365		
T051P5.0	104	4.80	2.73	6 7 66		1055	4382
T052P4.0	437	11.80	0.949	7 7 67		14	24
T053P5.0	1517	13.90	2.82	11 3 69	385		
T054P5.0	906	11.30	2.77	11 3 69	385		
T055P4.0	445	10.60	0.785	3 6 69		14	20
T056P5.5	501	11.20	3.73	29 7 69		7	48
T057P2.0E	618	49.40	0.0961	10 9 69	202		
T058P3.0E	573	52.30	0.291	10 9 69	202		
T059P3.0E	591	44.50	0.29	5 11 69	146		
T060P3.0E	567	45.20	0.314	6 1 70	84		
T061P2.0E	581	47.20	0.0983	6 1 70	84		
T062P2.0E	583	52.50	0.156	22 1 70	68		

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

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

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

T57, 60 and 61P2E and T58, 59, and 60P3E are St. Bernards.

DOG NUMBER	COMMENTS ON DEAD DOGS
T050P3.0	
T051P5.00	OSTEOSARCOMA
T052P4.0	SPECIAL STUDY
T053P5.0	
T054P5.0	
T055P4.0	SPECIAL STUDY
T056P5.5	SPECIAL STUDY
T057P2.0E	
T058P3.0E	
T059P3.0E	
T060P3.0E	
T061P2.0E	
T062P2.0E	

C. RADIUM-228 (MESOTHORIUM)*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T001M4.5	529	9.13	4.23	8 9 54	314	1598
T002M4.5	463	8.93	4.27	8 9 54	755	5097
T003M5.0	579	9.15	10.6	13 3 56	700	15867

* (μCi ^{228}Th/μCi ^{228}Ra) injected = 0.03.

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

D. THORIUM-228 (RADIOTHORIUM)*

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T001T5.0	490	9.30	4.88	1 12 53	23	1161
T002T5.0	501	8.48	2.56	8 2 54	77	1979
T003T4.0	429	10.40	0.87	8 2 54	820	4878
T004T5.0	455	8.92	2.59	28 9 54	113	2883
T005T5.0	455	10.10	2.32	28 9 54	65	1524
T006T4.0	591	7.01	0.884	18 10 55	651	4273
T007T3.0	591	9.23	0.298	18 10 55	910	1777
T008T3.0	606	9.23	0.293	14 10 58	1043	1883
T009T3.0	447	11.00	0.285	4 2 59	1	3
T010T3.0	447	14.20	0.289	4 2 59	8	24
T011T3.0	500	8.62	0.335	16 6 59	22	76
T012T3.0	514	10.60	0.302	7 7 59	22	69
T013T3.0	754	13.10	0.298	28 7 59	22	68

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

DOG NUMBER	COMMENTS ON DEAD DOGS
T001T5.0	DIED, SPECIAL STUDY
T002T5.0	SPECIAL STUDY
T003T4.0	CRIPPLING FRACTURES + NEPHRITIS
T004T5.0	THROMBOCYTOPENIA + PURPURA
T005T5.0	NEPHRITIS, THROMBOCYTOPENIA + PURPURA
T006T4.0	CRIPPLING FRACTURES
T007T3.0	SPECIAL STUDY
T008T3.0	OSTEOSARCOMA
T009T3.0	SPECIAL STUDY
T010T3.0	SPECIAL STUDY
T011T3.0	SPECIAL STUDY
T012T3.0	SPECIAL STUDY
T013T3.0	SPECIAL STUDY

E. STRONTIUM-90*

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T001S0.0	151	7.71		5 3 54	2	
T002S5.0	149	6.85	148.0	5 3 54	11	626
T003S5.0	144	6.19	148.0	5 3 54	18	957
T004S5.0	151	7.05	148.0	5 3 54	41	1945
T005S5.0	144	5.25	148.0	5 3 54	116	4863
T006S5.0	155	7.01	87.0	16 3 54	1/24	2
T007S5.0	155	6.74	87.0	16 3 54	2	84
T008S0.0	243	7.00				
T008S2.0H	67	3.69	2.74	27 9 55	66	31
T009S2.0H	67	2.79	3.62	27 9 55	66	40
T010S2.0H	67	3.11	3.25	27 9 55	132	93
T011S2.0H	67	3.85	2.62	27 9 55	132	75
T012S3.0	593	10.60	10.5	11 9 57	5	15
T013S4.0	324	10.50	19.1	8 7 60	8	50
T014S5.0	542	10.00	96.1	7 11 61	9	175
T015S5.0	595	9.43	98.4	7 11 61	30	423
T016S2.0	604	9.71	3.27	8 11 61	9	6
T017S6.0	670	7.18	295.0	19 1 62	14	689
T018S6.0	670	5.94	302.0	19 1 62	1369	18913
T019S6.0	670	5.43	284.0	19 1 62	24	637

DOG NUMBER	COMMENTS ON DEAD DOGS
T001S0.0	SPECIAL STUDY
T002S5.0	SPECIAL STUDY
T003S5.0	SPECIAL STUDY
T004S5.0	SPECIAL STUDY
T005S5.0	SPECIAL STUDY
T006S5.0	SPECIAL STUDY
T007S5.0	SPECIAL STUDY
T008S0.0	SPECIAL STUDY
T008S2.0H	SPECIAL STUDY
T009S2.0H	SPECIAL STUDY
T010S2.0H	SPECIAL STUDY
T011S2.0H	SPECIAL STUDY
T012S3.0	BREMSSTRAHLUNG PHANTOM
T013S4.0	BREMSSTRAHLUNG PHANTOM SAM MCGEE
T014S5.0	SPECIAL STUDY
T015S5.0	SPECIAL STUDY
T016S2.0	SPECIAL STUDY
T017S6.0	LEUKOPENIA, THROMBOCYTOPENIA + PURPURA
T018S6.0	HEMANGIOSARCOMA (ISCHIUM)
T019S6.0	LEUKOPENIA, THROMBOCYTOPENIA + PURPURA

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T020S4.0J	440	8.54	28.9	2 10 63	13	78
T021S2.5J	363	7.20	8.3	2 10 63	13	143
T022S5.0	545	9.01	99.0	1 4 69	364	
T023S5.0	545	11.60	100.0	1 4 69	364	

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

T20S4.0J received 0.5 μCi ^{85}Sr.

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

DOG NUMBER	COMMENTS ON DEAD DOGS
T020S4.0J	SPECIAL STUDY
T021S2.5J	SPECIAL STUDY
T022S5.0	
T023S5.0	

F. RADIUM-224*

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
T001Q3.0J	460	9.55	0.875	26 3 63		4/24	
T002Q4.0	466	12.00	2.91	27 3 63		2317	100
T003Q4.0	466	13.10	2.91	27 3 63	2561		
T004Q5.0	480	9.55	9.71	24 4 63		1462	400
T005Q5.0	455	9.67	9.59	24 4 63		1638	400
T006Q6.0	455	8.29	21.4	17 10 63		13	800
T007Q5.0	465	11.80	8.56	6 11 63		2053	400
T008Q5.0	475	9.77	8.62	6 11 63		16	300
T009Q4.0	503	9.80	2.57	4 12 63		1451	100
T010Q4.0	503	10.30	2.57	4 12 63		262	100
T011Q3.0	495	9.10	0.885	4 12 63	2309		
T012Q3.0	495	13.50	0.889	4 12 63	2309		
T013Q3.0	495	11.30	0.912	4 12 63	2309		
T014Q3.0	438	10.30	0.87	4 12 63	2309		
T015Q4.0	515	12.70	2.73	1 2 68	789		
T016Q2.0	515	9.36	0.31	1 2 68	789		
T017Q2.0	515	10.20	0.311	1 2 68	789		
T018Q2.0	502	9.68	0.306	1 2 68	789		

DOG NUMBER	COMMENTS ON DEAD DOGS
T001Q3.0J	SPECIAL STUDY
T002Q4.00	OSTEOSARCOMA
T003Q4.0	
T004Q5.0	OSTEOSARCOMA, EPIDERMOID CARCINOMA (FRONTAL SINUS)
T005Q5.0	OSTEOSARCOMA
T006Q6.0	PURPURA HEMORRHAGICA
T007Q5.0	OSTEOSARCOMA
T008Q5.0	PURPURA HEMORRHAGICA
T009Q4.0	STRANGULATION ON VOMITUS AND GRAND MAL
T010Q4.0	STATUS EPILEPTICUS
T011Q3.0	
T012Q3.0	
T013Q3.0	
T014Q3.0	
T015Q4.0	
T016Q2.0	
T017Q2.0	
T018Q2.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T019Q1.0	515	11.80	0.0475	1 2 68	789	
T020Q1.0	515	10.40	0.0472	1 2 68	789	
T021Q1.0	502	9.08	0.0447	1 2 68	789	

* The skeletal doses in rads are only from ^{224}Ra and its daughters. In at least some of these dogs, appreciable ^{210}Pb contamination was injected. The dose from the ^{210}Pb has not been included. (See the article ^{210}Pb Contamination of ^{228}Th: Its Contribution to Dose in Beagles in Our ^{228}Th Toxicity Studies in COO-119-237).

T001Q3.0J received 18.0 μCi ^{85}Sr.

DOG NUMBER	COMMENTS ON DEAD DOGS
T019Q1.0	
T020Q1.0	
T021Q1.0	

G. AMERICIUM-241*

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
T001W5.0	517	10.40	2.78	28 6 66		401	1636
T002W5.0	517	12.70	2.83	28 6 66		448	1734
T003W4.0	517	12.60	0.897	28 6 66	1372		
T004W4.0	517	9.40	0.911	28 6 66	1372		
T005W3.0	561	15.00	0.305	15 9 66	1293		
T006W3.0	561	11.90	0.31	15 9 66	1293		
T007W2.0	561	12.60	0.0952	15 9 66	1293		
T008W2.0	561	11.70	0.0957	15 9 66	1293		
T009W1.0	517	8.60	0.016	15 9 66	1293		
T010W1.0	517	9.90	0.0162	15 9 66	1293		
T011W0.5	526	8.17	0.00532	13 10 66	1265		
T012W0.5	526	11.90	0.00539	13 10 66	1265		
T013W0.2	526	10.60	0.00179	13 10 66	1265		
T014W0.2	526	14.90	0.00178	13 10 66	1265		
T015W5.5	858	11.50	4.53	23 10 67		1	10
T016W5.0	461	10.70	2.78	29 1 68		22	80
T017W4.0	523	9.87	0.924	21 3 68	740		

DOG NUMBER	COMMENTS ON DEAD DOGS
T001W5.0	LIVER DEGENERATION; KIDNEY DEGENERATION
T002W5.0	LIVER DEGENERATION; KIDNEY DEGENERATION
T003W4.0	
T004W4.0	
T005W3.0	
T006W3.0	
T007W2.0	
T008W2.0	
T009W1.0	
T010W1.0	
T011W0.5	
T012W0.5	
T013W0.2	
T014W0.2	
T015W5.5	SPECIAL STUDY
T016W5.0	SPECIAL STUDY
T017W4.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DEATH	DOSE TO SKELETON (RADS)
T018W3.0	525	8.60	0.307	21	3	68	740		
T019W2.0	515	13.40	0.097	21	3	68	740		
T020W1.0	513	10.80	0.0161	21	3	68	740		
T021W1.0	513	9.36	0.0166	21	3	68		235	5
T021W1.0A	552	11.40	0.0159	25	11	69	126		
T022W1.0	487	11.60	0.0164	21	3	68	740		
T023W0.5	487	12.20	0.0053	21	3	68	740		
T024W0.2	477	11.30	0.00181	21	3	68	740		
T025W4.0	475	10.50	0.927	8	5	68	692		
T026W3.0	473	12.40	0.31	8	5	68	692		
T027W2.0	473	12.70	0.0961	8	5	68	692		
T028W1.0	472	12.10	0.0156	8	5	68	692		

DOG NUMBER	COMMENTS ON DEAD DOGS
T018W3.0	
T019W2.0	
T020W1.0	
T021W1.0	ACCIDENTAL STRANGULATION
T021W1.0A	
T022W1.0	
T023W0.5	
T024W0.2	
T025W4.0	
T026W3.0	
T027W2.0	
T028W1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D	MO	YR	DAYS SINCE INJECTION 31/3/70	DEATH	DOSE TO SKELETON (RADS)
T029W0.5	472	10.40	0.00548	8	5	68	692		
T030W0.5	472	10.60	0.00538	8	5	68	692		
T031W0.2	472	10.90	0.0018	8	5	68	692		
T032W5.5	568	11.00	4.46	30	4	68		7	40
T033W5.5	593	10.50	4.47	30	4	68		8	46
T034W4.0	477	10.70	0.893	2	7	68	637		
T035W4.0	477	8.87	0.902	2	7	68	637		
T036W3.0	477	11.00	0.305	2	7	68	637		
T037W3.0	468	8.44	0.294	2	7	68	637		
T038W2.0	477	9.88	0.0945	2	7	68	637		
T039W2.0	468	9.21	0.0948	2	7	68	637		
T040W0.5	467	9.40	0.00528	2	7	68	637		
T041W0.2	467	11.90	0.0018	2	7	68	637		
T042W1.7	495	9.26	0.0484	30	7	68	609		
T043W1.7	492	10.40	0.0481	30	7	68	609		
T044W1.7	492	7.46	0.0473	30	7	68	609		
T045W1.7	492	11.90	0.0486	30	7	68	609		
T046W1.7	484	8.42	0.0479	30	7	68	609		
T047W1.7	484	11.10	0.0486	30	7	68	609		

DOG NUMBER	COMMENTS ON DEAD DOGS
T029W0.5	
T030W0.5	
T031W0.2	
T032W5.5	SPECIAL STUDY
T033W5.5	SPECIAL STUDY
T034W4.0	
T035W4.0	
T036W3.0	
T037W3.0	
T038W2.0	
T039W2.0	
T040W0.5	
T041W0.2	
T042W1.7	
T043W1.7	
T044W1.7	
T045W1.7	
T046W1.7	
T047W1.7	

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DEATH	DOSE TO SKELETON (RADS)
T048W0.2	484	11.00	0.00174	30 7 68	609		
T049W0.2	498	10.70	0.00175	25 11 69	126		
T050W0.5	552	11.80	0.00526	25 11 69	126		
T051W1.0	552	8.25	0.0163	25 11 69	126		
T052W1.7	552	9.57	0.0493	25 11 69	126		
T053W2.0	498	9.24	0.096	25 11 69	126		
T054W3.0	498	10.50	0.306	25 11 69	126		
T055W4.0	498	8.37	0.914	25 11 69	126		
T056W5.0	552	11.30	2.9	25 11 69		15	50
T057W5.0	496	7.01	2.77	26 1 70		15	47
T058W0.2	496	10.40	0.00168	26 1 70	64		
T059W0.5	496	11.50	0.00503	26 1 70	64		
T060W1.0	496	10.00	0.0157	26 1 70	64		

DOG NUMBER	COMMENTS ON DEAD DOGS
T048W0.2	
T049W0.2	
T050W0.5	
T051W1.0	
T052W1.7	
T053W2.0	
T054W3.0	
T055W4.0	
T056W5.0	SPECIAL STUDY
T057W5.0	SPECIAL STUDY
T058W0.2	
T059W0.5	
T060W1.0	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70 DEATH	DOSE TO SKELETON (RADS)
T0681W1.7	496	10.70	0.0458	26 1 70	64	
T0682W0.2	486	13.30	0.00175	24 2 70	35	
T0683W0.5	486	11.40	0.00524	24 2 70	35	
T0684W1.0	486	10.40	0.0158	24 2 70	35	
T0685W1.7	486	11.20	0.0471	24 2 70	35	
T0686W2.0	486	9.12	0.0935	24 2 70	35	
T0687W3.0	485	11.80	0.295	24 2 70	35	
T0688W4.0	485	11.80	0.89	24 2 70	35	

* Preliminary measurements indicate the liver dose to be approximately 4 times that to the skeleton.

DOG NUMBER	COMMENTS ON DEAD DOGS
T061W1.7	
T062W0.2	
T063W0.5	
T064W1.0	
T065W1.7	
T066W2.0	
T067W3.0	
T068W4.0	

H. LEAD-21A*

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
T001L5.0	522	9.78	10.7	24 6 69	280		
T002L5.0	522	9.16	10.7	24 6 69		28	180
T003L5.0	522	9.78	10.7	24 6 69	280		

DOG NUMBER	COMMENTS ON DEAD DOGS
T001L5.0	
T002L5.0	SPECIAL STUDY
T003L5.0	

I. ANCILLARY*

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi /KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
F001A						1383	
F002A						2492	
M003A						1451	
M004A						3345	
M005A						4713	
M006A						5266	
M007A						3896	
M008A						3746	
F009A						3719	
F010A						2605	
F011A						4198	
F012A						4219	
F013A						4527	
F014A						3777	
F015A						4874	
F016A						4415	
F017A						2145	
F018A						5921	
F019A						4166	
F020A						2464	
F021A						5508	
F022A						4350	
M023A						1741	
M024A						3074	
F025A						5646	
M026A						4133	
M027A						2130	
M028A						3114	
M029A						5382	
F031A						5631	
F032A						1990	
F033A						3282	
F034A						2564	
M035A						529	
M036A						1971	
M037A						4089	
F038A						3802	
M039A						4554	
M040A						4666	
F041A						4704	
M042A						1265	
F043A						3881	
F044A						5016	
F045A					5493		

DOG NUMBER	COMMENTS ON DEAD DOGS
F001A	SPECIAL STUDY
F002A	SPECIAL STUDY
M003A	SPECIAL STUDY
M004A	NOT DETERMINED
M005A	TRANSITIONAL CELL CARCINOMA, NEPHRITIS, PNEUMONIA
M006A	BRAIN HEMORRHAGE
M007A	LYMPHOSARCOMA
M008A	PROGRESSIVE PARALYSIS, CAUSE UNKNOWN
F009A	VAGINAL FIBROMA
F010A	SPECIAL STUDY
F011A	MAMMARY CARCINOMA
F012A	SEVERE OSTEOARTHRITIS
F013A	SPECIAL STUDY
F014A	OBTURATING EMBOLISM OF PORTAL VEIN
F015A	TRANSITIONAL CELL CARCINOMA URINARY BLADDER
F016A	SPECIAL STUDY
F017A	TRAUMA
F018A	NEPHRITIS
F019A	MAMMARY GLAND CARCINOMA
F020A	SPECIAL STUDY
F021A	MAMMARY CARCINOMA; THYROID CARCINOMA
F022A	LYMPHOSARCOMA
M023A	OBTURATING PULMONARY EMBOLISM
M024A	SPECIAL STUDY
F025A	ISLET CELL TUMOR; PNEUMONIA
M026A	SEMINOMA
M027A	SPECIAL STUDY
M028A	SPECIAL STUDY
M029A	MELANOMA ORAL CAVITY
F031A	
F032A	LYMPHOSARCOMA
F033A	OBTURATING PULMONARY EMBOLISM
F034A	SPECIAL STUDY
M035A	SPECIAL STUDY
M036A	SPECIAL STUDY
M037A	SPECIAL STUDY
F038A	MAMMARY CARCINOMA
M039A	OBTURATING PULMONARY THROMBO EMBOLISM
M040A	EPIDERMOID CARCINOMA (GINGIVA), PNEUMONIA
F041A	LEIOMYOSARCOMA (SPLEEN)
M042A	STATUS EPILEPTICUS
F043A	SPECIAL STUDY
F044A	ADRENAL CORTICAL CARCINOMA
F045A	

DOG NUMBER	AT INJECTION AGE (DAYS)	WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
F047A						1732	
F048A					4711		
F049A					4881		
M050A						2264	
F051A						1089	
F052A						509	
F053A					4645		
F054A						3190	
F055A					4301		
M056A						701	
F057A					4274		
M058A						767	
M059A						567	
M060A						4025	
M061A					4410		
F062A					4355		
F063A					4355		
F068A					4214		
F070A					3766		
M073A						5695	
F074A						5553	
M075A						5284	
F076A					3676		
F077A					3661		
F078A					3395		
F079A					3306		
F080A					3250		
F081A					3250		
M082A					3013		
F083A					2937		
M084A					2937		
M085A					3207		
M086A						499	
F087A					2445		
F088A					2445		
F089A					2601		
F090A					2385		
F091A					2375		
F092A					2375		
M093A					2320		
F094A					2320		
F095A					2305		
F096A					2288		
F097A					2288		

DOG NUMBER	COMMENTS ON DEAD DOGS
F047A	SPECIAL STUDY
F048A	
F049A	
M050A	SPECIAL STUDY
F051A	SPECIAL STUDY
F052A	SPECIAL STUDY
F053A	
F054A	SPECIAL STUDY
F055A	
M056A	VOLVULUS + PERITONITIS
F057A	
M058A	SPECIAL STUDY
M059A	SPECIAL STUDY
M060A	TRANSFERRED TO EXPERIMENTAL GROUP (SEE T071R50).
M061A	
F062A	
F063A	
F068A	
F070A	
M073A	DEGENERATION OF ADRENAL GLAND + DIABETES MELLITUS
F074A	LYMPHO SARCOMA
M075A	AORTIC THROMBUS
F076A	
F077A	
F078A	
F079A	
F080A	
F081A	
M082A	
F083A	
M084A	
M085A	
M086A	SPECIAL STUDY
F087A	
F088A	
F089A	
F090A	
F091A	
F092A	
M093A	
F094A	
F095A	
F096A	
F097A	

DOG NUMBER	AT INJECTION AGE (DAYS)	AT INJECTION WEIGHT (KG)	INJECTED (μCi/KG)	DATE INJECTED D MO YR	DAYS SINCE INJECTION 31/3/70	DAYS SINCE INJECTION DEATH	DOSE TO SKELETON (RADS)
F098A					2194		
F099A						479	
M100A						476	
M101A						290	
F102A						243	
M103A						217	
M104A						168	
F105A						157	
F106A					1889		
F107A					1798		
F108A						1969	
F112A					1114		

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

DOG NUMBER	COMMENTS ON DEAD DOGS
F098A	
F099A	SPECIAL STUDY
M100A	SPECIAL STUDY
M101A	SPECIAL STUDY
F102A	SPECIAL STUDY
M103A	SPECIAL STUDY
M104A	SPECIAL STUDY
F105A	SPECIAL STUDY
F106A	
F107A	
F108A	ENCEPHALOMALACIA (BACTERIAL)
F112A	

THE DYNAMICS OF LIFE

I. DEATH FROM AGING, CANCER, IRRADIATION, POISONS AND OTHER STRESSES

Betsy J. Stover and Henry Eyring

Abstract: Evidence is accruing that somatic mutations are important in aging and in the induction of the degenerative diseases as well as in oncogenesis and radiation damage (H. J. Curtis, Radiation and Aging, Symp. Soc. Exp. Biol., 21:51-64, 1967). The occurrence of a mutation reflects a change in the environment of a cell which may be an alteration in the concentration of a critical precursor to the gene. Since biological processes proceed near equilibrium, the occurrence of such a change results in counter changes. Thus the mutation rate is a steady-state phenomenon. Application of the theory of absolute reaction rates makes it possible to analyze the life-shortening and oncogenic action of irradiation. Death curves for nonirradiated beagles that die from aging and cancer and those for beagles that receive skeletal alpha irradiation leading to osteogenic sarcomata are similar in shape. They differ in that death occurs earlier in the case of the irradiated dogs, and the hastening of death is a function of the radiation dose.

Introduction

The very extensive program of observing the protracted biological effects of plutonium and other pertinent radionuclides in beagles at this University (1,2,3,4,5) indicates that the statistical effect of irradiation, in this case, is well described as one of premature aging. The statistical death curves have substantially the same shape for the irradiated dogs as for the controls except that death comes at an earlier age. The radiation specific or the degenerative diseases of old age thus simply take their toll earlier as a result of the weakening by irradiation.

The effect of poisons, i.e. disinfectants, has been interestingly treated as an irreversible attack on r separate sites with

death occurring when all these sites are incapacitated. Thus, if p is the probability that a site is operative and q that it is incapacitated, then $p + q = 1$ and further the term q^r in the binomial expansion of $(p + q)^r$ is the probability of death. If p is decreasing as the result of a chemical reaction with the rate constant k, then $p = e^{-kt}$ and $q = (1 - e^{-kt})$, so that $q^r = (1 - e^{-kt})^r$ and the fraction surviving at time t is

$$f = 1 - (1 - e^{-kt})^r \qquad (1)$$

In those cases in which the rate constant, k, varies with time, e^{-kt} should be replaced by $e^{-\int_0^t k\,dt}$ wherever e^{-kt} appears in the above discussion. This simple treatment gives a rather good account of disinfection (6).

Curtis has discussed radiation and aging in an interesting way in the context of the mutation hypothesis of aging and of the action of irradiation to accelerate the aging process (7).

Another quite successful way of considering the onset of disease or of death is to plot on a log log scale the rate of onset, $\dot{N}$, of death or of a particular disorder against the age of the subject, t. The result is frequently a fairly straight line. Thus

$$\dot{N} = ct^{r-1} \qquad (2)$$

and

$$N = \frac{ct^r}{r} \qquad (3)$$

In this case r is interpreted as the number of centers which must be changed to bring about the onset of death or diseases as the case may be. These centers have frequently been interpreted to be genes which are being modified with a consequent change in the nature of the affected cell. Burch has interpreted the onset of auto-immune diseases such as inflammatory polyarthritis, rheumatoid arthritis, and that of aging in terms of such somatic mutations in a parent cell or a stem cell (8). The quantity $c^{1/r}$ in equation (2) is a scale and must be proportional to a rate constant and thus reflect changes in the environment effecting the rate of mutation. Here the nature of the mutation process is unspecified.

A Steady State Theory of Mutation Rates

Typically biological processes proceed at near equilibrium so that, for example, a change in the concentration of some product may set in motion compensatory or reverse reactions which bring the system back into biological balance. In developing the Steady State Theory of Mutation Rates, we suppose that there are r sites in r or less than r cells, i.e. one or more per cell, subject to a critical change of which n of these have been changed at the time t. Then, if v_i is the rate at which a single unchanged site is changing, and v_j is the rate at which the change in one of the altered sites is disappearing, we can write

$$v_i (r - n) = v_j n \tag{4}$$

or

$$n = \frac{v_i r}{v_i + v_j} \tag{5}$$

and

$$\frac{n}{r} = \frac{1}{\frac{v_j}{v_i} + 1} \tag{6}$$

is the fraction of changed sites and the fraction of unchanged sites $p = 1 - q$ is

$$\frac{v_i + v_j}{v_i + v_j} - \frac{v_i}{v_i + v_j} = \frac{v_j}{v_i + v_j} = \frac{1}{1 + \frac{v_i}{v_j}} \tag{7}$$

Now, according to absolute rate theory,

$$v_i = \frac{\mathcal{H} k T}{h} e^{-\frac{\Delta G_{0i}^{\ddagger}}{RT}} c_1 c_2 \cdots c_i = \frac{\mathcal{H} k T}{h} e^{-\left(\frac{\Delta G_{0i}^{\ddagger}}{RT} - \sum_i \ln c_i\right)} \tag{8}$$

where c_i is the concentration of the i^{th} constituent, and, similarly,

$$v_j = \frac{\mathcal{H} k T}{h} e^{-\left(\frac{\Delta G_{0j}^{\ddagger}}{RT} - \sum_j \ln c_j\right)} \tag{9}$$

Some of these constituents, c_i, may be enzymes or other reactants that are being used up by irradiation, aging, poisons, or other stresses. Thus, we can write

$$\frac{dc_\ell}{dt} = -k_\ell c_\ell \tag{10}$$

Hence

$$\ln c_\ell = -k_\ell t + \ln c_{o\ell} \tag{11}$$

Since in a steady state such as this the process of modifying the site need not be the reverse of the process by which this site is mended. (For example, if a critical molecule is destroyed, the steady state is maintained by synthesis of another molecule of the same kind.) Changing an enzyme or other entity will alter the value of $q = n/r$. Combining equations (7,8,9, and 11) we obtain for the fraction of altered sites, n/r

$$q = n/r = (1 + e^{a-bt})^{-1} \tag{12}$$

where

$$a - bt \equiv \left[(\Delta G^{\ddagger}_{o\,i} - \Delta G^{\ddagger}_{o\,j})/RT - \sum_i \ln c_{o\,i} + \sum_j \ln c_{o\,j} \right] + \left(\sum_i k_i - \sum_j k_j \right) t \tag{13}$$

Then the probability of a site's being unaltered, p, is

$$p = (1 - q) = (1 + e^{-(a-bt)})^{-1} \tag{14}$$

Case A. Non-Survival from a Single Cause

The rate of mutation may be written as

$$\frac{dS}{dt} = - f\nu pq \tag{15}$$

Here ν is the sum of the rates of cell division of all the cells that cause non-survival and f is simply a scale factor which enables us to set S = 1 at the time t = 0. Since

$$\frac{dp}{dt} = -bpq \tag{16}$$

as is readily seen by carrying out the differentiation of p, we can write

$$\frac{dS}{dt} = \frac{f\nu}{b}\frac{dp}{dt} \tag{17}$$

so that

$$S = \frac{f\nu}{b}p + C \tag{18}$$

Since p is one at t = 0 and zero at t = ∞ and, since experiment shows that these are also the limits for S, it follows that in these cases C = 0 and

$$f\nu = b \tag{19}$$

Thus, we have for the fractional survival at time, t

$$S = p \tag{20}$$

Case B. Survival with Several Simultaneous Independent Mechanisms Contributing to Non-Survival

In this case we can write

$$S = \prod_i p_i \tag{21}$$

This follows from the fact that the probability of survival from independent mechanisms must be the product of the individual probabilities of survival, so that

$$\frac{dS}{dt} = \sum_j \frac{dp_j}{dt} \prod_{i \neq j} p_i = \sum_j -b_j p_j q_j \prod_{i \neq j} p_i \qquad (22)$$

Equation (21) reduces to equation (20) as it must when there is only one important independent mechanism of non-survival. At t = 0 the probability of survival is one and the sum of the rates for the different mechanisms for non-survival are simply additive. At subsequent times the rates for non-survival are still additive, but an individual rate, $\frac{dp_j}{dt}$, is multiplied by the probability that the system has not yet succumbed from other causes, $\prod_{i \neq j} p_i$. At infinite time S becomes zero as of course it must.

Case C. More than one Cause Acting through the Same Mechanism

In this case all the equations are formally the same as in Case A but

$$b = \sum_i b_i \qquad (23)$$

where b_i expresses the effect of cause i. It will be important in each case to establish whether a new cause acts through the same mechanism as in Case C or through an independent mechanism as in Case B, or in both ways. In the latter circumstance we would have Case C with possible changes in the cells' reserves, the a_i values, and also in the rates of change, the b_i values. The effect of

external environment exemplifies Case C. Notable examples are cell differentiation and the effect of carcinogens.

Case D. A Single Mechanism of Non-Survival Requiring m Like Unchanged Sites and n Like Changed Sites

Thus we have

$$\frac{dS}{dt} = -f\nu p^m q^n \tag{24}$$

Substituting equation (16) into equation (24) gives

$$\frac{dS}{dt} = \frac{f\nu}{b}\frac{dp}{dt}p^{m-1}q^{n-1} = g\frac{dp}{dt}p^{m-1}(1-p)^{n-1}$$

$$= g\frac{dp}{dt}p^{m-1}\sum_{r=0}^{n-1}\frac{(n-1)!(-p)^r}{(n-1-r)!r!} \tag{25}$$

Here we have written $\frac{f\nu}{b} = g$ for convenience. Hence

$$S = g\sum_{r=0}^{n-1}\frac{(-1)^r(n-1)!\,p^{m+r}}{(n-1-r)!\,r!(m+r)} + C \tag{26}$$

Since S = 1 at t = 0 and S = 0 at t = ∞, it is required that

$$C = 0 \tag{27}$$

and

$$g = \left(\sum_{r=0}^{n-1} \frac{(-1)^r (n-1)!}{(n-1-r)!\, r!\, (m+r)} \right)^{-1} \tag{28}$$

so that

$$S = \frac{\sum_{r=0}^{n-1} \frac{(-1)^r (n-1)!\, p^{m+r}}{(n-1-r)!\, r!\, (m+r)}}{\sum_{r=0}^{n-1} \frac{(-1)^r (n-1)!}{(n-1-r)!\, r!\, (m+r)}} \tag{29}$$

By solving g for successive values of n one readily sees that

$$g = \prod_{r=0}^{n-1} \frac{(m+r)}{(n-1)!} \tag{30}$$

and

$$S = \prod_{r=0}^{n-1} \frac{(m+r)}{(n-1)!} \sum_{r=0}^{n-1} \frac{(-1)^r (n-1)!\, p^{m+r}}{(n-1-r)!\, r!\, (m+r)} \tag{31}$$

is the solution of equation (25) which has the value S = 1 at t = 0 and S = 0 at t = ∞.

Case E. Non-Survival Due to Independent Action on Separate Systems of Sites

In this case

$$S = \prod_i S_i \tag{32}$$

where S_i may belong to either category A or D.

Case F. Survival when the Only Necessary Condition Is that One Site Must Be Changed to Permit Non-Survival

In this case

$$\frac{dS}{dt} = -\nu f q \tag{33}$$

But since

$$\frac{dp}{dt} = -bpq \tag{34}$$

we have

$$\frac{dS}{dt} = \frac{\nu f}{b}\left(\frac{dp}{dt}\Big/p\right) \tag{35}$$

so that

$$S = \frac{\nu f}{b} \ln p + C \tag{36}$$

In this case S takes the value C at t = 0 and - ∞ at t = ∞. Such a solution is not observed experimentally and so will not be considered further.

Relation of Dose Size to Survival Time

If we have Case A then

$$S = p = \frac{1}{1 + e^{-a+bt}} \tag{20,14}$$

and survival S will be one half when $(- a + bt_{S=1/2}) = 0$, but

$$a = (\Delta G^{\ddagger}_{o\,i} - \Delta G^{\ddagger}_{o\,j})/RT - \sum_i \ln c_{o\,i} + \sum_j \ln c_{o\,j} \qquad (37)$$

$$= (\sum_j k_j - \sum_i k_i)\, t_{S=1/2}$$

Now if a dose of radiation has the effect of changing some $c_{o\,i}$ to $(c_{o\,i} + c'_{o\,i})$ where $c'_{o\,i}$ measures the size of the dose, then we have

$$a' - \ln(c_{o\,i} + c'_{o\,i}) = bt_{S=1/2} \qquad (38)$$

where a' is a constant.

If $c'_{o\,i} >> c_{o\,i}$, we will get a straight line when the ln (dose) is plotted against $t_{S=1/2}$. On the other hand when $c'_{o\,i} << c_{o\,i}$ the result is that

$$\ln(c_{o\,i} + c'_{o\,i}) = \ln c_{o\,i}\,(1 + \frac{c'_{o\,i}}{c_{o\,i}}) = \ln c_{o\,i} + \ln(1 + \frac{c'_{o\,i}}{c_{o\,i}}) \qquad (39)$$

$$\approx \ln c_{o\,i} + \frac{c'_{o\,i}}{c_{o\,i}}$$

so that $t_{S=1/2}$ is a linear function of the dose. Both these cases have been observed experimentally as well as intermediate cases as the theory predicts (9). Similar results will be obtained if some concentration $c_{o\,j}$ is decreased to $(c_{o\,j} - c'_{o\,j})$ where $c'_{o\,j}$ measures the effect of the dose. In this case we have as our equation

$$a'' + \ln(c_{o\,j} + c'_{o\,j}) = bt_{S=1/2} \qquad (40)$$

The Probable Nature of Mutations Causing Cancer

There are between a hundred and two hundred known types of cancers depending on the method of classification. The fact that

radiation, the process of aging, and many types of carcinogens cause similar types of cancer suggests that they act non-specifically through a common mechanism. They thus presumably exemplify Case C, where they act by decreasing (a - bt), that is, they increase the difference between chromosome breaking as compared with the rate of repair bringing about a genetic change resulting in cancer (10, 11). The first possibility is that the critical chromosome break leads to the elimination of the gene for repressing growth and so leads to uncontrolled growth of the modified cell. The second possibility is that a gene is lost which controls the surface adhesion between like cells and so promotes growth through a looser structure which allows the escape of inhibitor and/or the diffusion in of nutrients and so leads to uncontrolled growth as well as promoting metastasis from the poorly fastened cells. In the process of evolution perfectly healthy unattached cells must have acquired genes which led to their attachment to form organs and with this clumping came inhibition of growth due to the slow escape of specific inhibitors, also genetically controlled, as well as poorer access of nourishment. The above types of mutation probably represent gene deletion or suppression. The third possibility is that cancer caused by a virus may also arise from addition of genes, promoting uncontrolled growth. However, a virus may also cause gene deletion. Since mutations involving both the gain and loss of genes occur it will not be surprising to find there are malignant conditions arising from both causes. Our formal theory does not distinguish between deletion or addition of genetic material but only requires that a

chromosome be modified to cause non-survival.

Application of the Steady State Theory of Mutation Rates

The experimental design of the study of the effects of ^{239}Pu, ^{226}Ra and other nuclides in beagles has been reported in detail by Dougherty et. al. (4), as has the incidence of osteosarcomata, a principal end-point of the experiment, by Mays et. al. (5). The beagle colony also includes a group of non-irradiated control dogs which are not only protected from irradiation but also from death from trauma, accident (e.g. anesthetic, drug allergy, etc.), infectious diseases, parasites, nutritional deficiencies, epilepsy, and readily operable cancer such as cancer of the skin.

The rightmost curves of Fig. 1 depict the fractional survival of a group of 32 of these control dogs. Five are still living but, as inspection of the graph will show, their life expectancy is short. The stair-step curve is the observed survival, and the smooth curve was calculated according to equations (14 and 20). In Table 1 are listed pertinent findings at autopsy for these dogs. The leftmost curves are the observed and calculated survival curves for 18 dogs whose deaths were the consquence of epilepsy. The center curves are those for 7 dogs that had a lymphosarcoma or leukemia. In all three cases, even when N is as small as 7, the fit is especially good in the region of $S = \frac{1}{2}$. This means that the death rate curves, $-dS/dt$ are sharply peaked about the value of t for S = 1/2.

The values of a and b and the extent of life-shortening are given in Table 2. The beagles are not an inbred strain and con-

siderable genetic variation should be expected, and it is strikingly apparent in the marked difference in the parameter, a, the cells' reserves.

The survival curves for groups of beagles at six different radiation dose levels are shown in Figs. 2 and 3, and those for the 32 controls are repeated in Fig. 2. Each of these irradiated dogs was given a single intravenous injection of ^{226}Ra in young adulthood. Some of the ^{226}Ra is retained throughout the life of the animal, and, since radium is an alkaline earth element, its principal site of deposition is the mineral of the skeleton. The osteoblast, the osteocyte, and the osteoclast, and also some cells in the bone marrow are thus subject to alpha irradiation. The mean value of the average radiation dose to the skeleton is given for each dose level. The dose unit is the rad, which means radiation absorbed dose, and is defined as

$$1 \text{ rad} \equiv 100 \text{ ergs/ g tissue} \qquad (41)$$

The meaning of average dose is total energy delivered per unit mass of tissue. However, the energy is not dissipated uniformly in the skeleton and there are regions of higher and lower dose.

The parameters of the survival curves of Figs. 2 and 3, the life shortening, and the incidence of osteosarcomata are given in Table 3. The correlation of both life-shortening and tumor incidence with radiation dose is highly significant. The highest dose level is the overkill situation in which there is massive skeletal damage and also significant hematopoietic injury. There is a marked increase

in b, the rates of change. The second highest dose level has an extremely sharply peaked death rate curve, and is a superb example of Case B in which death from osteosarcoma is the only important independent mechanism of non-survival and hence equation (22) reduces to equation (20) since all the other probabilities are still close to unity. The value of b is markedly elevated, and a is also very high. The high value of a, the reserves, means a high reserve against osteosarcoma which is consistent with the extremely low natural incidence of osteosarcoma in the beagle (12).

Similar results with ^{239}Pu are given in Figs. 4, 5, and 6 and in Table 3. Although the solution chemistry of plutonium is uniquely complex, the plutonium was administered in such a fashion that the main sites of deposition are the osseous surfaces and the parenchymal cells and reticuloendothelial cells of the liver. The average radiation dose to the liver is the same, within a factor of about two, as that to the skeleton. Thus the three above mentioned osseous cells, the hepatic cells, and, to a greater extent than in the case of radium, the hematopoietic cells are irradiated.

The highest dose level is an even greater overkill situation than in the case of radium. The depleted reserve as indicated by a could reflect the severe damage to three different groups of cells. Otherwise the same marked correlation of life-shortening, tumor incidence and radiation dose is observed. The second, third, and to a lesser extent, the fourth highest doses exhibit elevated reserves and rates and are again examples of Case B reducing to Case A.

The relationship of dose size to time to S = 1/2 is shown in

Fig. 7. In the case of ^{226}Ra the $t_{S=1/2}$ values for the highest tumor incidence dose levels are proportional to the ln(dose). The $t_{s=1/2}$ and ln(dose) values for the low incidence levels for which the life-shortening is very small also yield a straight line but it has a completely different slope. In the case of ^{239}Pu the logarithmic relationship is not observed. However, the $t_{S=1/2}$ values for the four lower dose levels and for the non-irradiated beagles are a linear function of dose. Thus, although both nuclides are effective in inducing the osteosarcoma, ^{226}Ra acts by inducing large changes in one or more c_{oi} or c_{oj} while ^{239}Pu at the four lower levels induces small changes which appear to be adjuvants to the changes occurring through the aging process. Thus at higher dose levels plutonium acts through an independent mechanism, Case B, while at lower levels it acts through the aging mechanism, Case C, and in both ways at intermediate levels.

Further use of the steady state theory of mutation rates to interpret the effects of irradiation should yield valuable guidelines. Two kinds of data should be particularly interesting to analyze, those from highly inbred strains of experimental animals and those from that highly outbred animal, man. The latter are the radium exposure cases, the atomic bomb survivors, the uranium miners, and the Marshallese islanders.

References and Notes

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Table 1

Findings in Control Dogs

No.	Finding
1.	Aortic body tumor
2.	Brain hemorrhage
3.	Lymphosarcoma
4.	Pulmonary embolism
5.	Seminoma, lymphosarcoma
6.	Pancreatic adenocarcinoma
7.	Transitional cell carcinoma
8.	Purulent meningoencephalitis
9.	Fibrosarcoma (spleen)
10.	Bronchogenic carcinoma
11.	Splenic rupture, metastatic seminoma
12.	Obturating pulmonary embolism
13.	Leukemia
14.	Circulatory failure
15.	Adrenal cortical carcinoma
16.	Testicular carcinoma
17.	Undetermined
18.	Obturating pulmonary embolism
19.	Living
20.	Vaginal fibroma
21.	Found dead - extreme debilitation

22. Living
23. Nephritis
24. Reticulum cell sarcoma (soft tissue)
25. Living
26. Thyroid carcinoma, nephritis
27. Chronic interstitial nephritis; thrombosis
28. Living
29. Pancreatic adenocarcinoma
30. Living
31. Living
32. Extreme debilitation

Table 2

Effects of Epilepsy and Lymphoma or Leukemia

No. Dogs	No. Living	a	b x 10^3 (d^{-1})	Life Shortening (d)	Category
32	5	10.9	2.28	0	aging and cancer
18	0	4.4	1.87	2443	epilepsy
7	0	7.8	2.11	1064	lymphoma or leukemia

Table 3

^{226}Ra and ^{239}Pu in Beagles

No. Dogs	No. Living	a	b x 10^3 (d^{-1})	Life Shortening (d)	Fraction with Osteosarcoma	Skeletal Radiation Dose (rads)
				^{226}Ra		
9	0	11.9	7.69	3233	1	15,074
12	0	21.8	10.57	2700	1	5,464
12	0	7.9	2.98	2124	0.92	2,122
12	0	9.9	2.20	300	0.42	948
10	3	~ 11.7	~ 2.60	≤ 272	~ 0.14	~ 500
9	1	~ 15.1	~ 3.25	113	~ 0	~ 170
				^{239}Pu*		
9	0	4.7	2.65	2990	0.78	5,830
12	0	16.1	8.67	2932	1	1,790
12	0	16.2	7.45	2612	1	≤ 720
11	0	18.4	5.42	1392	0.91	≤ 357
13	2	~ 13.0	~ 3.33	891	~ 0.73	~ 211
12	2	~ 9.3	~ 2.00	≤ 137	~ 0.40	~ 86

* Irradiation of liver is also significant.

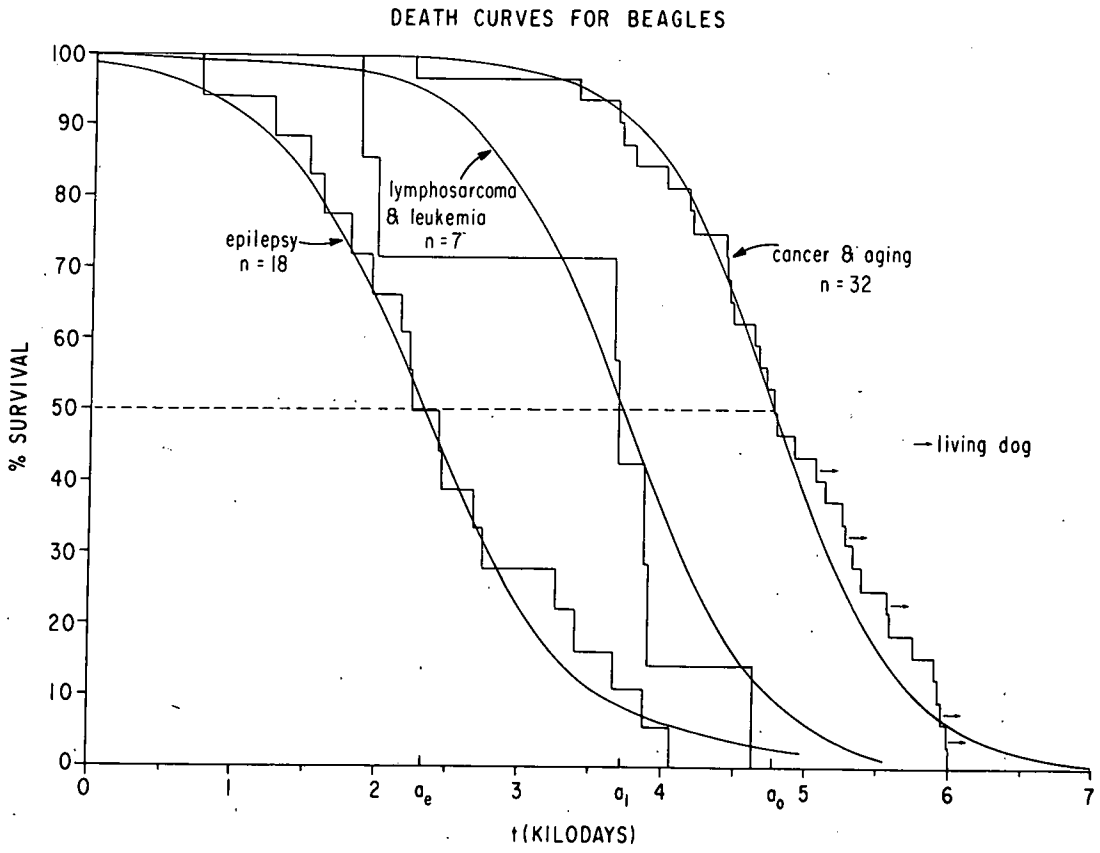

Figure 1. Theoretical and observed survival curves for controls and two disease categories.

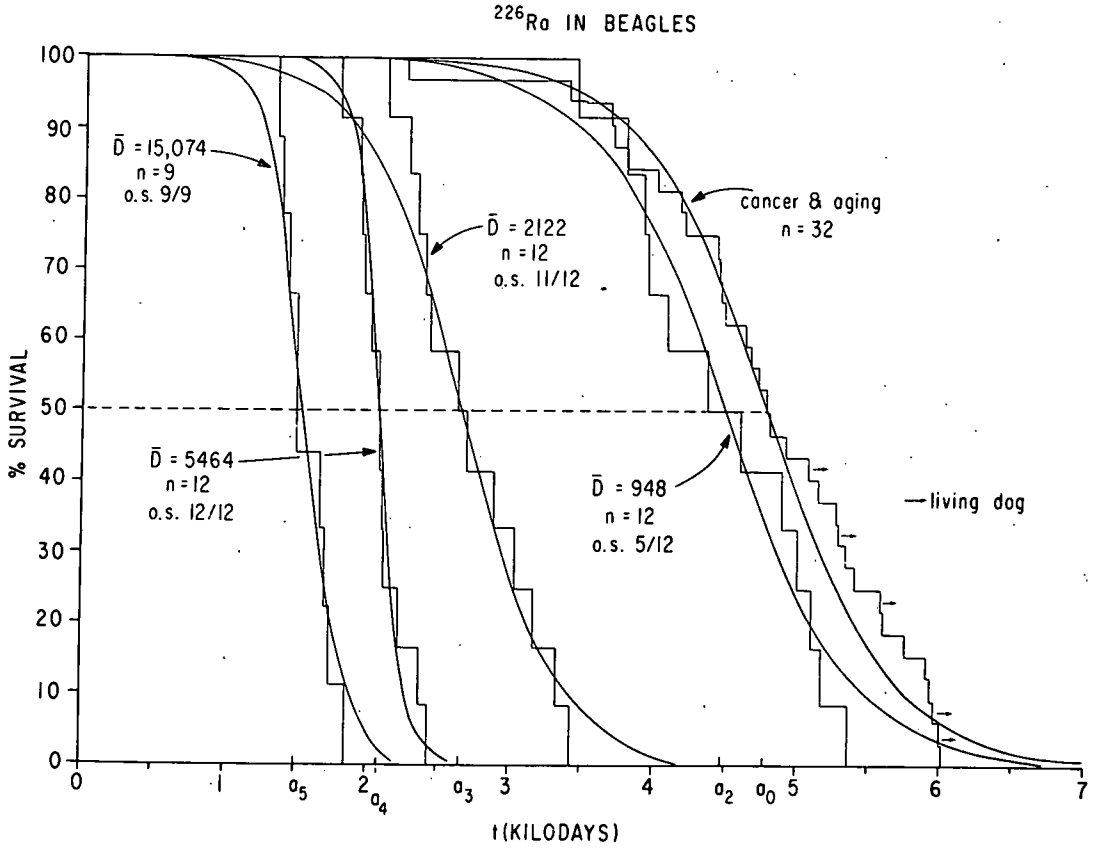

Figure 2. Theoretical and observed survival curves for controls and four dose levels of ^{226}Ra.

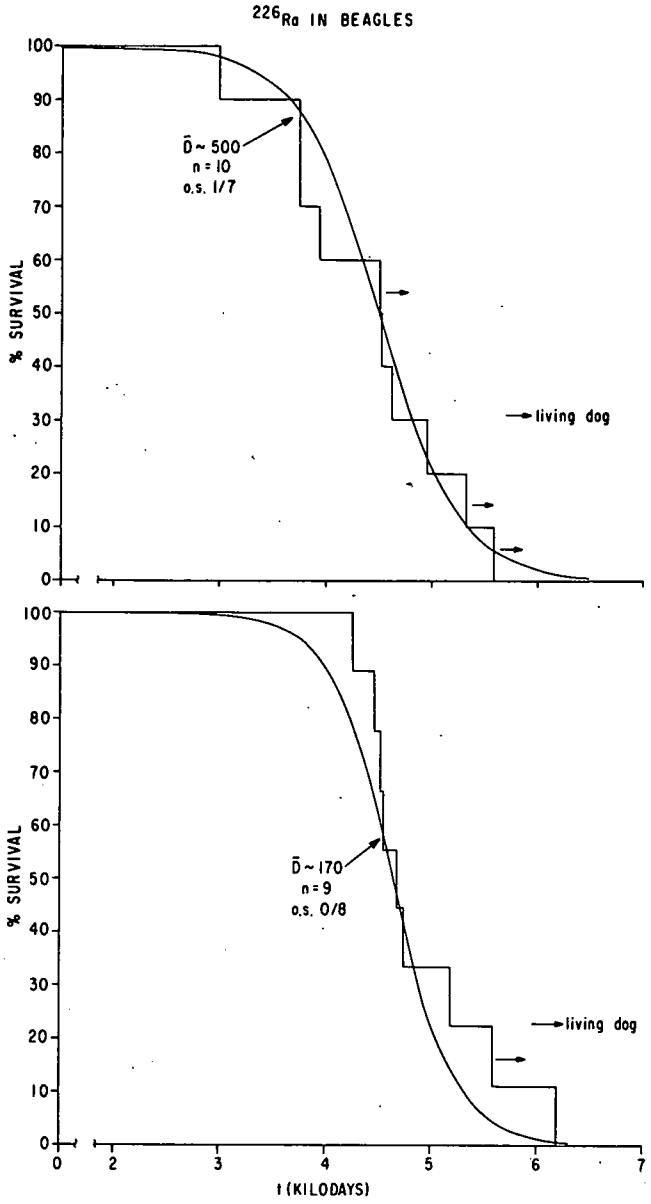

Figure 3. Theoretical and observed survival curves for two low levels of ^{226}Ra.

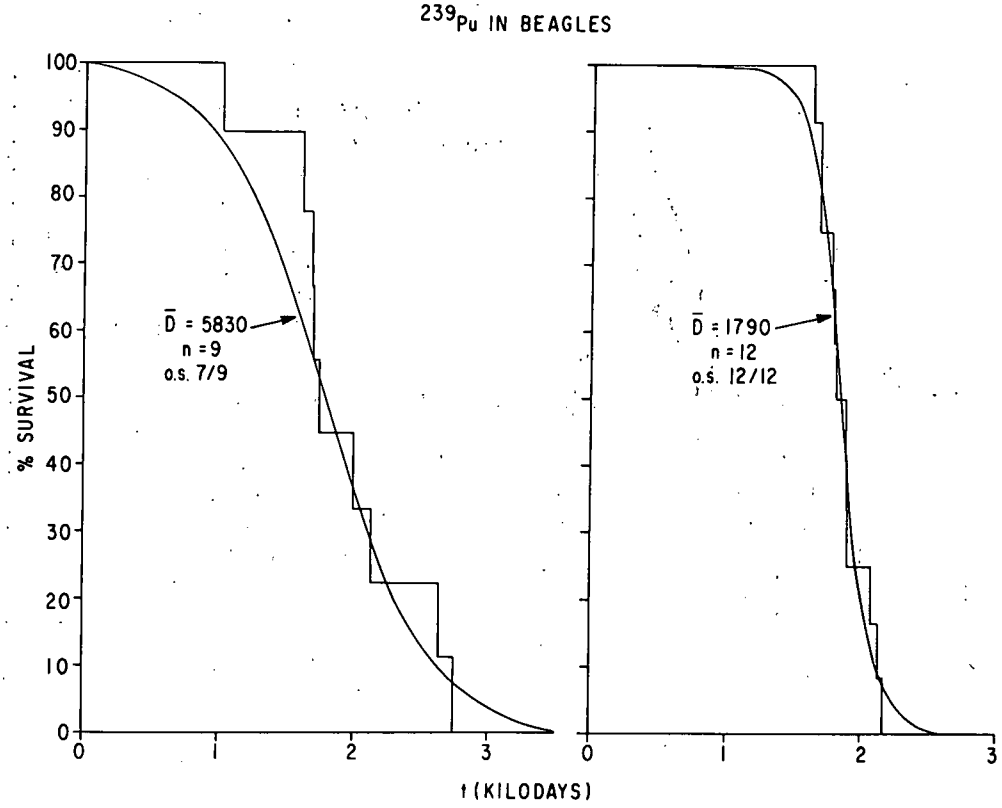

Figure 4. Theoretical and observed survival curves for two high levels of ^{239}Pu.

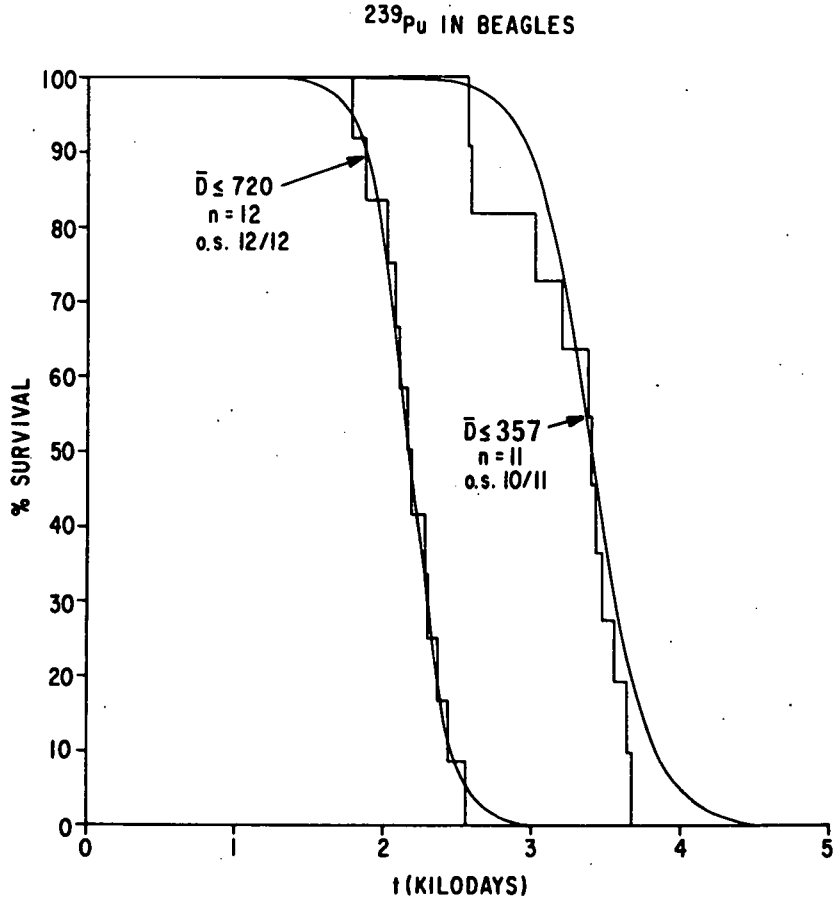

Figure 5. Theoretical and observed survival curves for two intermediate levels of ^{239}Pu.

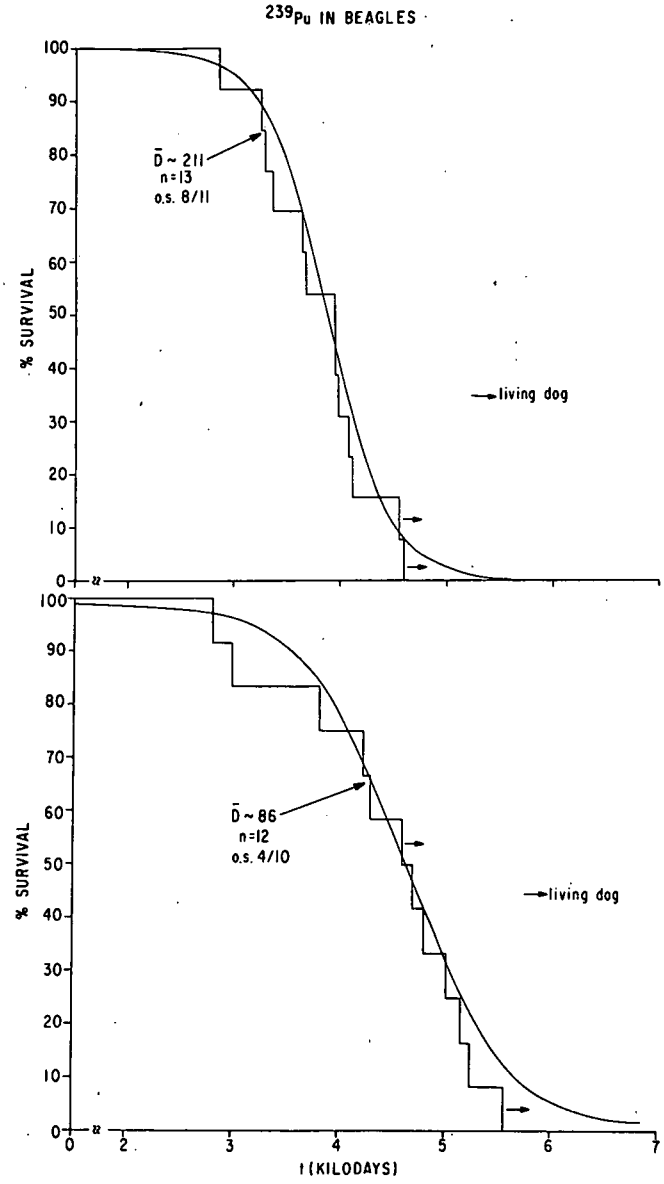

Figure 6. Theoretical and observed survival curves for two low levels of ^{239}Pu.

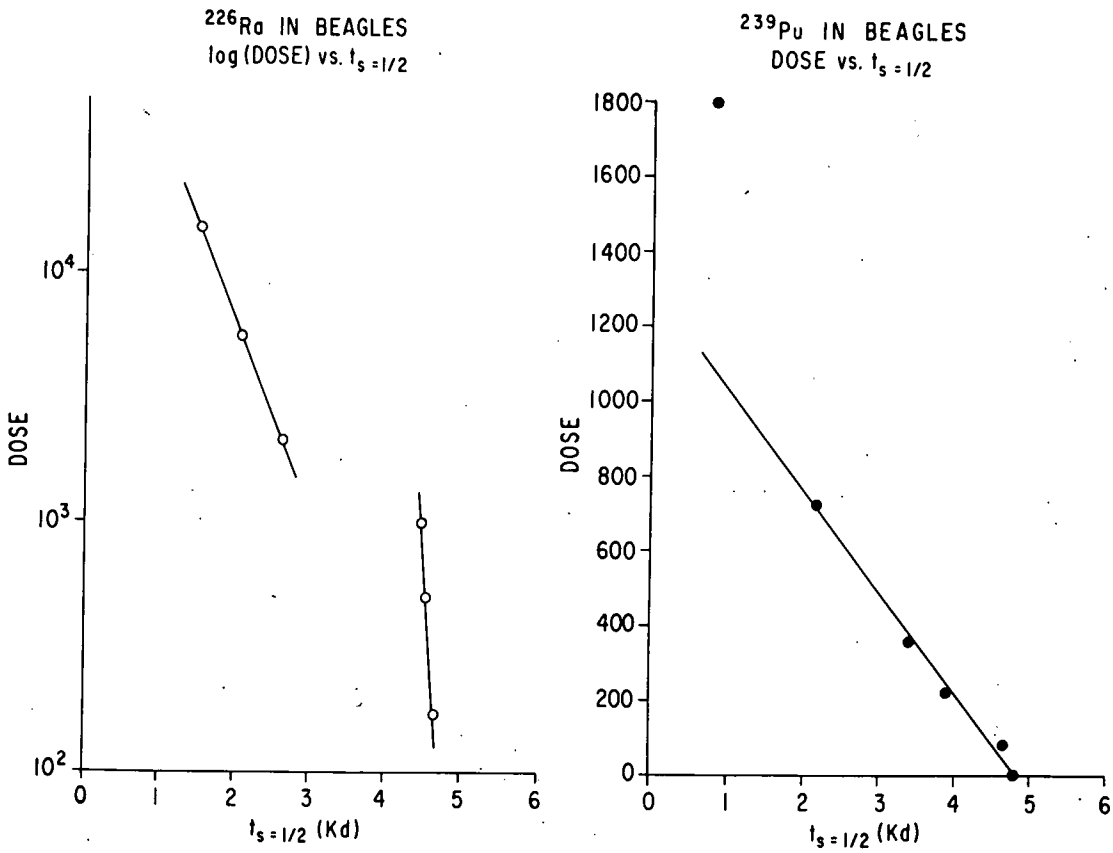

Figure 7. Relationships between time to half survival and skeletal radiation dose.

THE DYNAMICS OF LIFE
IVa. LENTICULAR CATARACTS INDUCED BY AGING AND IRRADIATION

Betsy J. Stover

Abstract: Our recently developed Steady State Theory of Mutation Rates has been used to compare the effects of aging and irradiation on the lens of the eye. A set of data for rats, which included animals irradiated by X-rays or neutrons and non-irradiated animals, was used. It was found that functional survival of the organ could be analyzed separately from survival of the animal. Results indicate that at high doses of radiation two cataractogenic mechanisms are operative, an early acting independent mechanism and an acceleration of the aging mechanism. At lower doses the latter mechanism is dominant.

Introduction

From the observation that the statistical nature of survival curves is the same for death from aging, irradiation, and other diseases, and the fact that biological processes proceed at near equilibrium, we have used Absolute Rate Theory to formulate a Steady State Theory of Mutation Rates. This theory has been successfully applied to the survival of beagles (1,2,3,4). The groups of dogs considered included protected control animals, groups experiencing intrinsic diseases, and groups subjected to internal irradiation from ^{239}Pu or ^{226}Ra.

Since the Steady State Theory of Mutation Rates is basic and thus not limited to a specific application, it should be applicable to many quantal phenomena in biology. In this paper the functional survival of a single organ, the lens of the eye, is considered.

There are several factors which make it interesting to examine the survival of the lens separately from survival of the animal. The lens becomes isolated from the vascular system early in fetal life, and thereafter depends on diffusion for its nutrients. It is an organ that grows

throughout life, and thus differs from other organs that mature at certain periods of development. Opacification of the lens, as well as embrittlement, is a part of the aging syndrome, and, since ionizing electromagnetic radiation and neutrons can also induce lenticular opacities, the lens is a system in which the effects of aging and irradiation can be compared. Further, there is the experimental advantage that the lens can be observed repeatedly by non-perturbing methods.

Effect of Aging and Irradiation on the Lens of the Rat

The relative biological effectiveness of neutrons, X-rays, and gamma-rays in the induction of opacities of the lens has been reported by Upton, Christenberry, Melville, Furth and Hurst (5). Several species and strains were compared. Their results on the effects of 250-kvp X-rays or cyclotron neutrons (contaminated with gamma-rays to the extent of 5 to 15% of the dose) have been used. This set was chosen since the lens of the rat was more sensitive to irradiation than were those of mice and guinea pigs, and since the controls showed a higher degree of opacifaction.

In the Steady State Theory of Mutation Rates it is assumed that there are r critical sites, one or more per cell, which, if altered, lead to a mutation. If v_i is the rate at which sites are being changed and v_j is the rate at which a changed site is disappearing, and n sites have already been changed, then the steady state equation is

$$v_i (r - n) = v_j n \qquad (1)$$

and the fraction of changed sites, q, is

$$q = \frac{n}{r} = \frac{1}{1 + \frac{v_j}{v_i}} \tag{2}$$

and the fraction that survives, p, is

$$p = 1 - q = \frac{1}{1 + \frac{v_i}{v_j}} \tag{3}$$

From Absolute Rate Theory we obtain expressions for v_i and v_j. Then, since the rate of non-survival from a lethal mutation is proportional to the chance that the change has occurred and the rate of cell division of the cells involved, we can calculate survival, S, as a function of age, t

$$S = (1 + e^{-(a-bt)})^{-1} \tag{4}$$

where

$$(a - bt) \equiv \left[(\Delta G^{\ddagger}_{o\,i} - \Delta G^{\ddagger}_{o\,j})/RT - \sum_i \ln c_{o\,i} + \Sigma \ln c_{o\,j} \right] + \left(\sum_i k_i - \sum_j k_j\right) t \tag{5}$$

The terms on the right side of Eq. (2) have their usual thermodynamic and kinetic meaning. The subscript i refers to processes in which a critical site is being changed and j refers to those processes acting to eliminate the altered site in order to maintain the biological steady state. If there are multiple independent mechanisms that lead to non-survival, then S is given by

$$S = \prod_i S_i \tag{6}$$

and, if one of these, S_i, acts early in time when all other $S_{i \neq j}$ are close to unity, then Eq. (6) reduces to Eq. (4). If multiple causes

act through the same mechanism, then b in Eq. (4) is given by

$$b = \sum_i b_i \qquad (7)$$

The functional survival, i. e. transparency, of the lens of the eye with increasing age is shown for control animals and for rats irradiated with X-rays (Fig. 1) and neutrons (Fig. 2). The points shown are those reported by Upton et. al. (5), (with the exception that three were interpolated). The age at irradiation is also shown. The degree of opacification was reported as grade + through grade ++++(complete opacification). This method places a limitation on the data, but, since there were 10 to 25 animals per group and there was little variation within a group, the data are probably as valid as is possible in this kind of observation. another limitation is that neither the controls nor the animals at the lower dose levels of X-rays reached complete opacification in the period reported, and it is presumed that the animals simply did not live long enough for complete opacification to occur. Further it was the survivors of the irradiated groups that were observed, and thus there was a selective factor in these groups that was not operative in the control group. The smooth curves shown are Eq. (4) fit to the experimental data. (In Fig. (1) the two broken curves were fit ignoring the last point for each of the two lower dose levels.)

Eq. (4) is symmetrical about the point S = 1/2 and thus describes data that are symmetrically distributed in time about $t_s = 1/2$. The two sets of data from the neutron irradiations and that of the 640 rep X-ray group are highly symmetrical, and pass through S = 1/2 at an age when the calculated value of S for controls is ≥0.9. The data for 480 rep X-ray group are less symmetrical but those for the controls and the 240 rep

X-ray group fit fairly well, especially considering that complete opacification did not occur during the period of observation.

Table I

Reserves, Rates, and Times to Half-Survival

Group	a	b x 10^3 d^{-1}	$t_{s=1/2}$ d
Control	4.6	7.0	660
X-ray 240 rep	4.8	9.9	485
X-ray 480 rep	4.2	12.7	329
X-ray 640 rep	3.8	13.7	276
Neutron 180 rep	4.7	13.7	344
Neutron 360 rep	6.9	25.0	276

The parameter a of Eq. (4) is a measure of cellular reserves and b is a measure of the rates of the reactions that maintain the steady state. An injurious factor can act to alter the reserves or the rates, or both. Thus, a decrease in (a - bt) represents the case in which the rate at which critical changes occur is increasing over the rate at which they disappear. The values of a and b for the curves of Figs. (1,2) are given in Table I. From this limited analysis two interesting findings emerge. First, the value of b increases with increasing dose of X-rays or neutrons, and the rate of increase is greater for the more damaging neutrons. Second, there is a change in the value of a at the highest dose of each radiation. This suggests that the cataractogenic effect of both X-rays and neutrons is one accelerating the aging mechanism, Eq. (7). But, at high doses there is also an early acting independent mechanism, Eq. (6). It is interesting that both 640 rep of X-rays and 180 rep of

neutrons result in approximately the same acceleration of the aging mechanism, but at 640 rep of X-rays the independent mechanism is also effective so that the reduction in time to half-survival is greater. Thus, the action of irradiation through multiple mechanisms to produce the same effect provides a possible explanation to the variation with dose of the relative effectiveness of different radiations. Further, these results on cataractogenesis are consistent with the concept that there is a threshold radiation dose for the independent mechanism, but that there is no threshold dose for the acceleration of the aging mechanism. The implication is that some environmental factors may be either threshold or nonthreshold or both.

In summary, this brief analysis suggests that the Steady State Theory of Mutation Rates is applicable to the survival of a single organ of an animal, and, in this case, served to relate the effects of aging and irradiation.

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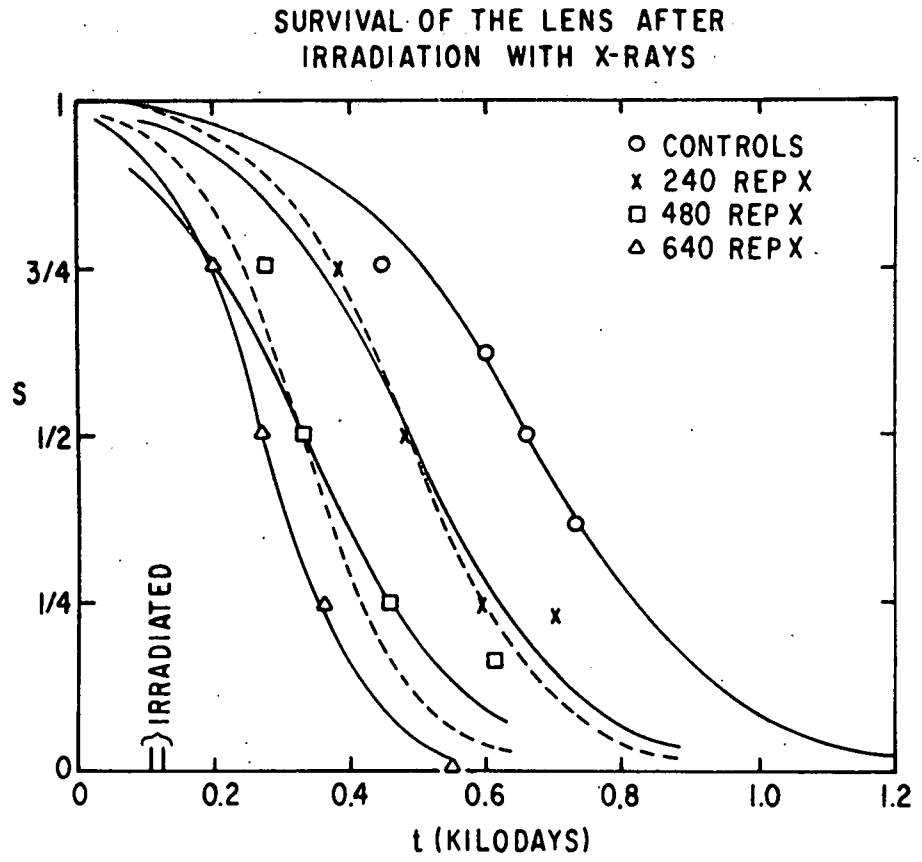

Figure 1. Survival of the lens after irradiation with X-rays.

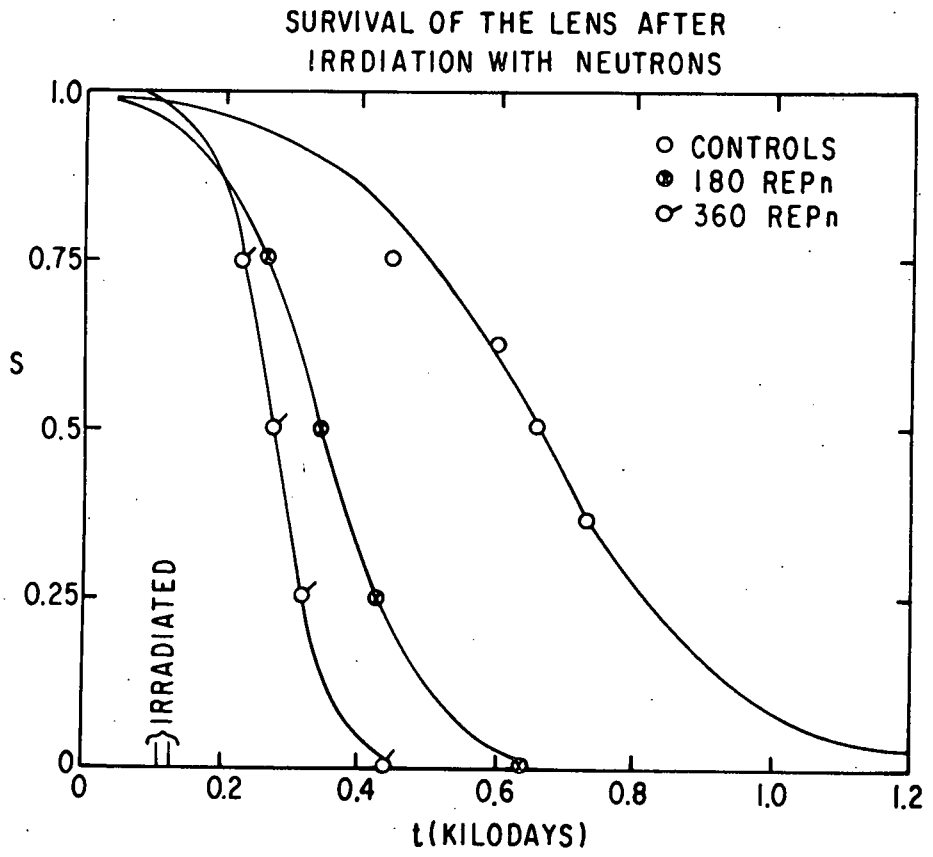

Figure 2. Survival of the lens after irradiation with neutrons.

THE SUBCELLULAR DISTRIBUTION OF PLUTONIUM IN THE LIVER AND ITS ASSOCIATION WITH FERRITIN

Betsy J. Stover, Friedrich W. Bruenger and Walter Stevens

Abstract - In canine livers ^{239}PuIV is distributed ubiquitously at the subcellular level. Livers from beagles injected with ^{239}PuIV in citrate buffer and sacrificed at times ranging from 7 to 1539 days after injection were separated by differential centrifugation. Significant concentrations of plutonium were found in the subcellular particles, the cytosol and the connective tissue. Concentrations in the mitochondrial-lysosomal fractions and microsomal fraction were the highest. Like AmIII most of the plutonium in the cytosol was bound to ferritin. Smaller fractions of plutonium were found in the very high molecular weight components (probably lipofuscin) and some with an unidentified low molecular weight material. Pure Pu-containing ferritin was separated by gel electrophoresis and by ultracentrifugation. The high concentration of plutonium in the microsomal fraction was due to contamination by ferritin which was heavily loaded with iron.

Introduction

The association of ^{241}Am with subcellular particles and the soluble fraction of the liver have been reported (1). ^{241}Am is a decay product of ^{241}Pu and, therefore, a contaminant of plutonium produced by reactors with high neutron fluxes. ^{241}Am emits a 60 Kev gamma in high abundance which makes it easy to measure and since the subcellular distributions of plutonium and americium in liver are similar, the methods for plutonium were developed using americium. The initial experimental procedures were developed by measuring a large number of ^{241}Am samples. Using these data methods were chosen which would yield a maximum amount of information about the distribution of ^{239}Pu in livers with a minimum of samples.

The distribution of AmIII and PuIV is related to the

reactions of proteins of the iron transport and storage system. PuIV-transferrin is more stable than the AmIII-transferrin complex. The simultaneous occurrence of plutonium and hemosiderin in macrophages of bone marrow was reported many years ago by Arnold and Jee (2). In addition, Taylor has demonstrated histologically that high concentrations of plutonium are found in areas of canine livers rich in hemosiderin several years after intravenous injection of PuIV (3). Recently we reported that a high molecular weight iron-containing protein from the soluble fraction of liver homogenates obtained after centrifugation at 105,000 xg was associated with plutonium. Also, in vitro Pu-transferrin and ferritin react to form free transferrin and Pu-ferritin. This and previous observations with ^{241}Am liver homogenates provided the basis for a more detailed study of the distribution of plutonium in liver homogenates.

Experimental

A beagle, 17 months old and weighing 11.2 kg received 4.5 μCi of $^{239}PuIV$ in 0.08 M citrate of pH approximately 3.5. This dog was designated as T56P5.5. Seven days later, the dog was sacrificed by exsanguination and the animal was perfused with cold 5% sucrose-physiological saline solution. The liver was excised, weighed and immediately cooled in ice. All subsequent work was done at a temperature between 0° - 5° C. Liver tissue was minced in a hand driven meat grinder and homogenized with three parts (v/w) of 0.25 sucrose containing 3 mM of Ca^{++}. The homogenate was strained through cheesecloth and was separated

by differential centrifugation into a nuclei-cell membrane fraction containing some debris, three successively heavier mitochondrial-lysosomal fractions, a microsomal and two soluble fractions. One fraction (cytosol 1) was prepared by centrifugation of the 25,000 xg supernatant at 105,000 xg, the other (cytosol 2) by centrifugation at 220,000 xg (Table 1). The identity of all fractions was confirmed by electron microscopy. Concentrations of plutonium based on wet weights were measured at each step. The cytosol was further separated by gel filtration on gels of different pore sizes, by ion exchange on DEAE-Sephadex, by heat denaturation and salt precipitation, and analytical and preparative gel electrophoresis. Additional data were gathered from livers of other beagles that had lived from 7 to 1539 days after injection. Some of these tissues had been frozen prior to analysis.

Results and Discussion

Freezing of tissue results in rupture of some subcellular membranes. Thus, data collected from tissue that had been preserved by freezing cannot exactly represent the actual in vivo distribution of the nuclide at the time of death. They do, however, provide a reasonable estimate of those values obtained from fresh tissue.

The plutonium concentration in the connective tissue was lower than the average concentration in the whole tissue, but plutonium was associated with the connective tissue elements. Listed in Table 2 are times after injection, concentration of

plutonium in the nuclei + debris, two mitochondrial fractions and the total liver retention of plutonium for T56P5.5 and five other dogs.

Table 2

Concentration of Plutonium Relative to Original Mince

Dog No.	Δt Days after Injection	Nuclei + Debris	Mitochondria 1	Mitochondria 2	% Inj. Dose Retained
T56P5.5	7	0.474	1.08	0.922	0.279
T42P5	13	0.431	0.28	0.723	
T52P4	14	0.611	0.425		0.247
T55P4	14		0.73		0.325
T51P5	1055		1.12	0.325	
T41P5	1227		0.545		0.172
F05P1	1539	1.049	0.402	0.825	0.26
T47P5*	69	1.724	0.826	0.172	
T48P5*	1327	0.816	1.05	0.724	
Average Concentration			0.72	0.615	

* Dogs received injections of Proferrin (Saccharated iron oxide)

The plutonium concentration in the washed nuclei + debris fraction varied from 43% up to greater than 170% of the concentration in the mince. Concentrations of plutonium in this fraction increase with time after injection, although more data should be collected to verify this trend. This increase may be

caused by the accumulation of iron (in the form of hemosiderin) and plutonium in areas fed by the portal vein. The phenomenon can be observed histologically at longer times after injection. Hemosiderin granules associated with plutonium are heavy enough to be sedimented under the weak centrifugal forces that are applied to separate the nuclei + debris fraction. Examination of this fraction by electron microscopy shows it was slightly contaminated with mitochondria.

Electron micrographs showed that mitochondria separated in sucrose solutions by centrifugation are swollen. The average swelling factor was conservatively estimated to be about 1.5. The average concentration of plutonium in the washed, heavy mitochondria of nine dogs was 72% of the concentration in the whole mince. If we multiply 72% by the average swelling factor, then the concentration in this fraction is greater than 108% of the concentration in the whole liver. In rats the mitochondrial fraction accounts for as much as 18% of the tissue as determined by Morphometric techniques (4). This means that as an average at least 20% of the plutonium retained by canine liver is associated with the mitochondrial fraction. This may not be true in livers undergoing pathological changes.

The highest concentration of plutonium was found in the microsomal fraction (> 1). As will be seen later, this is an artifact and is primarily due to accumulation of a soluble plutonium-carrying protein in the microsomal pellet. Some plutonium is found in purified microsomal fractions (but it is

not known at present how much plutonium is actually associated with microsomes.)

Since a soluble plutonium-containing material contaminates the subcellular particles, (demonstrated by loss of plutonium following washing), it is difficult to determine exactly how much plutonium is in the cytosol. The average concentration of plutonium in the soluble fraction of 9 dogs was 20% of that in the whole homogenate. If approximately 45% of the liver volume is cytosol then this fraction accounts for a minimum of 10%- 12% of the plutonium in the liver. It would probably be more realistic to triple this value in order to account for plutonium that sediments with the microsomal pellet or can be detached from the other fractions by washing.

The cytosols were subjected to standard gel filtration methods. First cytosol 1 was separated on Sephadex G-200 and BioGel A-1.5m (Fig. 1). With G-200, 2 peaks of plutonium were seen, one in the low molecular weight region, the other covering the region of void volume and adjacent area. The high molecular weight plutonium peak was separated into two peaks by chromatography on the BioGel A-1.5m resin. The molecular weight of the second peak was estimated as approximately 450,000. No protein peak was found with the plutonium eluting at this point.

Cytosol 2 contained much less protein (Fig. 2). The plutonium in the void volume appeared only as a shoulder, but the nuclide associated with molecules of molecular weight of approximately 450,000 formed a prominent peak. Low molecular weight

plutonium compounds were slightly enriched, relative to the distribution in 105,000 xg cytosol.

The 105,000 xg and 220,000 xg pellets were again homogenized and recentrifuged at 25,000 xg. The supernatant was of yellow-brown color. A plutonium-containing protein of molecular weight approximately 450,000 was obtained after gel filtration (Fig. 3). The density of this protein (which appeared heterogeneous) was high enough to cause collection in the high speed pellet (105,000 xg and 220,000 xg) and result in the contamination of the microsomal fraction by plutonium.

From our experience with ^{241}Am we expected that this protein was Pu-ferritin. After tagging the cytosol with ^{59}Fe, a fairly pure preparation of ferritin was prepared by heat denaturation followed by salt precipitation, dialysis and centrifugation at 105,000 xg in Tris-buffer (Fig. 4). Ion exchange chromatography of this preparation on DEAE-Sephadex using a linear salt gradient resulted in a simultaneous peak of protein, ^{59}Fe and ^{239}Pu.

Analytical gel electrophoresis of a ferritin preparation which was carried only through the salt precipitation, resulted in the appearance of a little plutonium in one of the front bands, but most of the plutonium was found in the ferritin region.

During preparative gel electrophoresis (Fig. 5) little plutonium was eluted with the fast moving proteins; the bulk was again associated with the brown ferritin.

Gel electrophoresis in discontinuous buffers leads to pure fractions and positive identification of Pu-ferritin was thus

possible.

The low molecular weight component has not yet been identified although it accounted for approximately 10% of the plutonium in the 105,000 xg supernatant. The plutonium in the very high molecular weight fraction is associated with a structureless material, probably lipofuscin.

In summary, after intravenous injection, plutonium in livers is ubiquitously distributed among the subcellular particles and is also found in the connective tissue. A variable amount is found in the cytosol. Most of the soluble plutonium is present as Pu-ferritin but a small amount is also associated with a low molecular weight and a very high molecular weight fraction.

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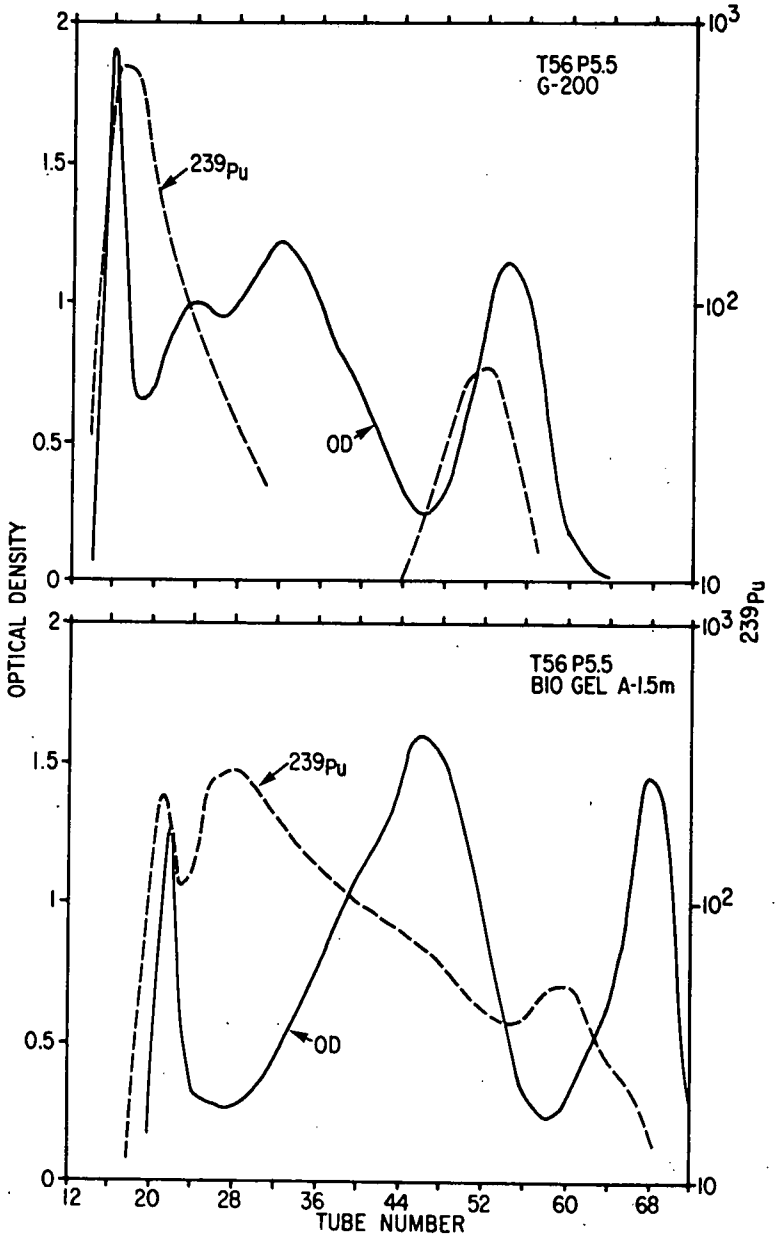


Figure 1 Distribution of ^{239}Pu in 105,000 xg liver supernatant (cytosol 1) after chromatography on Sephadex G-200 and BioGel A-1.5m. On G-200 only one peak of Pu-activity was resolved in the high molecular weight region and one peak of plutonium appeared in the low molecular weight region. On BioGel A-1.5m plutonium in the high molecular weight region was resolved into two distinct peaks.

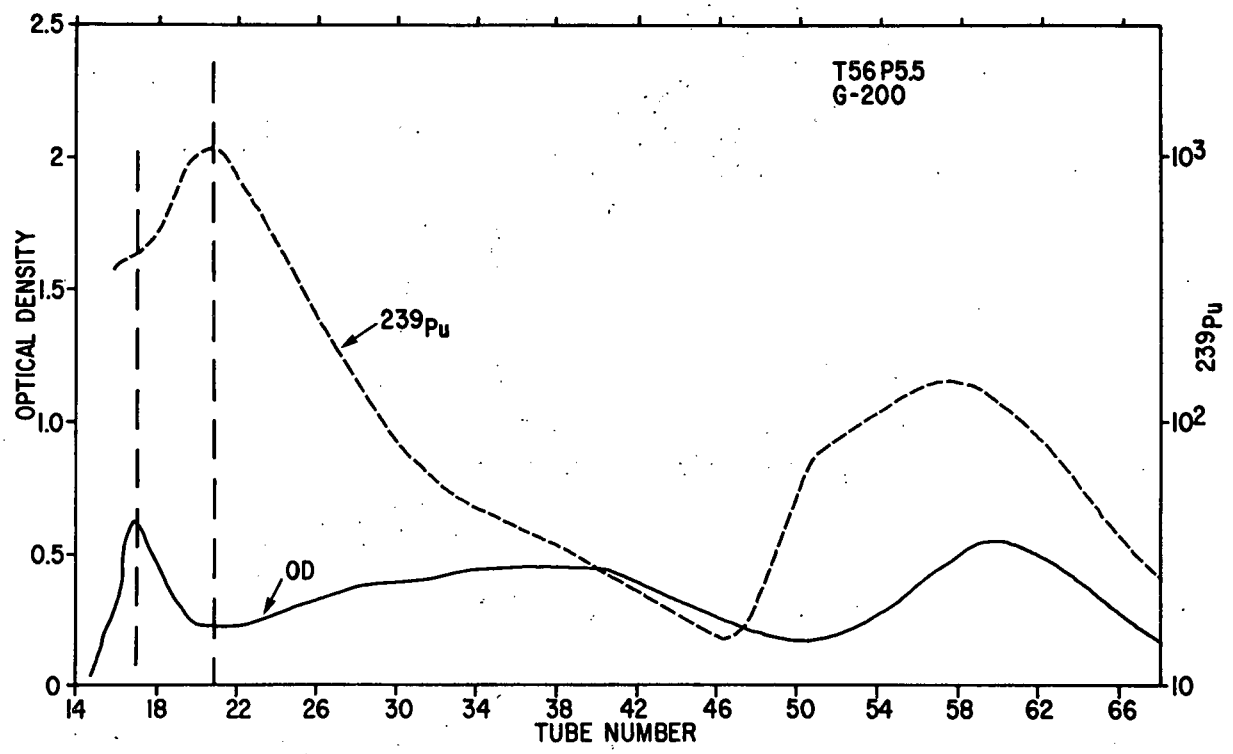


Figure 2 Distribution of ^{239}Pu in 220,000 xg liver supernatant (cytosol 2) after chromatography on Sephadex G-200. Some of the high molecular weight protein has been eliminated by the high g-forces. The peak of ^{239}Pu activity in the high molecular weight region is still present but the fraction of ^{239}Pu in the low molecular weight region is now larger relative to the combination seen in cytosol 1.

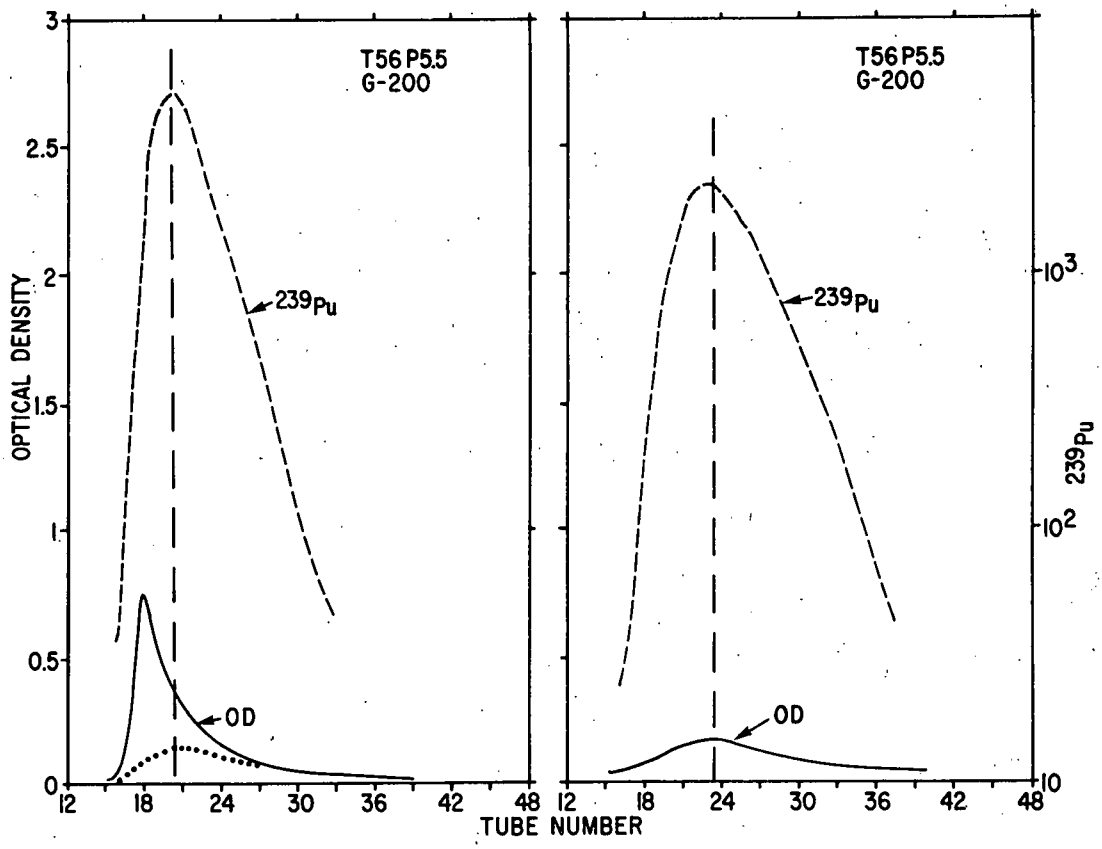


Figure 3 Spectra of U. V. absorbing material and ^{239}Pu after chromatography of a rehomogenized microsomal pellet of Sephadex G-200. The left side shows a chromatogram of the whole homogenate. The right side is the spectrum of the homogenate after elimination of non-ferritin material. The figure demonstrates that the high plutonium activity of the microsomal pellet is in part due to contamination by Pu-carrying ferritin.

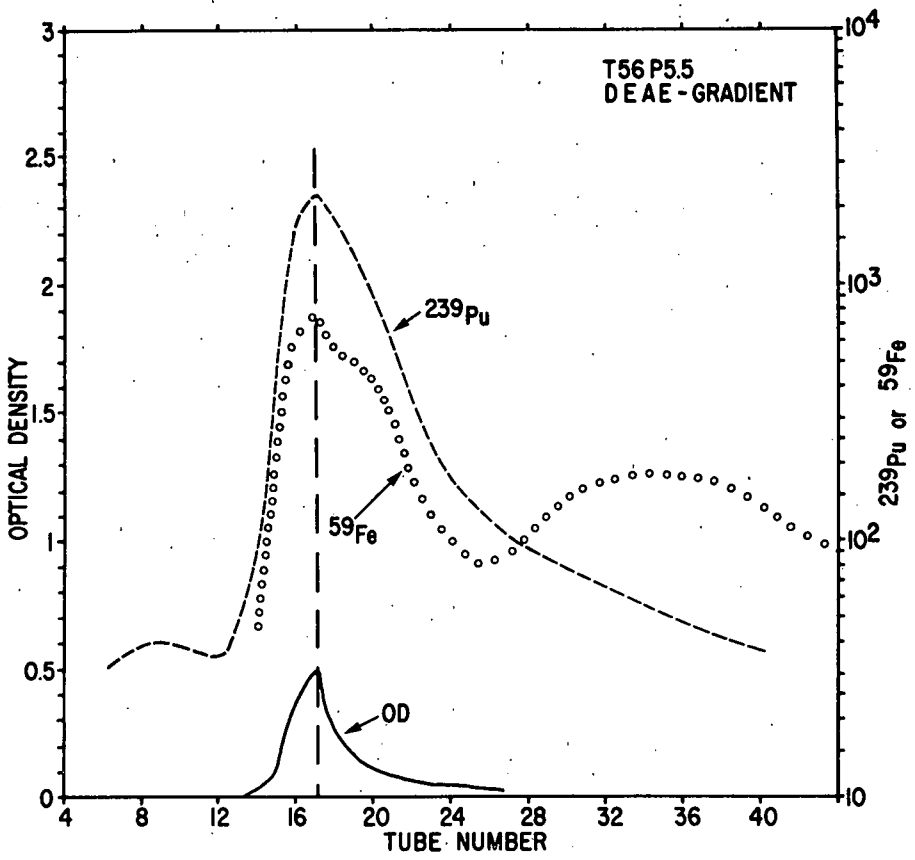


Figure 4 Spectrum of ^{59}Fe, ^{239}Pu and ferritin after gradient elution of a crude ferritin preparation obtained after tagging of a liver cytosol with ^{59}Fe. Peaks of the two nuclides and ferritin are coinciding.

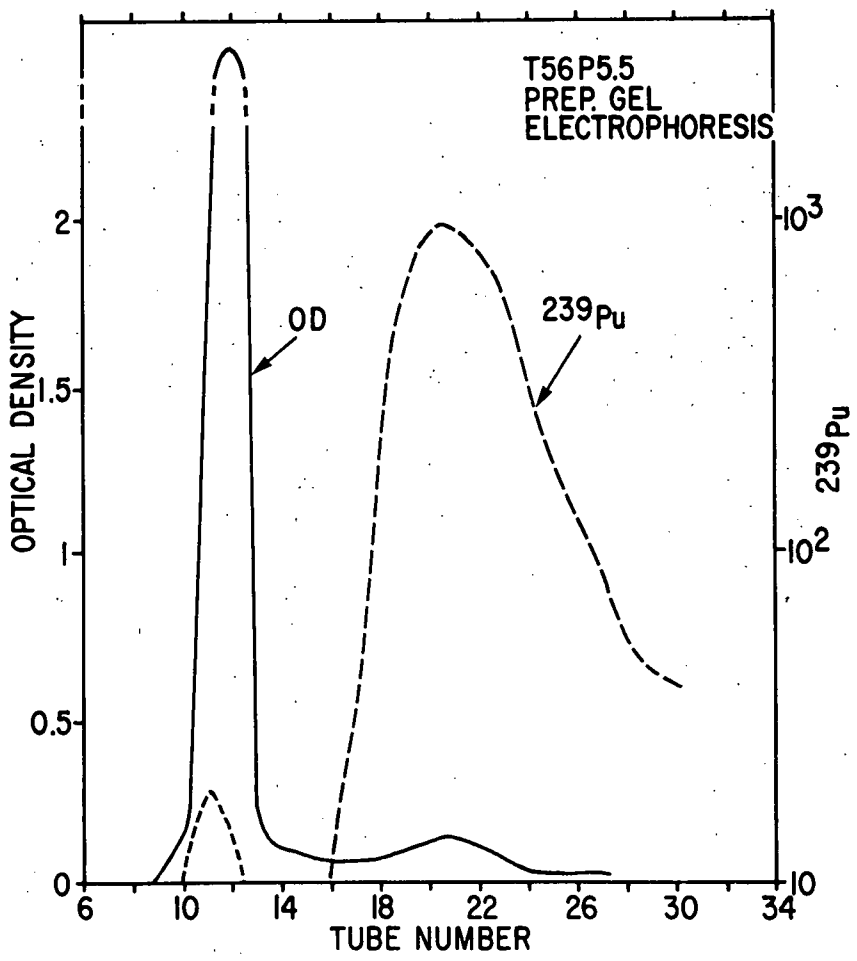
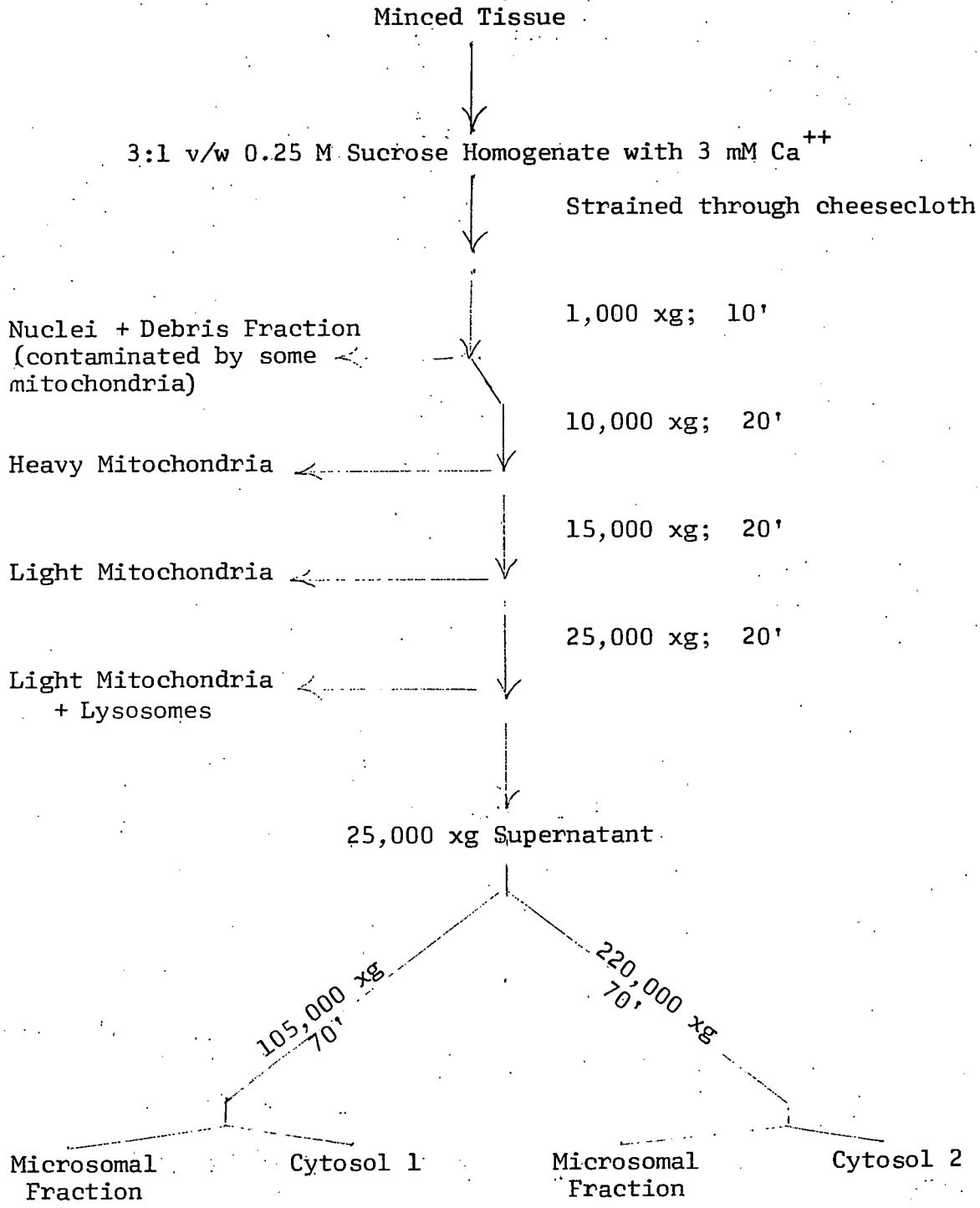


Figure 5 Results of preparative gel electrophoresis of a crude ferritin preparation obtained from T56P5.5. Only little plutonium was found in the front band; the bulk was bound to ferritin.

Table 1

Flow Sheet of Differential Centrifugation



STABILITY OF PuIV-TRANSFERRIN RELATIVE TO FeIII-TRANSFERRIN

Friedrich W. Bruenger, Betsy J. Stover and David R. Atherton

Abstract: To resolve an apparent conflict in the literature concerning the relative stabilities, in physiological environment, of the complexes of FeIII and PuIV with transferrin, specific experiments were designed to test the methods that had been used. Results confirm the greater stability of the FeIII complex, and further demonstrate that the utmost attention must be devoted to the choice of experimental methods in order to avoid hydrolysis of PuIV and to achieve meaningful separation of constituents.

Introduction

Both the blocking of the formation of PuIV-transferrin and the displacement of PuIV from the complex by FeIII have been reported (1). Human blood was used, and the proteins of the serum were separated by gel filtration and ion exchange chromatography. At about the same time, Turner and Taylor reported similar findings (2). Their experiments were nicely complementary, since in their work blood from a different species and different (electrophoretic) analytical methods were used. More recently there has been a contradictory report which appeared to result from experimental error (3). The experiments reported below were designed and executed to clarify the issue.

Experimental Procedures

A solution of PuIV nitrate was prepared in the manner described previously (4). This acidic solution was then mixed with a saturated solution of $NaHCO_3$, pH 8. Next the solution was filtered through a molecular filter (Aminco UM-2) that permitted passage of only that

material of molecular weight less than 1000.

Experiments were done with three proteins: A. α-globulin, B. human transferrin that contained no iron (apotransferrin), and C. human transferrin that was saturated with iron. In each case 20 mg protein was dissolved in 1.5 ml of a solution of 1 g. NaCl and 235 mg $NaHCO_3$ in 100 ml (the approximate concentration of HCO_3^- in serum (2.8 m equiv/100 ml)). The pH was maintained at 7.5. In Expt. C 30 μg Fe (210 μg $Fe(NH_4)_2(SO_4)_2$) in 0.05 ml was added to saturate the transferrin. This amount (1.5 μg Fe/mg protein) is based on two atoms of iron per molecule of transferrin and a molecular weight of 75,000 (5). The corresponding value for plutonium is 6.4 μg Pu/mg transferrin.

In each experiment 0.3 μCi ^{239}Pu in 0.1 ml, which is 4.9 μg of plutonium, was added to the solution of protein and incubated 20 minutes at 37-40° C. Then the solutions were analyzed chromatographically on identical columns of Sephadex G-200 (6.5g). The molecular weight range over which effective fractionation is obtained with this resin is 5000 to 500,000 (determined with globular proteins). The columns were eluted with a 0.1 M Tris (tris(hydroxymethyl) aminomethane) buffer of pH 7.4, that also was 4% in NaCl. Protein concentration was determined spectroscopically by measuring the optical density at 280 nm, and plutonium was measured by alpha scintillation counting.

Results and Discussion

The bicarbonate solution of plutonium was clear and, at the visual level of detection, homogeneous. However, filtration through

the membrane which permitted passage only of chemical entities of molecular weight less than 1000 showed that 29% of the plutonium was actually colloidal. The remaining 71% was in the form of a carbonate or bicarbonate complex of unidentified stoichiometry, and this filtered solution was used for the three experiments.

The 29% of the plutonium that did not pass through filter is a measure of the hydrolysis that took place when the acidic solution of PuIV was mixed with the alkaline bicarbonate solution. The rates for the reactions of plutonium ions with HCO_3^-, $CO_3^=$, and OH^- are fast and equal to the appropriate stoichiometric products of the concentrations and the specific rate constants. Thus, competitive reactions which lead to complexed or colloidal plutonium occur. Another kinetic factor is also involved, the rate of mixing. This affects the localized concentrations of the reactants, and, thus, affects the rates at which complexing and hydrolysis occur. The specific rate constants are constants in the thermodynamic sense, but rates of mixing depend on many factors, and are not readily subject to precise control. Thus, preparative procedures, should be sought that avoid small localized volumes of high pH.

The chromatograms that were obtained in the three experiments are compared in Fig. 1. Note that the protein concentration is plotted on a linear scale, while that of plutonium is plotted on a logarithmic scale since the range of detection is so great.

In Expt. A (Fig. 1 A) 55% of the ^{239}Pu was eluted as a stoichiometrically uncharacterized complex with carbonate or bicarbonate in the low molecular weight range. A trace was associated with

α-globulin, and another trace was excluded from the resin and eluted with an apparent protein impurity in the void volume. The remainder of the ^{239}Pu was lost in the chromatographic system. This occurred because the bicarbonate solution was diluted by the Tris buffer during the elution. The concentrations of $CO_3^{=}$ and HCO_3^{-} were reduced while that of OH^{-} remained essentially constant. Plutonium then dissociates from the ionic, low molecular weight, bicarbonate complex to maintain the equilibrium concentration determined by the stability constant of the complex and the concentrations of the various ions in the solution. Because of the high pH these dissociated plutonium ions are removed from the bicarbonate system through competitive hydrolysis reactions. This leads to further dissociation followed by further hydrolysis. The extent of hydrolytic loss of plutonium thus depends on the equilibrium constants for the competitive reactions, the varying concentrations of the complexing anion(s), and the pH.

In Expt. B (Fig. 1 B) essentially all of the plutonium was eluted as the transferrin complex. The peak concentrations of protein and plutonium coincide, and the peaks are symmetrically similar. No ^{239}Pu was detected ($\leq 0.2\%$) with the void volume, and the few counts measured in the low molecular weight range are of doubtful significance. Thus during the incubation the plutonium was essentially completely complexed by transferrin, and the stability of the complex is sufficiently greater than that with bicarbonate so that dissociation and hydrolysis during elution were insignificant.

In Expt. C (Fig. 1 C) in which transferrin saturated with iron

was used, the results are quite different. Only 1.5% of the ^{239}Pu was eluted with transferrin, 17.8% was recovered in the low molecular weight range, apparently as the carbonate or bicarbonate complex, and other 2.2% was excluded from the resin and eluted with the void volume. The rest of the plutonium was hydrolyzed and either remained on the resin or on surfaces through colloidal absorption.

These results clearly show that plutonium is not bound by transferrin that is already saturated with iron and confirm the previous reports that the iron complex is more stable than that of plutonium in the physiological pH range.

Gel filtration has proved to be a valuable method in determining the chemical binding of plutonium and americium in biological systems. However, Expt. C illustrates the need to select the proper resin. For example, had a resin of high crosslinkage with a fractionation range of 500 to 10,000 been used instead of Sephadex G-200, the transferrin would have eluted with the void volume and it would have been impossible to distinguish plutonium in large colloidal aggregates from that bound to transferrin. Thus, meaningful results are obtained only if the method separates the proteins and colloidal Pu.

In summary these experiments illustrate that hydrolysis is an ever present problem in biological studies with plutonium. It can be avoided by devising methods of preparing stable complexes at low pH and then adjusting the pH upward. Then the combination of small volumes of high pH and uncomplexed plutonium, which leads to hydrolysis, is avoided. An awareness of the conditions that

result in hydrolysis is absolutely necessary in choosing appropriate analytical methods, e. g., use of the G-200 rather than a resin of high crosslinkage in Expt. C.

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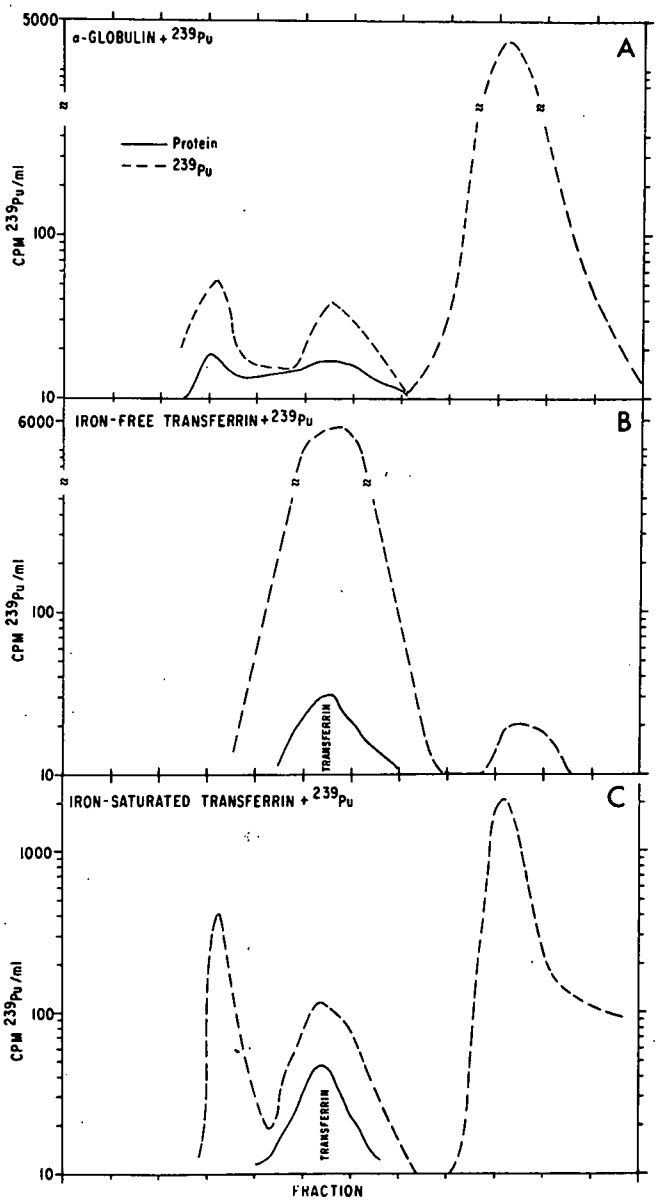


Figure 1. The figure depicts the elution spectra after gel-chromatography on sephadex G-200 of A.) α-globulin incubated with ^{239}PuIV, B.) apotransferrin incubated with ^{239}PuIV, and C.) iron-saturated transferrin incubated with ^{239}PuIV. Only the apotransferrin combines essentially quantitatively with the nuclide.

THE DISTRIBUTION OF ^{210}Pb IN CANINE BLOOD AFTER INTRAVENOUS INJECTION AND THE ASSOCIATION OF THE NUCLIDE WITH BLOOD CONSTITUENTS IN DOGS AND HUMANS

Friedrich W. Bruenger, Walter Stevens and Betsy J. Stover

Abstract: A preliminary report on the distribution of ^{210}Pb in blood after intravenous injection is presented. The continuation of the study will be published later. After intravenous injection of ^{210}Pb into beagles, the nuclide reached its maximum concentration in blood several hours after administration. Most of the ^{210}Pb was associated with blood cells. The concentration in blood was measured for 287 days after injection at which time 0.058% of the injected dose was still circulating. *In vitro* experiments with canine and human blood showed that about 90% of the nuclide combined with the red cells was associated with the red cell cytoplasm and less than 10% was bound to the stroma. Transfer across the red cell membrane was possible without prior binding of the nuclide to plasma proteins. After gel chromatography and disc electrophoresis, most of the lead was found in hemoglobin containing fractions. In the dog varying amounts were also found in fractions of high electrophoretic mobility. In humans the largest fraction of ^{210}Pb was found with a minor hemoglobin band. Only after saturation of this fraction were greater quantities found in the main hemoglobin band.

Introduction

Lead, a daughter product of several of the radionuclides used in this laboratory, has become a subject of increasing importance in the last few years. This is due to a number of factors. Environmental pollution by lead from the exhaust of engines using leaded gasolines has become a subject of controversy. Although no clinical proof exists at the present time that the level of lead in air is a health hazard to the public, the degree of lead pollution is still increasing and sufficient data on continuous exposure to such levels are as yet not available. In addition, uranium miners of the Colorado plateau are exposed to high levels of radioactive lead isotopes. In this case it is

not the chemical toxicity which imposes a health hazard but the radiotoxicity of inhaled radon and its daughter products. In our laboratory ^{210}Pb makes a significant contribution to the radiation dose in some of our dogs.

As data on the metabolism and distribution of tracer lead are very scarce it was decided to study the distribution of ^{210}Pb (half-life 22.5 years) and its association with biological molecules in greater detail. Evidence obtained from previous studies with ^{212}Pb (1) indicated that lead after intravenous injection was associated with red blood cells. The nature of this association is unknown. Therefore the following studies were performed.

Experimental

Three young, adult beagles were injected with approximately 10 μCi of ^{210}Pb/kg of body weight. One dog was sacrificed 28 days after injection. The other two dogs are being kept to study the late-effects. Data on the distribution of ^{210}Pb in the sacrificed animal are found elsewhere (2). The level of circulating ^{210}Pb in the blood following injection was measured at frequent intervals for 28 days in one dog and for 72 days in the other two dogs. A final measurement was taken at 287 days. ^{210}Pb was determined in whole blood, cells and plasma. Cells and plasma were separated by centrifugation for 20 minutes at 3000 RPM (r = 18.1 cm). Plasma was removed but cells were not washed. A sample of red blood cells was lysed and ^{210}Pb was determined in the soluble fraction (obtained after 40' at 20,000 xg) and in the ghosts after they were washed in 310 mOsm buffer.

Results obtained from the above investigation encouraged us to do a number of in vitro experiments with human blood from several individuals and with canine blood. In each case, blood was drawn with a heparinized syringe and was incubated for 20 minutes at 40° C with up to 3 μCi of ^{210}Pb dissolved in physiological saline for each 20 ml of blood. Two or three 1 ml aliquots of the washed, packed cell fraction were lysed by osmotic shock with 4 ml of distilled water and 1 ml of toluene was added. Mixing was achieved by intermittent shaking. Toluene was drawn off after low speed centrifugation and the stroma was separated from the soluble proteins by centrifugation at 27,000 xg for 30 minutes. The supernatant red cell cytoplasm (in this text simply called cytoplasm) was analyzed by gel filtration and disc electrophoresis.

Using a separating gel with 7% acrylamide, analytical disc electrophoresis was carried out in duplicate for 70' at 200 V. The gel cylinders had a diameter of 11 mm and held 10 ml of the separating gel. One gel cylinder was stained for detection of protein bands, the other was cut into slices of equal thickness (~1.3 mm) and counted.

Preparative gel electrophoresis was performed in 6 ml of 10% separating gel and 8 ml of spacer gel at a constant 400 V. Red cell cytoplasm was applied to the gel in all cases as a sample of high density (using sucrose). Some of the conditions for these experiments and variations from the given procedure will be described in the result section.

High and low molecular weight components of the red cell cytoplasm were separated by chromatography on Sephadex G-25 and/or by dialysis through Visking Nojax casing using a 0.05 M Tris-HCl buffer of pH 7.5.

The transfer of ^{210}Pb from plasma to cells was studied in the following manner. Twenty ml of human or canine blood was introduced into a solution of ^{210}Pb in physiological saline solution. The mixture was briefly agitated with a Vortex mixer and 3 ml aliquots were taken and transferred to Corex tubes. Plasma and cells were separated by centrifugation after the following periods of incubation at 37°-40° C : 2', 5', 10', 20', 60'. At each step, cells were separated and washed 3 times with 3 ml of saline each and counted. A final count was made after the third washing.

All counting was done with a NaI(Tl) scintillation detector (3) using the 47 keV gamma emission of ^{210}Pb.

Results

The concentrations of ^{210}Pb in the whole blood, cells and plasma are listed in Table 1 for times from 30 minutes to 72 days after injection. Also listed are the ratios of % D/gm in cells to the % D/gm in plasma. Shortly after injection, the concentration of the nuclide in blood went through a minimum. No measurements were made before 30 minutes after injection. From 30 minutes to 6.5 hours after injection the concentration of ^{210}Pb increased in the whole blood and in the cell fraction. In plasma, the concentration was continuously decreasing and

Table 1

Distribution of ^{210}Pb in Blood, Cells and Plasma after Intravenous Injection

Δ T (Time after injection)	% of ^{210}Pb Dose/g of blood ($\times 10^3$)	cells ($\times 10^3$)	plasma ($\times 10^3$)	% D/g cells / % D/g plasma
30 min	52.70	94.1	2.51	37.5
1 hr	53.24	98.3	1.33	73.9
3 hr	56.17	101.8	0.67	151.9
6.5 hr	57.34	102.0	0.53	192.4
1 day	42.32	81.6	0.28	291.4
2 day	33.19	61.8	0.20	209.0
3 day	26.11	50.5	0.17	297.0
7 day	14.43	26.4	0.07	*
13 day	7.98	15.2	0.05	*
21 day	5.63	10.1	0.05	*
36 day	2.16	4.2	0.01	*
55 day	1.02	1.8	0.01	*
72 day	0.57	1.0	*	*

* Large counting errors make computation of this ratio meaningless.

reached very low levels a few days after injection. The same data are presented in Fig. 1, showing the respective curves for the first 24 hours after injection.

The amount of ^{210}Pb circulating in the whole blood at times from 1 day to 72 days is given in Fig. 2. Data were calculated by multiplying the % D/g in whole blood by 99.7 (g of blood/1 kg of weight in the beagle (4)) times the weight of the animal. At 30 minutes after injection, an average of 50.3% of the injected ^{210}Pb was circulating. This amount increased gradually to 54.7% at 6.5 hours. At one day after injection 40.4% was still in circulation. Most of the nuclide was associated with the red cells, only 0.27% of the injected dose was plasma-bound at this time. The ^{210}Pb circulating in the blood declined rapidly with a half life of approximately 3 days during the early time after injection, followed by a less precipitous decline out to 72 days. Another measurement was made at T = 287 days. An average of 0.058% of the injected dose was circulating at that time.

Various cell fractions, obtained between 30 minutes and 3 hours after injection, were lysed in order to determine the distribution of ^{210}Pb between the stroma and the soluble fraction of the red cell (the supernatant after 40 minutes at 20,000 xg). Between 85% and 86% of the ^{210}Pb could be separated from the stroma and after washing only 1.4% of the lead was still bound to the membrane fraction. The majority of the balance was associated with the soluble fraction.

The results of the <u>in vivo</u> experiment prompted a more

detailed in vitro study of the association of ^{210}Pb with constituents of the red cell cytoplasm and of the transport of the nuclide through the red cell membranes. Dog and human blood was used in these studies. First the distribution of ^{210}Pb between plasma, the RBC-cytoplasm and stroma was studied in blood from dogs of two different breeds, a Beagle and a St. Bernard. The distribution of ^{210}Pb between the plasma and cell fractions varied over a considerable range. After incubation, in the beagle 32% of the ^{210}Pb was found in the plasma and 68% was with the cells and 90.6% of the ^{210}Pb in the red cell was in the cytoplasm and 9.4% in the unwashed stroma. In the St. Bernard 18% of the lead was left in the plasma and 82% was with the cells. In the red cells, 90.7% was associated with the cytoplasm and 9.3% was found in the stroma. In human red cells under identical conditions, 87.6% was found in the red cell cytoplasm, the balance in the unwashed stroma.

Transfer of ^{210}Pb from plasma to cells was faster in human blood than it was in the beagle. It was assumed that equilibrium was reached after 1 hour of incubation time. At this time approximately 80% and 60% of the ^{210}Pb had entered the red cell in human and canine blood, respectively (Fig. 3). After incubation of human blood with 0.3 mg of stable lead + approximately 0.2 μCi of ^{210}Pb/ml of blood, more than 1/2 was found in the stroma.

Studies were made to see if constituents of plasma were necessary for the transport of ^{210}Pb across the red cell membrane.

Human blood was separated into a plasma and a cell fraction. The cell fraction was then washed three times with saline to remove plasma residues and 2 ml aliquots of red cells were taken for the experiment. Meanwhile 10 ml of the original plasma and 10 ml of saline had been incubated with an equal quantity of ^{210}Pb. The 2 ml aliquots of cells were then added to the plasma and saline, respectively, and incubated. Separation of cells and subsequent washings were carried out in the usual manner. After incubation 84% of the initial ^{210}Pb in the saline medium and only 48% of the initial ^{210}Pb in the serum medium were found with the washed cells. Other data from this experiment are found in Table 2.

Table 2

Distribution of ^{210}Pb in Red Cell Fractions after Incubation of Cells in Saline or Plasma Media*

Sample	Saline Medium	Plasma Medium
Clear cytoplasm	85.3%	78.8%
Cloudy cytoplasm	7.6%	8.1%
Stroma before 1st washing	8. %	14.5%
Stroma after 3rd washing	5.5%	10.6%

*Data are % of ^{210}Pb in fractions relative to ^{210}Pb in cells (100%).

A sample of ^{210}Pb tagged red cell cytoplasm was chromatographed on Sephadex G-100. Elution was carried out with a buffer containing 0.1 M Tris + 4% NaCl, pH 7.5. The elution spectrum is

seen in Fig. 4. ^{210}Pb-activity reached its peak slightly after the hemoglobin peak.

Treatment of hemoglobin with 0.88 volumes of a cold ETOH-chloroform mixture as used in the preparation of erythrocuprein (5) from red blood cell cytoplasm resulted in the precipitation of 95% of the nuclide. Crystalization of hemoglobin with concentrated phosphate solutions according to the method of Drabkin (6) rendered the ^{210}Pb diffusible through Visking bags.

Fractions were separated by disc electrophoresis in a discontinuous buffer system (Fig. 5). To avoid misinterpretation of data, a sample of ^{210}Pb without any protein was analysed and positions of ^{210}Pb-activity coming from unbound lead were recorded. The R_f value of lead when applied as an inorganic salt with no protein present varied and was dependent on the buffer which was used. It was not sufficiently different from that of the dominant hemoglobin fraction to make a positive distinction between their exact positions possible. Elimination of unbound ^{210}Pb from ^{210}Pb that was associated with high molecular weight components of the cytoplasm was done by column chromatography and dialysis. In Table 3 products of these two procedures are compared with the original cytoplasm. Curves of Hb content and ^{210}Pb activity obtained from the G-25 columns had the same shape. Electrophoresis of these samples showed that ^{210}Pb was still present in the hemoglobin fractions although the ratio of ^{210}Pb in the main band to that in the faint band had changed somewhat.

Table 3

Elimination of ^{210}Pb Not Bound to High Molecular Weight Material by Dialysis or Column Chromatography

Sample	CPM/ml	CPM/mg Hb*	% of Original ^{210}Pb Retained
Original cytoplasm	13670	136.7	100.
Dialyzed cytoplasm	10250	123.5	90.3
G-25 cytoplasm	2000	105.3	77.

* Hb was determined using the extinction of a 1% Hb solution

$$E^{1\%}_{1\ cm} = 8.4 \quad \text{(at 540 nm)}$$

Addition of phosphate ions (at a concentration of 1.5 moles/liter) to the cytoplasm before electrophoresis detached the nuclide from its binding protein such that the bulk of it migrated with the front band. The same phenomenon was observed when electrophoresis was carried out in the presence of high concentrations of urea.

Electrophoresis of red cell cytoplasm obtained from dogs produced a number of protein bands, but showed only one rather broad band of hemoglobin. The R_f of the peak of dog Hb is ~ 0.41. Contrary to this, human red blood cell cytoplasm separated into two hemoglobin fractions, a very broad band having an R_f of ~ 0.47 and a faint band with an R_f of ~ 0.25. Gel electrophoresis of ^{210}Pb-tagged dog cytoplasm showed two peaks of radioactivity. ^{210}Pb was found in the front band and a second peak was in the leading edge of the hemoglobin ($R_f \approx 0.44$). There was a small rise in ^{210}Pb-activity trailing the main Hb peak. In human cytoplasm little or no ^{210}Pb appeared in the front band. Again

some ^{210}Pb was found at the leading edge of the broad Hb band and a second peak occured in the area of the minor Hb band. In the first human cytoplasm analysed by disc electrophoresis, 35% of the ^{210}Pb was with the main Hb-fraction, 65% was associated with the faint Hb-band. Displacements of ^{210}Pb from Hb-bands was greater when either voltage or time was increased.

In order to study the interaction of ^{210}Pb with samples of human blood more thoroughly, blood specimens were obtained from 5 individuals. These were randomly chosen and consisted of 2 males and 3 females. Samples were incubated with ^{210}Pb *in vitro* and subjected to the usual analysis. As a control, another sample of dog blood was analysed with the five human blood specimens. The distribution seen in Table 4 was the result of the electrophoresis. As indicated in the table, the major fraction of ^{210}Pb was again found with the minor Hb-band. When the time of electrophoresis was increased, the lead trailed the minor band by approximately 1 mm in the gel cylinder. Analysis of beagle cytoplasm which does not contain the Hb type seen in the minor band of human cytoplasm showed only little ^{210}Pb in the area of the missing peak.

The addition of stable lead (0.3 mg Pb/ml of blood) caused a shift in both Hb and lead toward higher R_f values. The previously prominent peak of lead became small and most of the lead was detected in the area of the major peak of hemoglobin.

During preparative Disc-Electrophoresis of ^{210}Pb-tagged human cytoplasm, the peak of ^{210}Pb-activity trailed the hemoglobin

Table 4

Distribution of ^{210}Pb in Human Red Blood Cell Cytoplasm

Blood Sample	% of ^{210}Pb in Major Hb band	% of ^{210}Pb in Minor Hb band	Ratio Major/Minor
B. F. male	39	61	0.64
R. J. male	25	75	0.33
E. B. female	33	67	0.49
D. B. female	27	73	0.36
I. Z. female	43	57	0.75
Beagle	74	26*	2.9

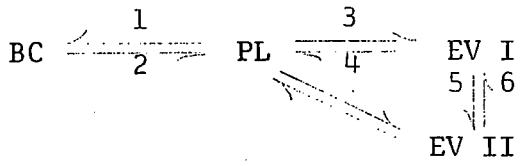
* No minor Hb band was present but counts were collected in same area.

peak by one tube. The shapes of the hemoglobin- and the ^{210}Pb-elution curves were very similar. The two bands of hemoglobin did not separate as much as during analytical gel electrophoresis.

Discussion

The concentration of the ^{210}Pb nuclide in blood increased for the first several hours after injection which confirms earlier observations with ^{212}Pb. Lead did not leave the circulation immediately, but redistributed itself within the first

24 hours after injection. Data obtained using ^{210}Pb also confirmed the findings that most of the ^{212}Pb was associated with the red blood cells. Immediately after injection, 100% of the injected dose should be circulating. This, of course, cannot be observed experimentally since it requires some time before the injected material is mixed well enough to show a uniform distribution throughout the whole vascular system. During this period some of the lead was bound by extravascular components. They can be designated as EV I and EV II (1). The reaction describing the distribution of lead can be written as:



Reaction 3 seems very fast but EV I is metastable. Reaction 1 is fast but slower than reaction 3 and BC is stable. Lead may be transferred to EV II either through EV I as an intermediate or directly from PL. EV II is more stable than BC. The increase in ^{210}Pb concentration of the cell fraction during the first 6 - 7 hours is a result of the high reaction rate of 3 and the instability of EV I. Reaction 2 can be neglected at short times after injection. The concentration of ^{210}Pb in blood cells decreases slowly and can be described by a sum of exponentials for up to 72 days. The biological half-life of ^{210}Pb in red cells, however, is appreciably shorter than the biological half-life of normal dog red cells (4).

When lead is present in tracer amounts, only a very small fraction of the nuclide can be found with the stroma. This indicates that some mechanism is actively engaged in transporting lead across the red cell membrane into the cytoplasm, where it is bound to one of the protein constituents, or the chemical potential of the cell interior is such that very little lead can diffuse out of the cell.

In vitro experiments were performed to study some of the mechanisms involved in the transfer of lead to red cells and to learn more about the association of lead with the red cell and its constituents. Information obtained from identical experiments, done simultaneously with human and canine blood in vitro should allow extrapolation of data obtained in vivo from dogs. Dual experiments were done in some, but not in all cases. Extrapolations of data, however, are complicated by variations found within the different species. Although the distribution of the nuclide varied between plasma and cells, more than 90% of the lead associated with the red cell had penetrated through the red cell membrane when tracer quantities were used. In vitro studies showed that the rate of transfer of lead from plasma across the red blood cell membrane was high initially, but that the reaction slowed within a few minutes after the incubation was started. The reason for this reduction in rate is not known, nor is it known why the transfer does not proceed quantitatively, but it was seen that the red cell membrane had a substantial influence on the final distribution pattern of ^{210}Pb in the

cytoplasm. No comparison with the distribution in vivo is possible due to concurrent reactions with the extravascular pool.

Contrary to results obtained when lead is present in tracer concentrations, an increase in lead concentration to 0.3 mg/ml of blood changed the distribution of lead between stroma and cytoplasm from approximately 10% to more than 50% in the stroma. A saturation effect was observed earlier by Schubert and White (7), who have shown that the number of cellular binding sites for lead atoms was limited when milligram quantities of lead were used. Our data not only confirm this observation but they indicate that a membrane barrier exists and that the membrane itself can bind lead.

It became evident from the incubation in a protein-free medium (saline) that plasma proteins are not necessary for the attachment of lead to red cell membranes or the transport across the membrane. The speed of reaction makes it doubtful that the transfer of lead across the red cell membrane is due to diffusion alone. The higher viscosity of the plasma medium, however, will slow down the reaction. Since under the controlled conditions of the experiment more lead was transferred into the cell from the plasma-free medium than from the plasma medium, this indicates that plasma bound some of the nuclide and made it unavailable for the direct transfer. Chromatography of ^{210}Pb tagged plasma on Sephadex G-200 demonstrated that lead was bound to several protein constituents of light and medium molecular weight, although most of the nuclide eluted as material of very

low molecular weight.

Since most of the ^{210}Pb was associated with the cytoplasm, the distribution of the nuclide in this fraction was determined by gel-chromatography on Sephadex G-100. Elution patterns demonstrated the presence of a Pb-protein complex having a molecular weight similar to hemoglobin. No pure protein fractions can be obtained by this method and therefore disc-electrophoresis was used. In humans there is a faint band of Hb trailing the main Hb band. This band was not observed in canine samples. In canines the majority of the lead was found in the area of the broad Hb band. The appearance of ^{210}Pb in the fastest moving bands of canine cytoplasm either indicated the presence of a highly charged lead component in that cytoplasm or pointed to great instability of a protein component which dissociated under the influence of the voltage gradient. No second, minor band of Hb was present in canine cytoplasm and no significant quantity of ^{210}Pb was found trailing the broad Hb band. In humans, however, the minor band of Hb (having lower electrophoretic mobility) was present, and in the area of this minor band most of the nuclide was found. This band probably represents HbA_2 ($\alpha_2\delta_2$) and it accounts for approximately 2% of the hemoglobin in human blood.

The fact that peaks of protein and lead sometimes did not exactly coincide is not inconsistent with the view that a Hb-Pb compound is present. The introduction of lead into a protein molecule such as Hb may well alter the surface (effective) charge of the protein and may also influence the size or shape

of the molecule. It is known that very small differences in the amino acid composition of hemoglobin can lead to drastic changes in the physical properties of the molecules. An excellent example of this is the greatly diminished solubility of the reduced form of HbS* in which only one amino acid residue per monomer is replaced. The delta chain of HbA_2 differs from the beta chain in the substitution of 6 amino acids. Thus, major differences in the chemical and physical properties can be expected.

There were differences in the relative distribution of ^{210}Pb between the major and minor Hb-bands in the five human samples tested, but in all cases more ^{210}Pb was found in the minor Hb-band than in the main Hb-band. In the dog, however, no minor Hb-band was present and correspondingly no significant quantity of ^{210}Pb was found in that area of the electrophoretogram. Due to the introduction of milligram quantities of lead the relative distribution of lead was shifted from a maximum amount in the small band to a maximum at the broad band. Therefore we must conclude that the binding protein in the minor component has been saturated and additional lead now is associated with a constituent of the major band.

The quantity of lead used in the latter experiment had no stoichiometric relationship to Hb. 2.85×10^{-5} moles of hemoglobin was incubated with 1.45×10^{-5} moles of lead, thus there

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*Sickle Cell hemoglobin

were 2 molecules of hemoglobin for each atom of lead. This quantity not only saturated the minor component but it also attached a greater fraction to the stroma.

At the present time, we cannot positively identify the protein molecule(s) with which lead is associated in the red blood cell cytoplasm. It is clear however that lead was bound to a high molecular weight compound and the presence of ^{210}Pb in the area of the A_2 band, observed in humans but not in canines, suggests its association with Hb. Since silver and mercury undergo a strong binding with the -SH groups of Hb, a reaction of lead with Hb does seem likely. The lack of any stoichiometric relation and the relatively low stability are in contrast to the behavior of mercury and Hb. If binding to Hb occurs, then it is weaker than with either mercury or silver.

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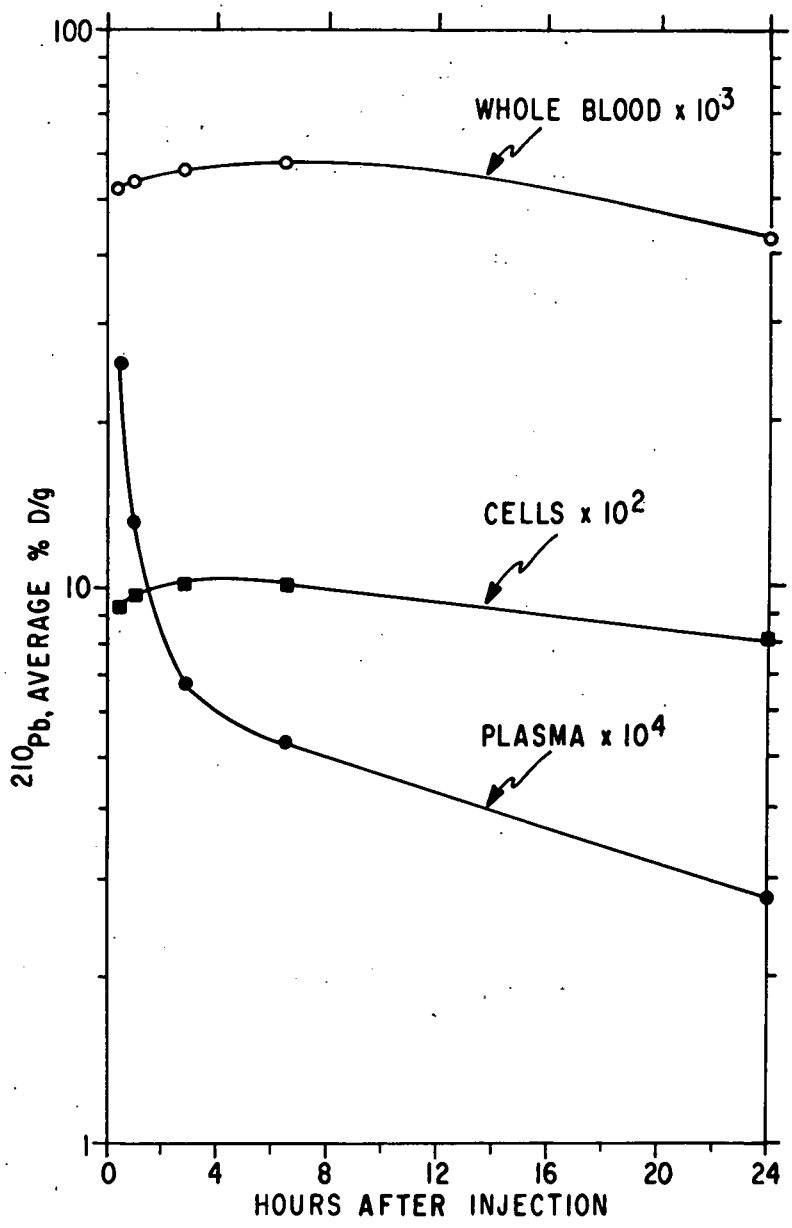

Figure 1. Concentration of ^{210}Pb in whole blood, cells and plasma are presented as a function of time. The concentration in plasma drops rapidly and continuously, ^{210}Pb concentrations in whole blood and cells however are increased during the first several hours after injection.

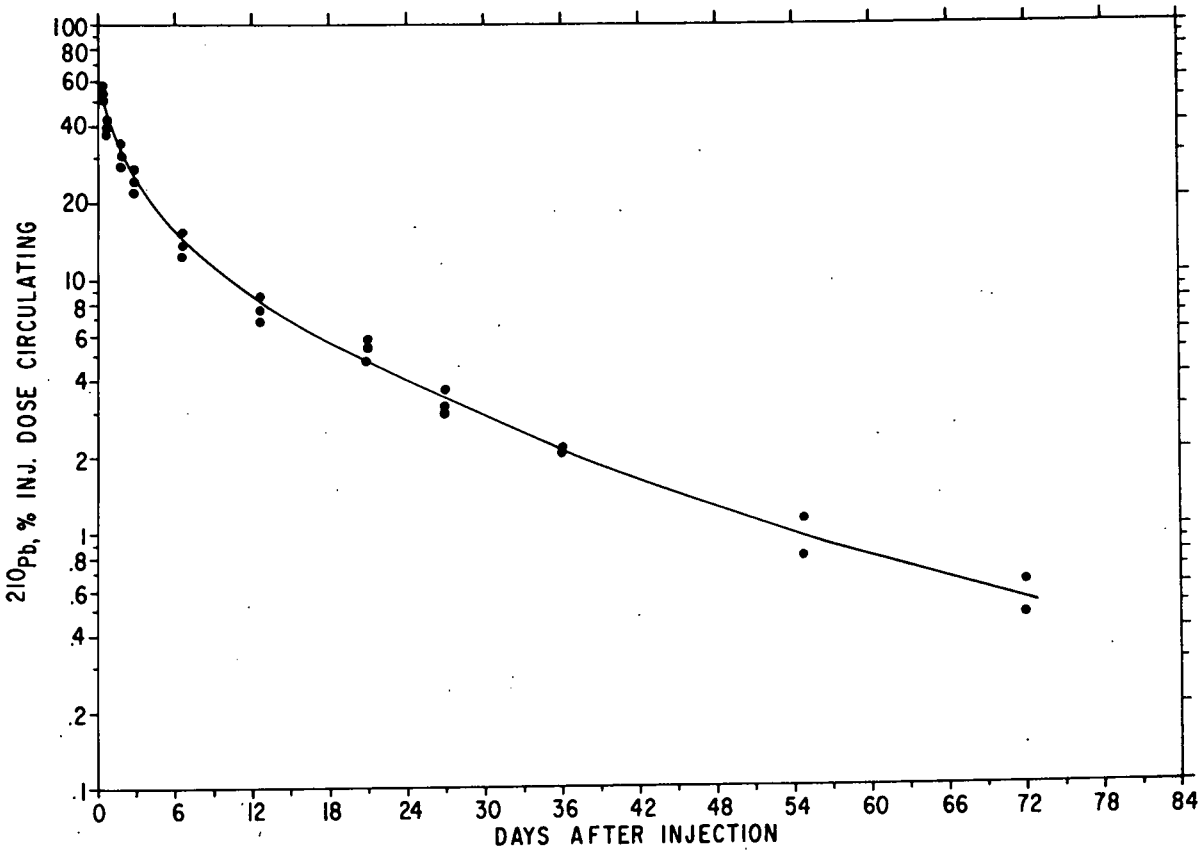

Figure 2. Percent of injected ^{210}Pb circulating in the whole blood as a function of time up to 72 days after injection.

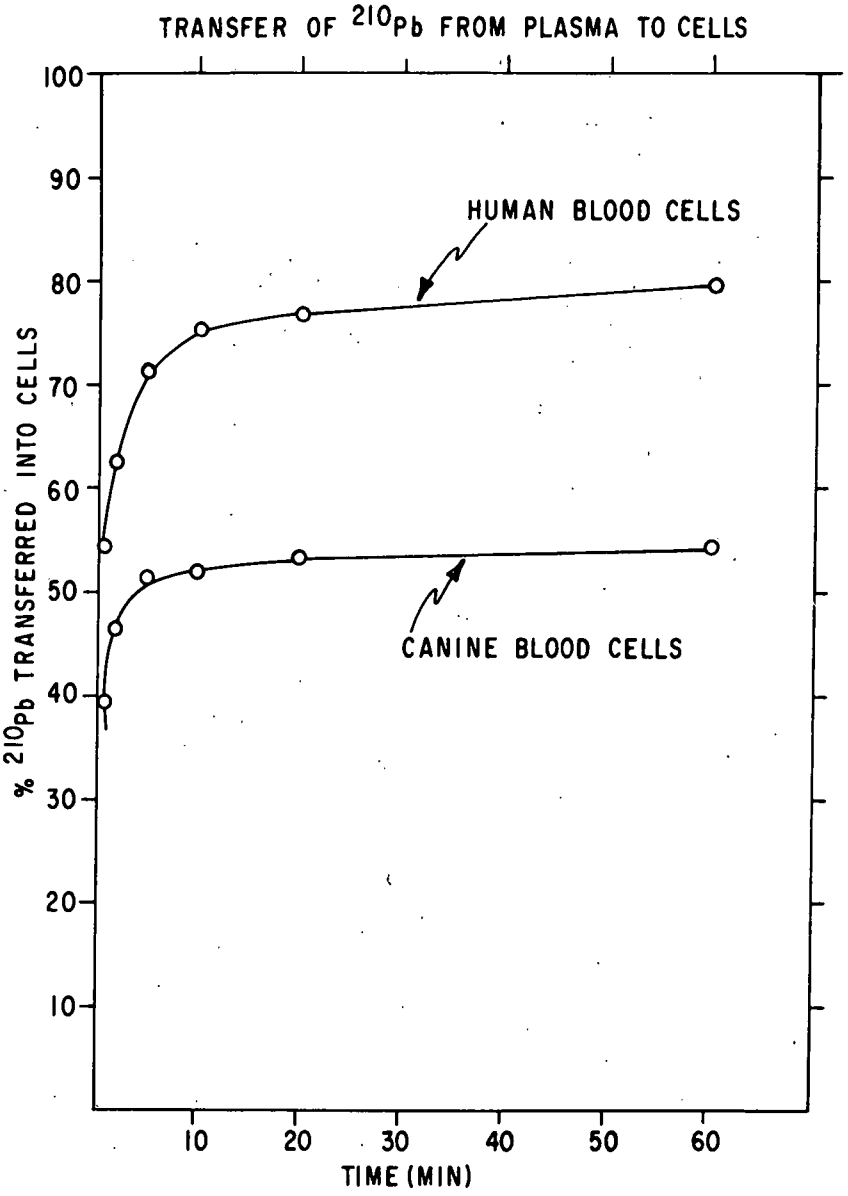

Figure 3. A comparison of transfer of ^{210}Pb from plasma to red cells in human and canine blood.

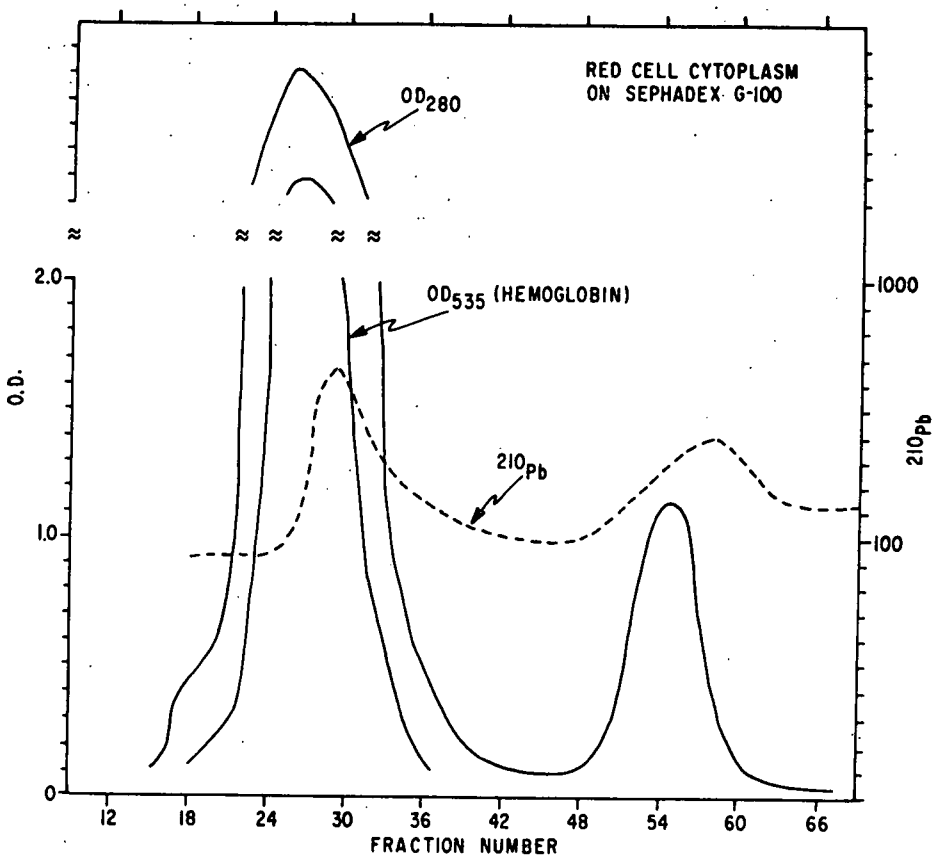

Figure 4. Elution spectrum of ^{210}Pb tagged dog red cell cytoplasm on Sephadex G-100. ^{210}Pb is associated with the Hb-peak and also with low M.W. components.

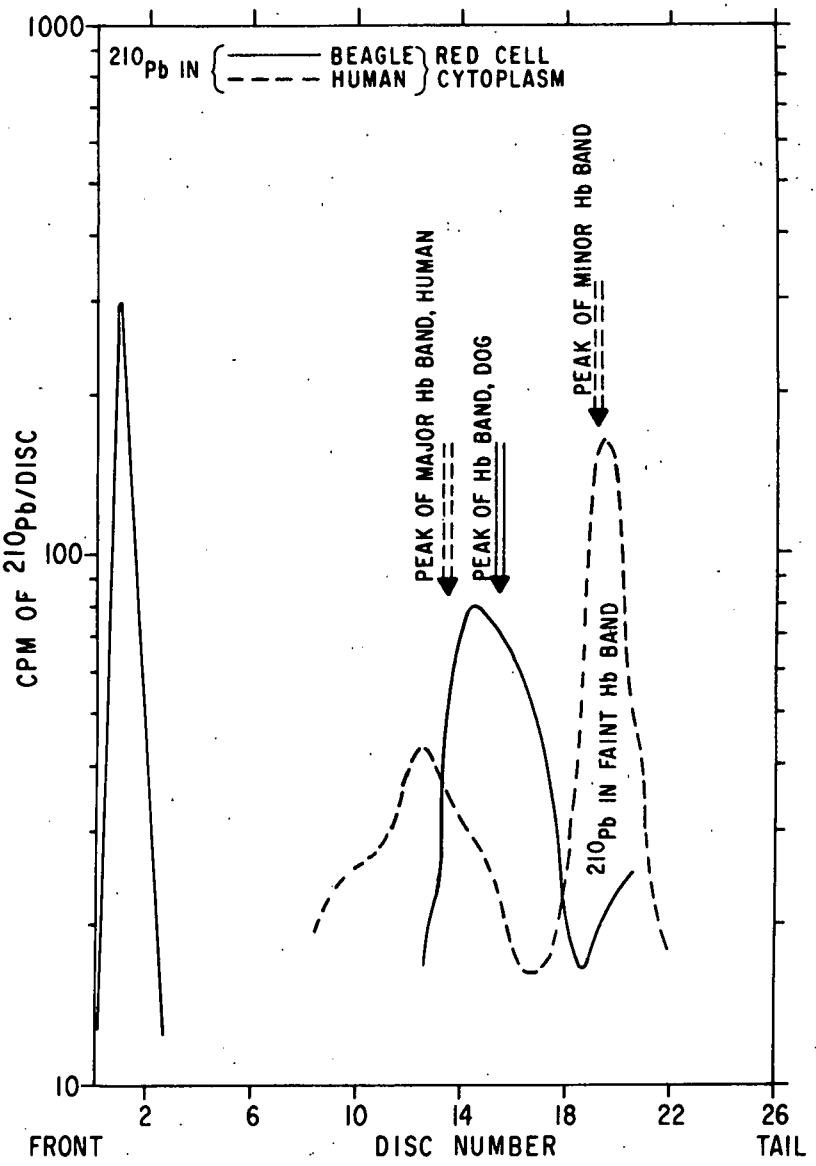

Figure 5. Spectra obtained by disc electrophoresis of canine and human red cell cytoplasm. The figure shows the various positions of ^{210}Pb and hemoglobin and the strong association of lead with the faint Hb band of human cytoplasm.

PROTRACTED HEPATIC, SPLENIC, AND RENAL RETENTION OF ^{239}Pu IN THE BEAGLE

Betsy J. Stover, David R. Atherton and Dawn S. Buster

Abstract: Retention of plutonium in liver, spleen, and kidneys of beagles injected intravenously with ^{239}PuIV in citrate buffer at injected dose levels of 0.016 and 0.048 μCi/kg has been measured from young adulthood to death. Dogs at 0.016 μCi/kg suffer no life-shortening, and those at 0.048 μCi/kg, 18%. Hepatic retention is independent of dose level at these and the previously reported level of 0.096 μCi/kg, and, thus, at these levels the radiation damage is not sufficiently extensive to result in a significant dose level effect. Sets of hepatic retention equations have been calculated and those most appropriate for calculation of radiation dose have been designated. Surprisingly, splenic and renal retentions were higher than anticipated from results at higher levels. This may be the consequence of more nearly normal skeletal biology at low levels.

Introduction

It has been previously reported from this laboratory that there is a significant dose level effect on the canine hepatic retention of plutonium, and that this effect is a consequence of radiation damage (1, 2). Further, it is well established that the time dependence of hepatic retention of plutonium in man is much more like that in the beagle than that in rodents (3,4,5,6,7). Thus, in obtaining information to use in the assessment of plutonium as an environmental hazard, the lower bound of the dose level effect is required. In this report we establish the needed lower bound.

The concentrations, and, hence, dose rates, of plutonium in

the spleen and the kidneys are considerably lower than that in the liver, and consequently no dose level effect resulting from intrinsic radiation damage of either spleen or kidneys had been observed previously (1). Data presented herein suggest an indirect dose level effect.

Materials and Methods

The basic design of the long term experiment to evaluate the effects of plutonium in beagles has been given, as have the specific chemical procedures (8,3,9,10). Each young adult dog received a single intravenous injection of ^{239}PuIV in 0.08 M citrate buffer of pH 3.5.

Results

The amount of ^{239}Pu in the livers of 23 dogs, and the concentration in 22, at the Pl-level, which is 0.016 μCi/kg were measured at times ranging from 5 to 4810 days after injection. At the Pl.7-level, 0.048 μCi/kg injected, 10 livers were available for measurement from dogs that lived 467 to 3430 days after injection. Both sets of data are given in Fig. 1. At these levels the retention decreases slowly, and it requires the remainder of the animal's life to lose half of that deposited in young adulthood. Interestingly, there is a difference in the rates at which amount and concentration decrease, and it appears that the weight of the liver increases gradually from youth to senility. Unfortunately it was not possible to calculate the rate because precise duplication of exsanguination techniques over 18 years is not possible. Single

exponentials were calculated for the P1-level and for P1- and P1.7-levels combined as shown in Fig. 1 and in Table 1.

Similar sets of measurements were made on the spleens and kidneys from these dogs and the results appear in Figs. 2 and 3. There is no readily apparent difference between the two dose levels for either spleen or kidneys, and there is considerable variation in both sets of data. For comparison, equations that were calculated from measurements on 17 dogs at the P3-level (0.30 μCi/kg) which lived 40 to 1950 days after injection are shown. The P3-level equations appear adequate to about 2000 days, but from then on underestimate the data from the P1- and P1.7-levels.

Discussion

This completes the presentation of retention of ^{239}Pu in the liver of the beagle at six different injected doses ranging from 0.016 μCi/kg to 2.8 μCi/kg, i.e. from a level at which life-shortening is negligible, through levels in which induction of osteosarcomata is the principal mechanism of non-survival, to a level which is the over-kill situation (11). We have previously reported that retention in the liver decreases more rapidly as the dose level is increased and that this correlates with the extent of radiation damage (1,2,12,13). Similar observations have also been made on ^{241}Am in the liver (14). Current and prior retention equations are summarized in Table 1. Measurements at the P1-, P3-, and P5-levels include both early serial sacrifice dogs and the toxicity dogs whose deaths are the consequence of irradiation and aging, and con-

stitute the framework of the analysis since the other levels include only the toxicity beagles. The values of α for several groupings of dose levels range from 30 to 35% of the injected plutonium, and are consistent with our earlier observation that the dose level effect does not arise from variation in initial deposition but rather from the ensuing radiation damage. All the values of β are significantly greater than zero ($p < 0.01$). No pair of the first three values of β nor of β' differ significantly. Therefore, the P2-level, even though suffering a life-shortening of 34%, can be grouped with the lower two levels in the evaluation of hepatic retention (11). This does not mean that there is no radiation damage to the liver at this level, but more likely that the effect of injury on retention is masked by the dog to dog variation.

A second approach to the analysis of these sets of data was based on the fact that there is no dose level effect on the value of alpha. An overall value of α (= 32.6 $\pm$ 2.0) was calculated from the weighted values of β for P1-, P3-, and P5-levels as follows:

$$\overline{\ln \alpha} = \frac{(n \ln\alpha)_{p1} + (n \ln\alpha)_{p3} + (n \ln\alpha)_{p5}}{n_{p1} + n_{p3} + n_{p5}} \qquad (1)$$

A least squares determination of β when α is fixed was done for the several sets of data. The results are given in Table 2 and Fig. 4, which also include the interval for each set. In this way separate equations are obtained for the P1.7-, P2-, and P4-levels for which there are no early data. And, although the errors are high, values of β for these levels are consistent with all of our other obser-

vations.

Next we must choose from this array of equations those most appropriate for dosimetry. They are shown in Table 3. Because of the absence of detectable dose level effect, the three lower levels can be grouped. There are experimental equations for both L (retention) and λ (concentration) at the P3-level, and for L at the P5-level. The P4-level is clearly bracketed by the P3- and P5-levels, and in the absence of early data, the choice for L is the equation from Table 2. In the equations of λ for the P4- and P5-levels, α is 32.6%/265 g at injection, calculated from the other four dose levels. Actually the equation for the P5-level is valid only for a short period after injection since the ensuing gross damage results in marked changes in the weight of the organ.

The equations for the concentration of ^{239}Pu in liver that are given in Table 3 have been used to calculate average radiation dose to the liver from the time of injection to the time of half-survival, $t_{s=1/2}$, of the dose level, and the results are given in the rightmost column of Table 3. The average dose to the liver is approximately the same as that to the skeleton in these dogs, and the relationship between hepatic dose and time to half-survival is linear at the lower dose levels (Fig. 5), which was the case with skeletal dose (11). Further, the point for the control dogs is essentially on the same line. This is again evidence that plutonium acts through the aging mechanism by accelerating the rate of induction of small critical changes in genetic material or its precursors (11,16,17). At higher levels the independent mechanism

that leads to non-survival through induction of osteosarcoma is important. In contrast, the lower dose levels of ^{226}Ra showed little life-shortening while the independent mechanism that leads to osteosarcoma was effective at higher levels. Since it is mainly the skeleton that is irradiated in the case of ^{226}Ra, the possibility is suggested that irradiation of the skeleton by ^{239}Pu leads to non-survival through the independent mechanism of osteosarcoma and that irradiation of the liver hastens death through the aging mechanism. Further, the former is dominant at higher levels but gives way to the latter at lower levels. If this be the case, then clearly the latter mechanism should receive careful attention in assessing the effects of plutonium at low exposure levels.

Using the equations of choice, (Table 3), liver doses for each of the beagles in our chronic ^{239}Pu toxicity series has been calculated. The calculations were made as follows:

$$\text{Dose (Cumulative rads)} = C \times W \times I \times \int_0^t \ell \, dt$$

$$= C \times W \times I \times \frac{\alpha'}{\beta'} (1 - e^{-\beta' t}) \qquad (2)$$

where C is a constant to convert energy to rads, W. is the dog's weight (kg) at the time of injection, I is the μCi of ^{239}Pu/kg injected; α' is the <u>fractional</u> retention of plutonium per gram of liver (Table 3); β' is the rate at which α' is diminished; and t is the interval from injection to death. The results of these calculations appear in Table 4 and are compared to the cumulative dose to the liver of the average beagle in a specific dose level group

at the time of 1/2 survival of that group.

Of lesser importance, but still of basic interest, is the apparent increase in splenic and renal retention in the lower dose levels at long times following plutonium administration. This apparent increase is artificial in the sense that the reference point is an extrapolation from results on more severely injured dogs. Yet, in spite of this artificiality, the finding is completely consistent with histological observations on bone remodeling (15). Thus, at the lower levels bone remodeling proceeds at a more nearly normal rate than at higher levels, and more plutonium is released to deposit in spleen and kidneys. It should be noted, however, that this is a small effect, and that the skeletal deposition is not significantly reduced.

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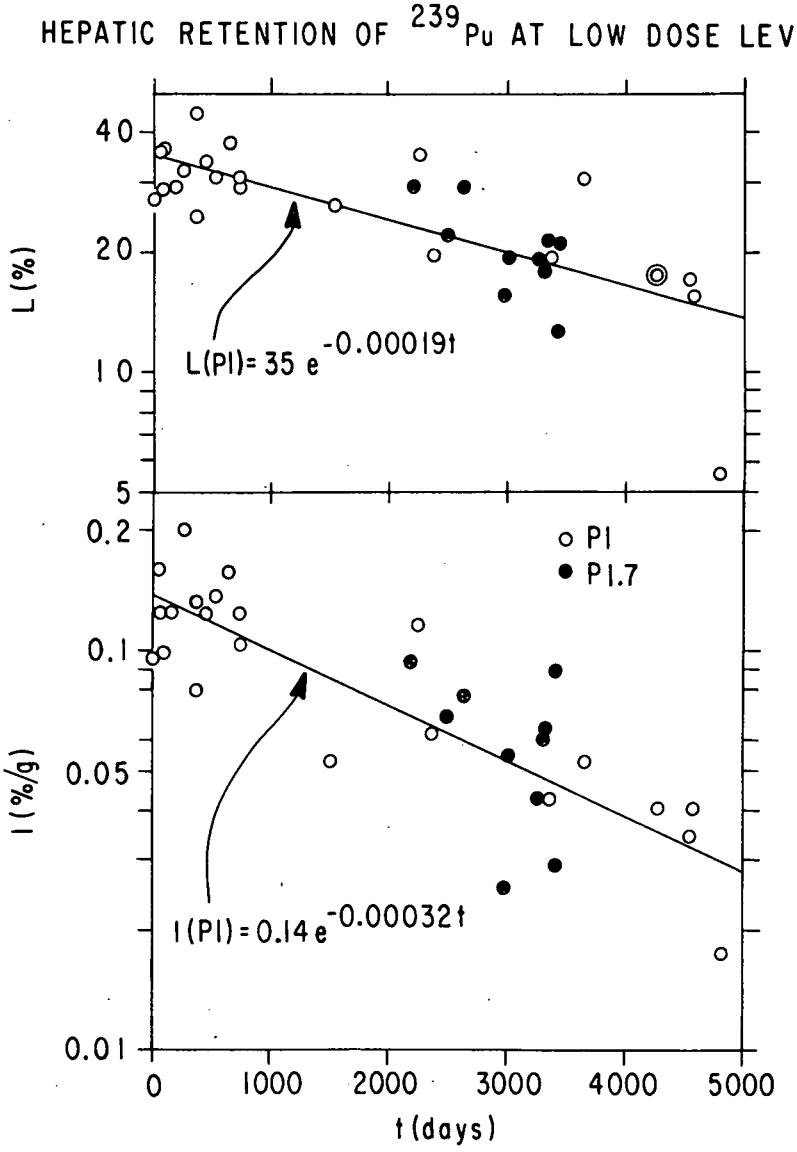

Figure 1. Hepatic retention and concentration of ^{239}Pu at low dose levels (0.016 and 0.048 μCi/kg) measured at times ranging from 5 to 4810 days following injection.

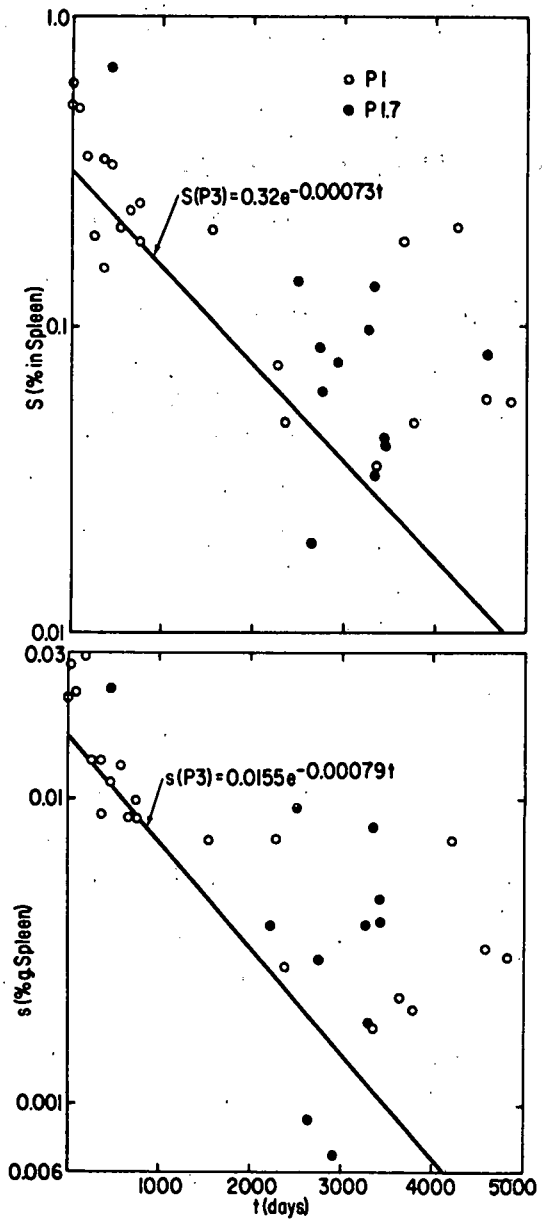

Figure 2. Comparison of splenic retention and concentration of ^{239}Pu in beagles given low dose levels (0.016 and 0.048 μCi/kg) with that in beagles receiving an intermediate level (0.9 μCi/kg). Times of measurements in low level animals ranged from 5 to 4810 days following injection.

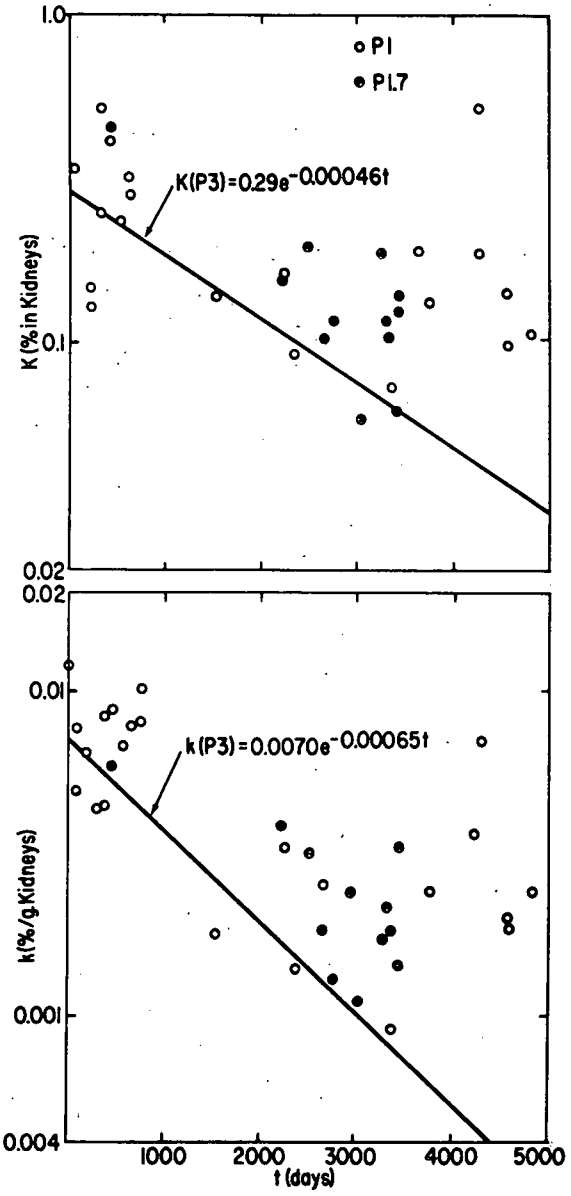

Figure 3. Comparison of renal retention and concentration of ^{239}Pu in beagles given low dose levels (0.016 and 0.048 μCi/kg) with that in beagles receiving an intermediate level (0.9 μCi/kg). Times of measurements in low level animals ranged from 5 to 4810 days following injection.

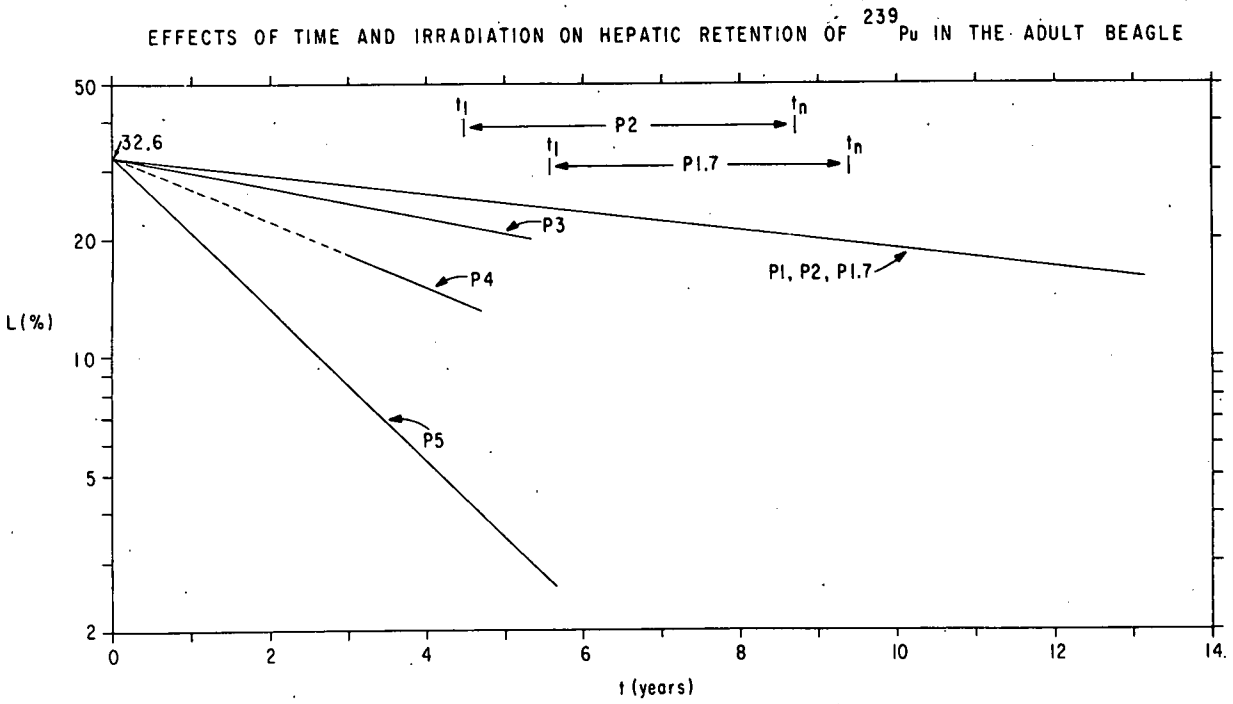

Figure 4. Effects of time and irradiation on retention of ^{239}Pu in the liver of the adult beagle, calculated using the equations of Table 2.

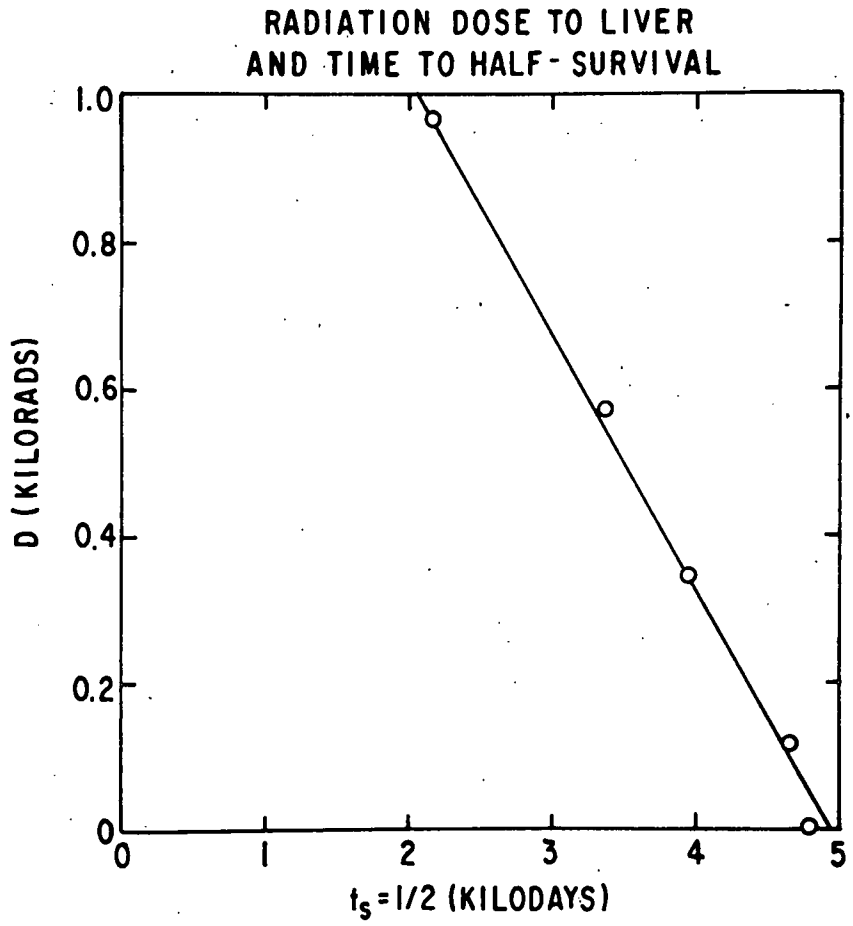

Figure 5. Relationship between time to half survival and hepatic radiation dose at low and intermediate injection levels of ^{239}Pu.

Table 4

Liver Dose
Cumulative Rads

Sex & Group No.	Dose Level 1 0.016 µCi/kg	1.7 0.048 µCi/kg	2 0.096 µCi/kg	3 0.30 UCi/kg	4 0.90 µCi/kg	5 2.8 µCi/kg
M1	122	287	446	725	2323	5261
F2	99	277	569	893	3102	6890
M3	111	318	705	933	2536	3033
M4	119	296	578	1036	2279	6916
F5	54*	365	413	833	1843	6269
F5A	145	---	---	----	----	----
F6	86	268	464	872	2388	4323
F7	86	344	454	1050	2373	5819
M8	134	473	445	1146	2326	5469
F9	76	289	518	943	2602	5387
F10	140	80*	520	954	1680	----
F10A	---	308	---	----	----	----
M11	> 127	370	443	965	2718	----
M12	95*	317	576	1180	3194	----
M13	> 161	> 416	---	----	----	----
n	12	13	12	12	12	9
Ave. Dose $\sigma \overline{D}$	> 117	> 333	511	961	2447	5485
# $t_s = 1/2$	> 120	> 342	571	970	2462	5385

* Not included in $\overline{D}$ - Died of causes unrelated to radiation.

> M11 and 13P1 and M13P1.7 are alive at times since injection exceeding 5076, 4214 and 4214 days respectively.

\# Dose for average beagle for the interval: injection to time of 1/2 survival.

Table 1

Parameters of Equations of the Form $L = \alpha e^{-\beta t}$ for Hepatic Retention and Concentration of ^{239}Pu

Level(s)	n	$\alpha \pm \sigma_\alpha$ (% injected)	$(\beta \pm \sigma_\beta) \times 10^4$ (d^{-1})	$\alpha' \pm \sigma_{\alpha'}$ (% inj/gm)	$(\beta' \pm \sigma_{\beta'}) \times 10^4$ (d^{-1})
P1	23	34.7 ± 3.0	1.86 ± 0.35	0.138 ± 0.012	3.18 ± 0.37
P1,1.7	33	35.1 ± 2.7	1.89 ± 0.29	0.139 ± 0.013	3.14 ± 0.37
P1,1.7,2	44	34.7 ± 2.8	1.84 ± 0.31	0.131 ± 0.014	3.06 ± 0.43
P3,	17	31.3 ± 3.7	2.25 ± 0.83	0.123 ± 0.023	4.45 ± 1.30
P1,1.7,2,3	61	32.3 ± 3.8	1.69 ± 0.27		
P5	22	29.8 ± 3.8	11.6 ± 1.4		

Table 2

The Parameter β and Interval of Observation by Dose Levels when $\alpha = 32.6 \pm 2.0$ in $L = \alpha e^{-\beta t}$

Level(s)	n	$(\beta \pm \sigma_\beta) \times 10^4$ (d^{-1})	t_1 d	t_n d
P1	23	1.68 ± 0.35	5	4810
P1.7	10	1.64 ± 1.78	2221	3430
P2	11	1.51 ± 2.12	1617	3185
P1,1.7,2	44	1.60 ± 0.32	5	4810
P3	17	2.51 ± 0.84	40	1950
P4	12	5.35 ± 5.92	1066	1724
P5	22	12.3 ± 3.4	15	2059

Table 3

Equations of Choice for Hepatic Retention of ^{239}Pu by Dose Level

Level	Injected Dose ($\mu Ci\ ^{239}Pu/kg$)	Equations		Dose (rads) at $t_s = 1/2$
		Retention	Concentration	
P1	0.016			118
P1.7	0.048	$L = 34.7e^{-0.000184t}$	$\ell = 0.131e^{-0.000308t}$	342
P2	0.096			566
P3	0.30	$L = 31.3e^{-0.000225t}$	$\ell = 0.123e^{-0.000445t}$	970
P4	0.90	$L = 32.6e^{-0.000535t}$	$\ell = 0.123e^{-0.000535t}$	2445
P5	2.8	$L = 29.8e^{-0.00116t}$	$\ell = 0.123e^{-0.00116t}$	5385*

* Over-estimate since radiation induced cell death decreases weight of liver.

CORTICOSTEROID BINDING BY CYTOSOL MACROMOLECULES FROM RAT BRAIN

B. I. Grosser,[1] W. Stevens,[2] F. W. Bruenger,[2] and D. J. Reed[3]

Abstract: The administration of ^{3}H-corticosterone by ventriculo-cisternal perfusion to adrenalectomized rats results in a significant amount of steroid binding by cytosol derived macromolecules. Further evidence for macromolecule-steroid interaction was obtained by incubating ^{3}H-corticosterone (B), ^{3}H-cortisol (F) and ^{3}H-11-deoxycortisol (S) with brain cytosol *in vitro*. The amount of each steroid bound after gel-chromatography, equilibrium dialysis and/or ultrafiltration was B > F > S.

Introduction

Although corticosteroids exert a profound effect on brain (1), little is known of the mechanisms by which they influence neurochemical events. An attractive concept of steroid action is that the hormone interacts with specific receptor molecules to produce a steroid-receptor complex which is responsible for the unique effect of the hormone on the target tissue (2). The testing of this hypothesis has resulted in the isolation of receptors for estradiol in the uterus (3), for dihydrotestosterone in the prostate (4) and for cortisol in cultured fibroblasts (5) and liver (6). Estradiol binding molecules have been found in the hypothalamus and anterior pituitary (7); however, only indirect evidence has been obtained for the binding of testosterone, corticosterone and cortisol in brain (8). These findings prompted the present investigation, the results of which provide direct evidence for the existence of glucocorticoid

Departments of Psychiatry,[1] Anatomy,[2] and Pharmacology,[3] University of Utah College of Medicine, Salt Lake City, Utah 84112

receptor molecules in rat neural tissue.

In three separate *in vivo* studies, a ventriculo-cisternal perfusion (9) was employed to circumvent the blood-brain barrier and thereby permit maximal uptake of steroid by brain. In each experiment, two male 400-500 g Sprague Dawley rats (adrenalectomized 1 to 4 weeks) were anesthetized with pentobarbital sodium (50 mg/kg I.P.) and connected to a small animal respirator through a tracheal cannula. ^{3}H-corticosterone (8.7 μc/0.1 ug) in synthetic CSF (0.1 ug/ml) was perfused into the left lateral ventricle at rates ranging from 0.53 - 2.0 ml/hr, and was collected from a cannula in the cisterna magna. The animals were immediately killed by exsanguination and the whole brains were rapidly removed and freed of superficial blood vessels. After two washes in ice cold buffer, each brain was homogenized in 10 mls 0.05 M EDTA, 0.278 M sucrose buffer, pH 7.0, and an aliquot of each homogenate was counted. The total radioactivity in the homogenates averaged 7.4×10^5 - 1.8×10^6 dpm. The homogenates then were centrifuged for 1 hr at 105,000 x g and the supernatants were decanted. The pellets were resuspended in buffer and fractionated by differential centrifugation. The distribution of the radioactivity in the resulting fractions expressed as a percent of the total dpm in the homogenate was as follows: nuclei and cell membranes (1000 x g for 20 min.) 11.7 ± 3.2*; heavy mitochondria (15,000 x g for 1 hr) 0.81 ± 0.2; light mitochondria and membrane fragments (25,000 x g for 1 hr) 0.11 ±

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* (Mean ± S.E.)

0.04; microsomes (25,000 x g supernatant)-6.9 ± 1.4. The 105,000 x g supernatant contained 76.0 ± 6.4% of the radioactivity found in the whole homogenate. The percent of activity in these fractions is in good agreement with the values obtained elsewhere following systemic injection of corticosterone (10).

The cytosol fractions were frozen, lyophilized overnight and analyzed by gel-chromatography. The results obtained with chromatography of the cytosol proteins from two in vivo experiments are shown in Fig. 1. In both experiments, significant quantities of radioactivity were associated with macromolecules which eluted in the first peak on Sephadex-G-25. Over 53% of the corticosterone present in the cytosol in the first experiment (1 A) and approximately 24% in the second experiment (1 B) eluted with high molecular weight macromolecules after G-25 chromatography. Lesser amounts of radioactive steroid were eluted with low molecular weight compounds in the two experiments and represent "free" steroid. Subsequent chromatography on G-100 of steroid-macromolecule complexes obtained by chromatography on G-25 demonstrated two peaks of radioactive steroid, one associated with macromolecules of high molecular weight and the other with low molecular weight materials (1 C, D). A similar G-25 sample was taken from the first experiment and was dialyzed for 48 hours at 0-4°C against an equal volume of the same buffer. The ratio of "bound" radioactivity to that of the buffer (free steroid) was 20, which indicated that little of the steroid was free to diffuse across the membrane.

Two in vitro experiments then were performed to obtain further

evidence of macromolecule-steroid interaction. Equivalent amounts of tritiated corticosterone (B), cortisol (F) and 11-deoxycortisol (S) were incubated separately with protein fractions derived from cytosols of rat brain. The elution profiles for protein and radioactive steroids in each experiment are shown in Fig. 2. In each case, distinct differences were found in the amount of individual steroid associated with molecules eluting at the void volume. In the first experiment (2 A) 24 percent of the corticosterone, 10 percent of the cortisol and 2 percent of the 11-deoxycortisol placed on the column were eluted with the macromolecules, whereas in the second experiment (2 B) these values were 17, 4 and 1 percent respectively. This difference was substantiated by recovering the balance of these steroids in the free remainder (i.e., S > F > B). Recoveries from the column ranged from 70-105 percent.

A portion of the incubation mixture from the first experiment was chromatographed on DEAE-cellulose using a linear NaCl gradient (0.0 → 1.0 M) for the eluting buffer. This resulted in the elution of all the steroid as a free compound which indicated a dissociable interaction between the macromolecule(s) and the steroid.

In the second <u>in vitro</u> experiment, a portion of the incubation mixture as well as a sample of the steroid-macromolecule complex previously chromatographed on G-25 were rechromatographed on Sephadex G-200 and eluted in 0.1 M Tris buffer pH 7.0. In both cases, most of the steroid was eluted as a free compound although some binding occurred with the high molecular weight material. This provided additional evidence for dissociable interaction between steroid

and macromolecule(s).

In the two in vitro experiments, further evidence for the existence of a receptor-steroid complex was obtained when equilibrium dialysis and ultrafiltration was carried out on the protein-steroid complex excluded from the gel phase on G-25. Equilibrium dialysis was performed against the buffer used for elution (Sucrose-EDTA or Tris) and against bovine serum albumin in the case of one of the corticosterone samples. Results of these studies are shown in Table 1. The ratios of bound to free corticosterone are similar in the two experiments but they are not identical. However, they are generally higher than those obtained for the other steroids with the exception of 11-deoxycortisol in sucrose. The average amount of corticosterone associated with the macromolecules in ug/mg protein was five times greater than cortisol which was three times greater than 11-deoxycortisol. The corticosterone-macromolecule complex was dialyzed against bovine serum albumin (BSA) in one case since BSA is capable of binding steroid non-specifically. The presence of BSA did not change the ratio of bound to unbound steroid after dialysis indicating a brain protein-steroid interaction which was stronger than the BSA-steroid interaction.

The ratio of bound to free steroid after ultrafiltration of protein-steroid samples was in close agreement with that obtained with equilibrium dialysis, thus providing further evidence for the existence of steroid-receptor molecules.

Extraction of corticosterone and cortisol from the dialysate followed by paper chromatography and crystallization to constant

specific activity indicated that no metabolism of either molecule had occurred as a result of steroid-protein interaction. The small amount of 11-deoxycortisol present prevented reliable crystallization to constant specific activity; therefore, it was authenticated by paper chromatography only.

The foregoing data demonstrate significant binding of corticosteroids to brain protein. Furthermore, some specificity is present as indicated by the fact that corticosterone, the natural adrenal corticoid of the rat, was bound to protein in an amount 15 x greater than 11-deoxycortisol, a structurally similar but inactive steroid. Although the full significance of these findings is not known, further experimentation may provide insight into the mechanism of corticosteroid action on brain functions.

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13. We thank Dr. L. T. Samuels, S. Krowas and G. Howerton for their assistance.

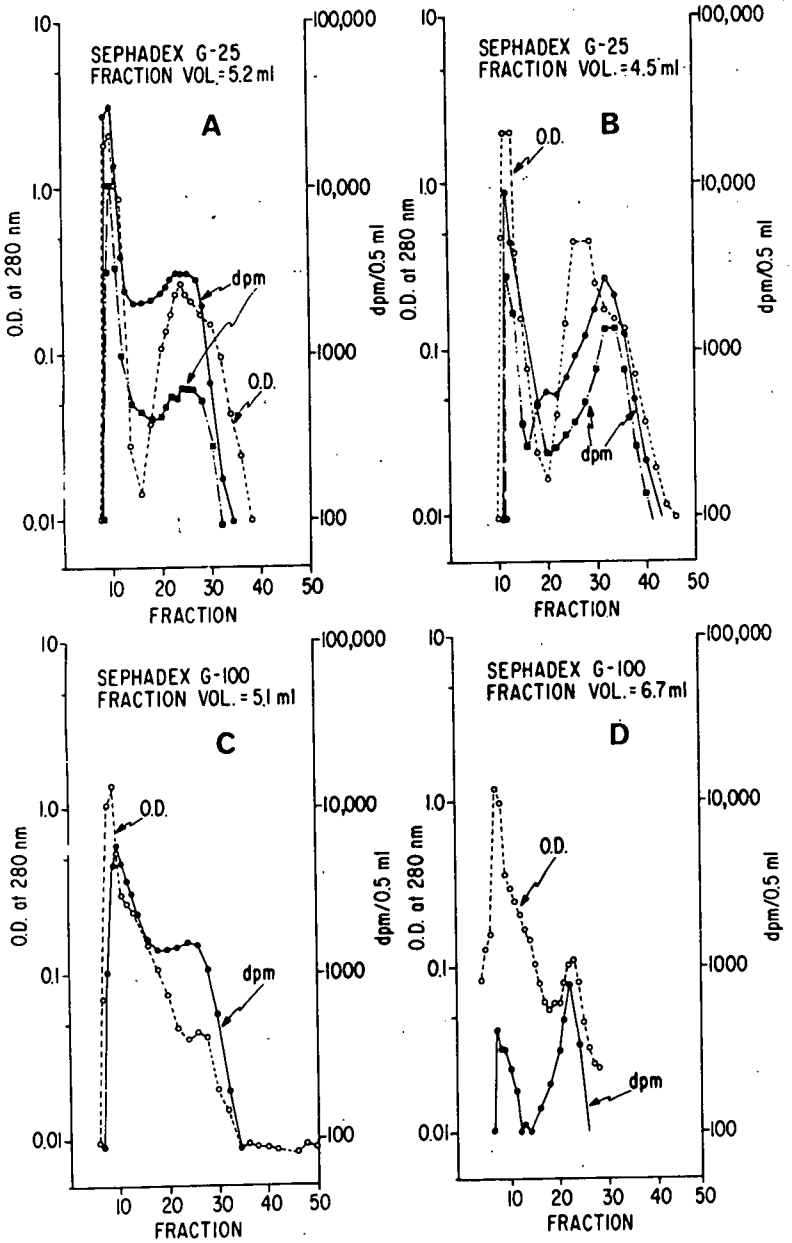

Figure 1. ^{3}H-corticosterone-macromolecule elution profiles from two <u>in vivo</u> experiments. The OD readings from two separate brains in each experiment (1A, 1B) were averaged and plotted as a single curve. The two dpm curves in each experiment represent the ^{3}H-corticosterone from individual rat brain cytosols. Cytosols were chromatographed on Sephadex G-25 columns (3.5 x 11 cm) at 0-4° C using 0.278 M sucrose, 0.05M EDTA, pH 7.0 buffer for elution. Sephadex G-100 chromatography was done using identical columns and buffers. In each graph the large OD peak on the left represents molecules excluded from the gel-phase while the OD peak on the right represents low molecular weight peptides and other small molecules. These elution characteristics were determined using Dextran Blue (MW 200,000) and "free" cortisol for reference compounds. In the first experiment the protein-steroid complex from one brain was re-chromatographed on G-100 (1C). The sample for G-100 chromatography was taken from the single G-25 tube with the highest OD reading whereas in the second experiment, G-100 chromatography (1D) was performed on pooled samples from the entire G-25 protein peak. Otherwise, the two experiments were performed in an identical manner. Protein content of each fraction was determined by optical density at 280 nm. Radioactivity was measured by liquid scintillation counting of 0.5 ml samples dissolved in 2.0 ml of tissue solubilizer and 12.5 mls of toluene containing 6 g PPO and 75 mg POPOP per liter.

Figure 2. Elution profiles for two experiments in which protein and radioactive steroids were incubated in vitro and chromatographed on Sephadex G-25. In each experiment (2A, 2B), brains from 9 adrenalectomized rats were homogenized in 0.278 M sucrose, 0.05 M EDTA buffer pH 7.0, and centrifuged at 105,000 x g for 1 hr. The resulting supernatants were pooled, frozen, and lyophilized overnight. The lyophilized proteins were dissolved in distilled water and dialyzed for 5 hrs to remove sucrose. The protein concentration of the dialysate was adjusted to 4.3 mgs/ml. Five ml of protein solution was incubated with 0.02 µg of either ^{3}H-corticosterone (1.84 µCi), ^{3}H-cortisol (2.5 µCi) or ^{3}H-deoxycortisol (2.0 µCi) for 1 hr at 37° C. Portions of each incubate (3 ml for the 1st experiment and 2 ml for the second experiment) were chromatographed on Sephadex G-25 (3.5 x 11 cm) at 0-4° C using either sucrose: EDTA buffer (first experiment) or 0.1 M Tris pH 7.0 buffer (second experiment) for elution.

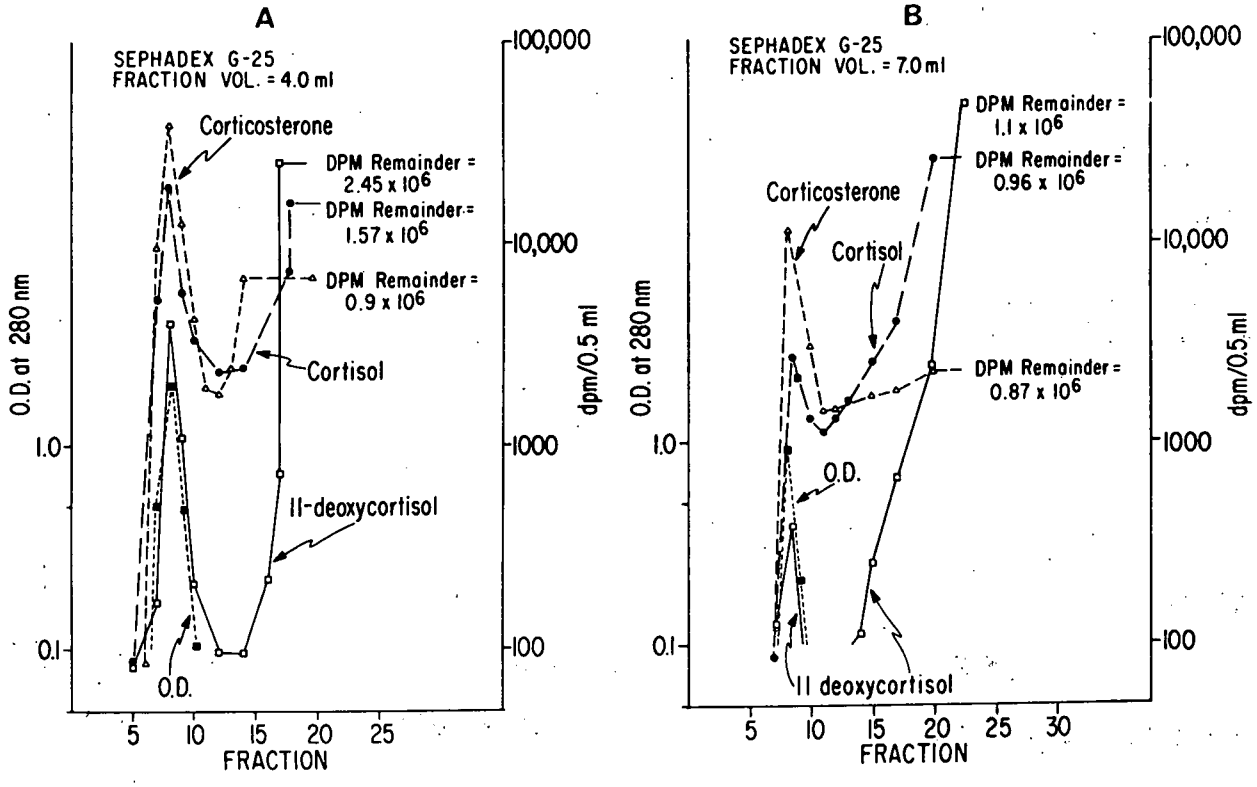

Table 1. Steroid-protein complexes that were eluted from the gel-phase in the void volume on Sephadex G-25 after *in vitro* incubation were subjected to equilibrium dialysis in plexiglass chambers that held 3.0 mls on each side of the membrane. Dialysis was performed for 48 hrs against either 0.278 M sucrose, 0.05 M EDTA buffer, pH 7.0, or 0.1 M Tris buffer, pH 7.0 with constant shaking at 0-4° C. After dialysis 0.5 ml of the protein solution and 0.5 ml of the buffer were counted. The protein content of the dialysate was determined using the method described by Lowry *et. al.* (11).

Ultrafiltration was done using Amicon Centriflo filter cones (CM-50). Five ml of the pooled protein-steroid complex excluded from Sephadex on G-25 was centrifuged for 90 min. at 1000 x g. At the end of this time about 2.5 ml of ultrafiltrate had formed. Equal aliquots of the retentate and ultrafiltrates were counted. Ultrafiltration was performed only on samples from the second experiment. The concentration of bovine serum albumin (BSA) was 4.5 mg/ml. A control of ^{3}H-corticosterone dissolved in sucrose buffer and dialyzed under identical conditions had equal concentrations of ^{3}H-corticosterone on either side of the membrane at the end of 48 hrs. In the case of BSA dialysis, the free steroid actually represents an equilibrium between unbound, dialyzed corticosterone and that associated with bovine serum albumin.

BINDING OF STEROID TO PROTEIN FOLLOWING DIALYSIS OR ULTRAFILTRATION

STEROID	BUFFER	DIALYSIS		ULTRAFILTRATION
		dpm BOUND / dpm FREE	µg STEROID / mg PROTEIN	dpm BOUND / dpm FREE
CORTICOSTERONE	TRIS	5.0	1.7×10^{-4}	3.35
	SUCROSE	3.2	1.3×10^{-4}	4.7
	SUCROSE	5.2	1.5×10^{-4}	---
	BSA:SUCROSE	3.4	2.1×10^{-4}	---
CORTISOL	TRIS	1.8	4.8×10^{-5}	2.04
	SUCROSE	2.4	2.0×10^{-5}	---
11-DEOXYCORTISOL	TRIS	2.3	0.7×10^{-5}	2.0
	SUCROSE	8.3	1.5×10^{-5}	---

Submitted to: 2nd International Congress of International Radiation Protection Association, Brighton, England (May 1970)

THE SUBCELLULAR DISTRIBUTION OF ^{239}PuIV AND ^{241}Am IN THE CANINE LIVER

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

The liver is a principal deposition site for both ^{239}PuIV and ^{241}AmIII. In the beagle the rate of decrease of hepatic retention of both nuclides is slow unless the radiation dose rate is sufficiently high to destroy cells. Thus, chemical bonds of high stability must be formed between these nuclides and chemical entities in the liver. By differential centrifugation methods it was found the highest concentration of ^{241}Am was in the microsomal fraction. Successively lower, but nevertheless significant concentrations were measured in the mitochondria, the cytosol, the nuclei, and the connective tissue. Extensive analysis of the cytosol revealed that both ^{241}Am and ^{239}Pu were strongly bound to ferritin, the iron storage protein. Further, the major fraction of these nuclides in the liver is in association with ferritin.

Presented at the 54th Annual Meeting FASEB
Atlantic City, New Jersey (1970)

CORTICOSTEROID BINDING BY BRAIN PROTEINS

W. Stevens and B. I. Grosser

The profound effects of corticosteroids on brain suggest the existence of intracellular steroid receptor molecules. To investigate this hypothesis, ^{3}H-corticosterone (8.7 μc:0.1 μg) in synthetic CSF was administered by ventriculo-cisternal perfusion for 1 hr to adrenalectomized rats. Brains were removed and homogenized in 5 vol. cold EDTA-sucrose buffer pH 7.0 and centrifuged at 105,000 x g for 60 min. The supernatant was lyophilized and placed on a G-25 Sephadex column. The eluted protein fraction contained about 50% (> 100,000 DPM) of the radioactivity present in the whole brain homogenate. The protein was then concentrated by pervaporation and chromatographed on G-100 Sephadex. Approximately 30% of the radioactivity in the homogenate was found with the proteins which eluted immediately after the void volume. A fraction of the protein eluted from the G-25 column also was subjected to equilibrium dialysis for 48 h at 0°-4° which resulted in no significant loss of radioactivity into the buffer. Similar evidence for protein-steroid interaction was obtained by incubating corticosterone (B), cortisol (F) and 11- deoxycortisol (S) with brain protein *in vitro*. The degree of binding was in the order B> F>> S.

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PRELIMINARY STUDY OF KIDNEY FUNCTION IN BEAGLE DOGS: AGING CONTROL DOGS

Charles J. Nabors, Jr. and Walter Stevens

Abstract: Preliminary studies of kidney function have been carried out on beagles ranging in age from 7 months to 16 years. The animals were grouped into two year periods for purposes of analysis. Blood urea nitrogen (BUN) and blood creatinine determinations were made. The data show a decrease in average blood levels of BUN and creatinine with increasing age of the animals. The decrease in both of these measurements was more marked in females than in male animals. In future studies BUN and blood creatinine will be measured in these same animals as they age and compared to similar measurements obtained from beagles injected with either ^{239}Pu, ^{241}Am or ^{228}Th, nuclides that deposit significant concentrations in the kidney.

Introduction

Previous reports from this laboratory have shown that ^{239}Pu deposits in the kidneys of beagles.[1] Injected ^{241}Am is also found in kidneys of beagles. Both of these nuclides are deposited in significant concentrations in other soft tissues such as liver, spleen and thyroid. It has been shown that both plutonium and americium are capable of inducing profound changes in serum enzymes which are indicative of liver damage.[2] Because of these findings, a study was initiated to determine the effects of these radionuclides on renal function. The first step in this investigation was to establish control values for purposes of comparison with radionuclide bearing dogs. Blood urea nitrogen and serum creatinine values were determined on animals from 7 months to 16 years of age.

Materials and Methods

The experimental animals were pure-bred beagles that were

bred and raised in our laboratory. These animals were either zero level animals from the different nuclide-injected groups or aging control dogs that showed no clinical pathology. All the dogs used in this study were judged to be in good health. The determinations were made using routine clinical laboratory procedures. Blood urea nitrogen was measured using urease. Creatinine was measured using the method of Folin and Wu. For the purposes of data presentation, the animals were grouped into two year periods.

Results

The results obtained for blood urea nitrogen measurements in our aging control animals are shown in Table 1. Note that the average BUN value for all dogs shows a decrease with increasing age. Reference to the measurements for male and female dogs will show that BUN decreases are more marked in females than in males. The decrease in BUN appears to be a non-linear function.

Table 2 illustrates the creatinine measurements in the aging population. The same animals used for the BUN study also were used for the creatinine study with the exception of a few animals which were added and some older dogs which died. The two studies were conducted approximately three months apart. Again, we see a slight decrease in the average creatinine value for all animals with increasing age. The decreases in male and female animals appear to be approximately the same. No statistical analysis of the data is available at this time.

Discussion

The decrease in BUN and creatinine values with increasing age was quite different from what was expected at the beginning of this study. Human values generally increase with increasing age. Several explanations are possible. In previous studies [3] we have shown mild increases in alkaline phosphatase and serum glutamic pyruvic transaminase in zero level dogs over a period of months post-injection. The present study was conducted on the basis of age from birth rather than months post injection. It is possible that some increase in urine flow with increasing age would be related to a decrease in blood urea nitrogen measurements. Decreases in muscle mass and activity may relate to the changes in creatinine. Future studies will follow the individual animals involved in the present report as well as a comparison of these values to those obtained with low levels of thorium-228, plutonium-239 and americium-241.

Acknowledgment

The authors wish to thank Miss R. Imelda Zizumbo and Mr. Robert Firmage for their technical assistance.

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Table 1. BUN values in aging control dogs

Age Range	Sex	n	Average BUN[1]	Average BUN[1] All Dogs
7mo.-2yr.	Male	14	20.3±0.9	
	Female	5	17.9±9.6	
				19.7±0.7
2yr.-4yr.	Male	10	18.5±0.8	
	Female	12	16.7±0.8	
				17.5±0.6
4yr.-6yr.	Male	5	17.4±0.7	
	Female	14	16.9±0.7	
				17.0±0.5
6yr.-8yr.	Male	4	18.5±0.7	
	Female	6	14.9±0.8	
				16.4±0.8
8yr.-10yr.	Male	3	20.3±0.6	
	Female	6	15.4±0.8	
				17.0±1.0
10yr.-12yr.	Male	5	16.1±0.9	
	Female	6	15.2±1.0	
				15.6±0.7
12yr.-14yr.	Male	2	22.3 --	
	Female	4	14.2±1.8	
				16.9±2.1
14yr.-16yr.	Male	2	20.4 --	
	Female	2	15.5 --	
				15.2±1.4

[1] Mean ± 1 standard error

Table 2. Creatinine values in aging control dogs

Age Range	Sex	n	Average Creatinine[1] mg %	Average Creatinine[1] all dogs
10mo.-2yr.	Male	13	1.3±0.03	
	Female	5	1.3±0.07	
				1.3±0.03
2yr.-4yr.	Male	6	1.3±0.03	
	Female	10	1.3±0.04	
				1.3±0.03
4yr.-6yr.	Male	9	1.4±0.03	
	Female	13	1.4±0.05	
				1.4±0.03
6yr.-8yr.	Male	5	1.2±0.04	
	Female	5	1.2±0.04	
				1.2±0.03
8yr.-10yr.	Male	2	1.1±0.05	
	Female	4	1.0±0.05	
				1.1±0.05
10yr.-12yr.	Male	3	1.0±0.04	
	Female	8	1.1±0.03	
				1.1±0.02
12yr.-14yr.	Male	3	1.1±0.05	
	Female	3	1.1±0.12	
				1.1±0.06
14yr.-16yr.	Male			
	Female	1	0.9 --	--

[1] Mean ± 1 standard error

RADIATION INDUCED INTRAOCULAR MELANOMAS

G. N. Taylor, R. D. Lloyd, D. R. Atherton, C. W. Mays,
L. Shabestari and J. Williams

Abstract: Prolonged retention of ^{226}Ra, ^{228}Ra or ^{228}Th has resulted in a significant number of intraocular melanomas in beagles. Thus far, such tumors have occurred below the lowest presently observed osteosarcoma dose by factors of 3 and 6 for ^{226}Ra and ^{228}Ra, respectively. They have developed at the lowest osteosarcoma dose in ^{228}Th dogs, but not below this level. Intraocular melanosis was induced by ^{90}Sr and ^{239}Pu but, thus far, intraocular neoplasms have not been produced by these radionuclides.

Introduction

Studies by Stover, et. al., have shown the retention of ^{226}Ra, ^{228}Th, ^{239}Pu and ^{90}Sr in the canine eye to be significantly elevated above the plasma level. (1) They also found the burden to be principally in the vascular tunic. Autoradiographic studies further indicated that within this site most of the burden was localized in the melanin component of melanocytes and melanophores and in the iridiocytes of the tapetum. (2) Bruenger, et.al., have shown that radium, strontium and thorium retention within the melanin granules is in the indole polymer and not in the protein component. (3) High levels of radium retention in these sites produced variable degrees of depigmentation and some focal areas of melanosis. Marked pigment cell hyperplasia was produced by some of the lower doses. Strontium-90 and ^{239}Pu induced principally the hyperpigmentation syndrome, but some equivocal tapetal changes were occasionally seen.

During the past few years, intraocular melanomas have become

a very significant endpoint in some of the lower dose levels, usually at relatively long survival times. Thus far, such tumors have occurred only in dogs treated with ^{226}Ra, ^{228}Ra and ^{228}Th. It is the purpose of this report to summarize the present incidence of eye melanomas and to present some of the factors related to their induction.

Methods

All of the dogs used in this study were purebred beagles from a moderately inbred colony and were maintained under comparable conditions throughout their lifespan. They were injected with a single intravenous (I.V.) injection of the radionuclide during young adulthood according to procedures described in another report. [4]

The chinchillas were obtained from local ranchers and were given a single I.V. injection of radium. Most were approximately 19 months of age, but a few were significantly older at the time of injection. [5,6] The radionuclide burden of the eyes and eye components was determined in a side-well gamma ray detector. [7]

Results

Detailed descriptions of the intraocular radiotoxicity syndrome of ^{224}Ra, ^{226}Ra, ^{228}Ra and, to a lesser extent, ^{228}Th, have been presented in earlier studies. [8-11] Grossly the lesions were characterized by depigmentation and loss of the tapetum at the higher levels and hyperpigmentation at the lower doses. The earliest changes at the high level were seen clinically in the tapetum, whereas the first apparent clinical change in the lowest levels was

hyperpigmentation in the iris (Fig. 1). The approximate time when iris hyperpigmentation was first observed is given in Table 1. These data also indicate the relatively wide variability in the latent period of such changes, as observed clinically. Similar hyperpigmentation also occurred as part of the aging syndrome but usually at significantly longer latent periods, although there was some overlap. The radiation induction of such melanosis appeared to be the acceleration or enhancement of a spontaneously occurring event and not the production of a feature unique to radiation.

Microscopically, the radiation induced melanosis involved the various parts of the vascular tunic; however, the iris and the ciliary body were the most reactive sites. In some extreme cases, the hyperplastic changes obscured most of the normal structure of the iris and ciliary body (Figs. 2, 3). The principal hyperplastic cell in the melanotic sites was a large, melanin packed cell which resembled the "clump" cells seen normally--especially in the iris and ciliary body (Fig. 3). This type of pigmented cell was also seen at the high dose levels but in much lower numbers (Fig. 5). Such high level cases also had a significant reduction in the melanophore syncitium of the iris, which appeared to be more radiosensitive than the pigment cell epithelium or the melanocyte component (Figs. 3, 4). The ratios of melanotic to non-melanotic components were, within general limits, dose dependent and these ratios will be summarized in a subsequent report.

The origin of the hyperplastic component of the pigmented foci was not unequivocally determined. However, at least some

of the cells appeared to arise from the pigment epithelium of the ciliary body. An apparent succession from this epithelium could sometimes be seen (Fig. 6). In many cases there appeared to be a migration of such cells from the ciliary body into the iris and the anterior aspect of the choroid. The usual location of the pigmented iris plaques near the periphery of the ciliary region of the iris is consistent with this possibility. In relatively advanced cases, movement into the trabecular region of the anterior chamber also occurred (Fig. 7). Proliferation of the pigment epithelium of the iris and passage of cells through the dilator muscle were not seen. However, it should be noted that the pigment epithelium of the iris and ciliary body are not homologs.

Of the various eye changes induced, the most serious was the induction of intraocular tumors which occurred principally in the lower dose levels (Tables 2-5) (Figs. 8-12). The depigmentation and tapetal changes of the higher doses were very striking, but these did not appear to threaten vision or life and have not been associated with intraocular tumors. Eye neoplasia has not been observed above the 1.07 μCi ^{226}Ra/kg dose, however, one such tumor has been reported in a high level ^{226}Ra dog in the Davis studies. [12]

The development of intraocular tumors was associated with the hyperplastic changes and occurred only in those levels in which hyperpigmentation was a significant feature. However, the succession from the hyperplastic to the neoplastic changes was not established. Thus far, such neoplasms have not been observed in any of the controls.

All of the tumors, except one, arose in the ciliary body. The exception occurred in the choroid immediately underlying the tapetum (Fig. 9). They were each classified as melanomas, and all but one were moderately to densely pigmented. The exception was a very lightly pigmented tumor which occurred at the 3-level ^{226}Ra dose -- the highest level in which eye tumors developed.

Microscopically, the melanomas were generally characterized by large, densely pigmented cells of variable size plus spindle cells with a much lesser degree of pigmentation (Fig. 13). The presence of the latter was usually one of the most obvious differences between the apparently non-neoplastic hyperplastic changes and the frank neoplasms. The incidence of mitotic figures was low and the observed growth rate was quite slow. Contingent invasion into the ciliary processes, iris and fibrous tunic became extensive if the tumors were allowed to reach significant size before enucleation. Metastases have been observed in two cases, but the post-surgical survival times are still too short to make an accurate evaluation of this feature. Nevertheless these tumors tentatively appear much less extreme in their metastatic and growth rate tendencies than the melanomas arising in other sites such as the mouth and skin. It has been speculated that tumors from the skin are possibly of a different origin. [13] Certainly their clinical behavior is different. Secondary glaucoma was observed in a high percentage of the tumors which arose in the ciliary body and which were allowed to reach significant size.

Retention of radium in the beagle eye was a function of both

the post injection time and the injected dose level (Fig. 14). A similar relationship was also observed in our long-term chinchilla studies and the eye retention half-time for the one comparable dose level (Fig. 15) was roughly similar to the beagle.

Based on the half-times, as shown in Fig. 14, the rad doses on both an average and a local basis are summarized for post-injection times of 2000 and 3000 days (Table 6). These intervals were selected because of their relationship to the time of neoplasia but are not intended to imply true latent periods. The calculations for the local cumulative dose are based on (a) no significant retention of radon, [2] (b) a non-uniformity factor of 29, (c) absorption of 50% of α energy in the pigmented tissue, (d) total eye and eye fraction weights as shown in Table 7 and average total body weight of 10 kg. Similar dose estimates for the other two radionuclides producing intraocular tumors, ^{228}Ra and ^{228}Th, have not been prepared because of the various unknowns related to translocation and retention of daughter products.

It is significant that intraocular melanomas have thus far arisen at levels considerably below the osteosarcoma induction dose--lower by factors of 3 and 9 in the case of ^{226}Ra and ^{228}Ra respectively (Tables 2-3). Such tumors have developed at lower injection levels than any other radiation induced neoplasms observed thus far in our radium studies. Eye melanomas have occurred down to, but not below, the bone tumor dose in ^{228}Th treated dogs (Table 4).

The retention half-times of ^{239}Pu in the canine eye appear to be relatively long, but the data are insufficient to establish

Table 7. Average total eye weights and average weights of major pigmented components (Grams), based on 7 beagles

Total eye wt.	Ciliary body	Iris	Choroid*
5.6309	0.2232	0.0515	0.1647

* Also includes the pigment cell layer of retina.

retention curves.[1] The eye retention data for ^{90}Sr, which is very limited, tentatively suggests a pattern more nearly like that of ^{226}Ra.[1]

Pigmentary hyperplastic changes in the eye also occurred in a significant number of the ^{239}Pu dogs, 0.0951 μCi/kg and above, and in part of the higher level ^{90}Sr dogs. However, melanomas have not yet occurred in these instances.

Discussion

One of the most significant differences in the radium toxicity syndrome in man as compared to the beagle is the eye syndrome. Pigmentary lesions, including an abnormal incidence of intraocular melanomas, have not been reported in the human ^{226}Ra and/or ^{228}Ra cases.[14-16] However, pigmented plaques have been observed on the iris and skin of patients treated with multiple injections of ^{224}Ra.[17] We are presently trying to determine the reason for this difference and plan to study eyes from several human patients injected with low doses of radium shortly before death. If human eye melanin does not selectively retain radium, it differs in this

respect from not only the dog but also the chinchilla, hamster, mouse and synthetically produced melanin. (3, 5, 6)

In any event, it is very fortunate that the response to radium in the human eye has not paralleled that of the dog, for the induction of intraocular melanomas is one of the most serious endpoints in radium treated beagles at the relatively low levels.

Acknowledgements

The authors wish to thank Klaus Voigtlaender and Walter Angus for their help in this study.

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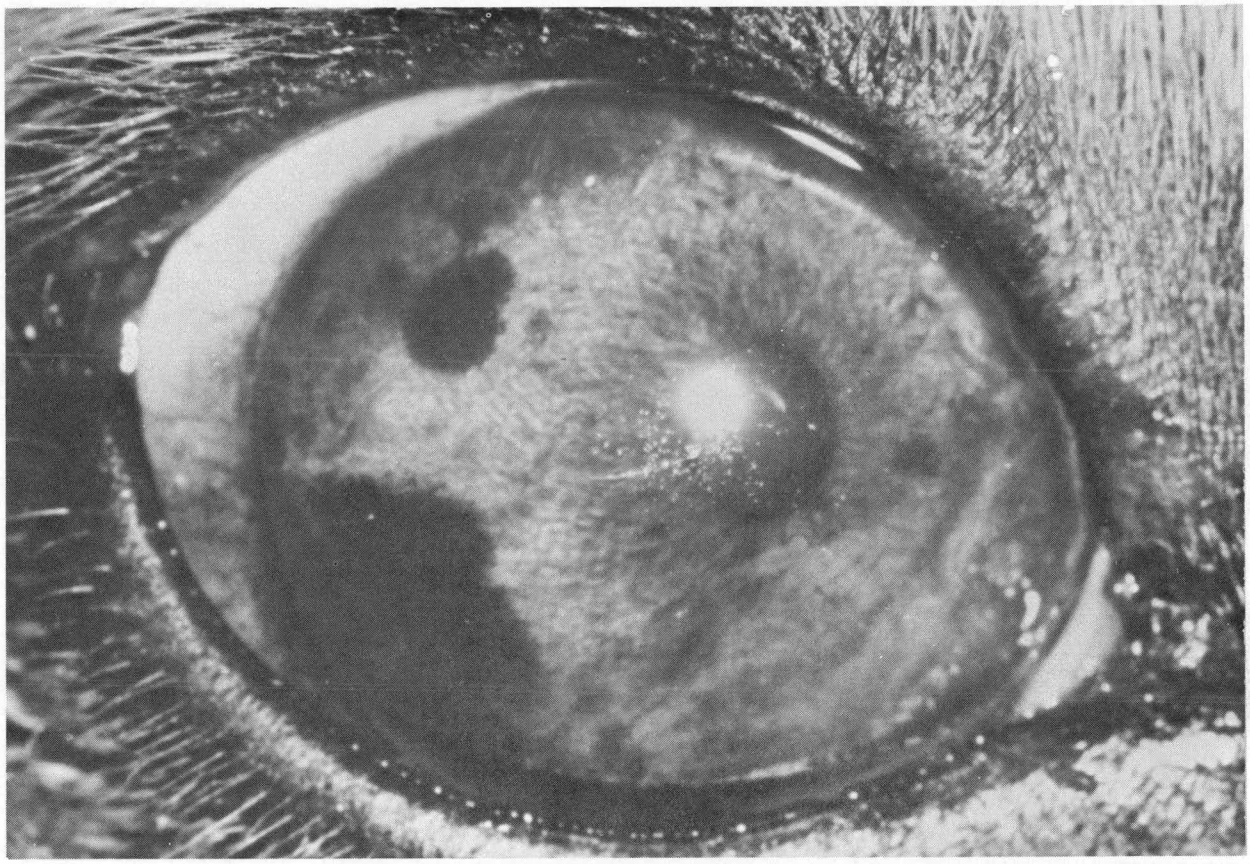

Figure 1. Beagle eye showing ^{226}Ra induced hyperpigmented plaques 1932 days following injection with 0.154 μCi ^{226}Ra/kg. X 7.

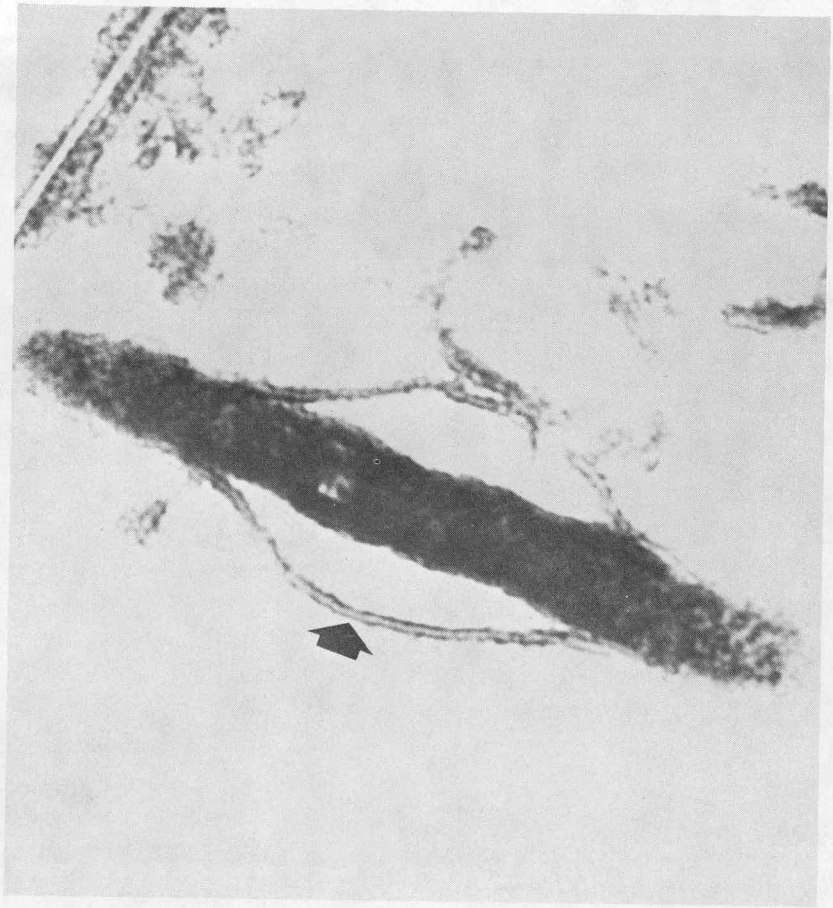

Figure 2. Microphotograph of beagle iris which shows ^{228}Ra induced hyperpigmentation adjacent to the dilator muscle (white arrow). The pigment epithelial layer (black arrow) of the iris is normal. 2746 days following injection of 0.146 μCi ^{228}Ra/kg. X 180.

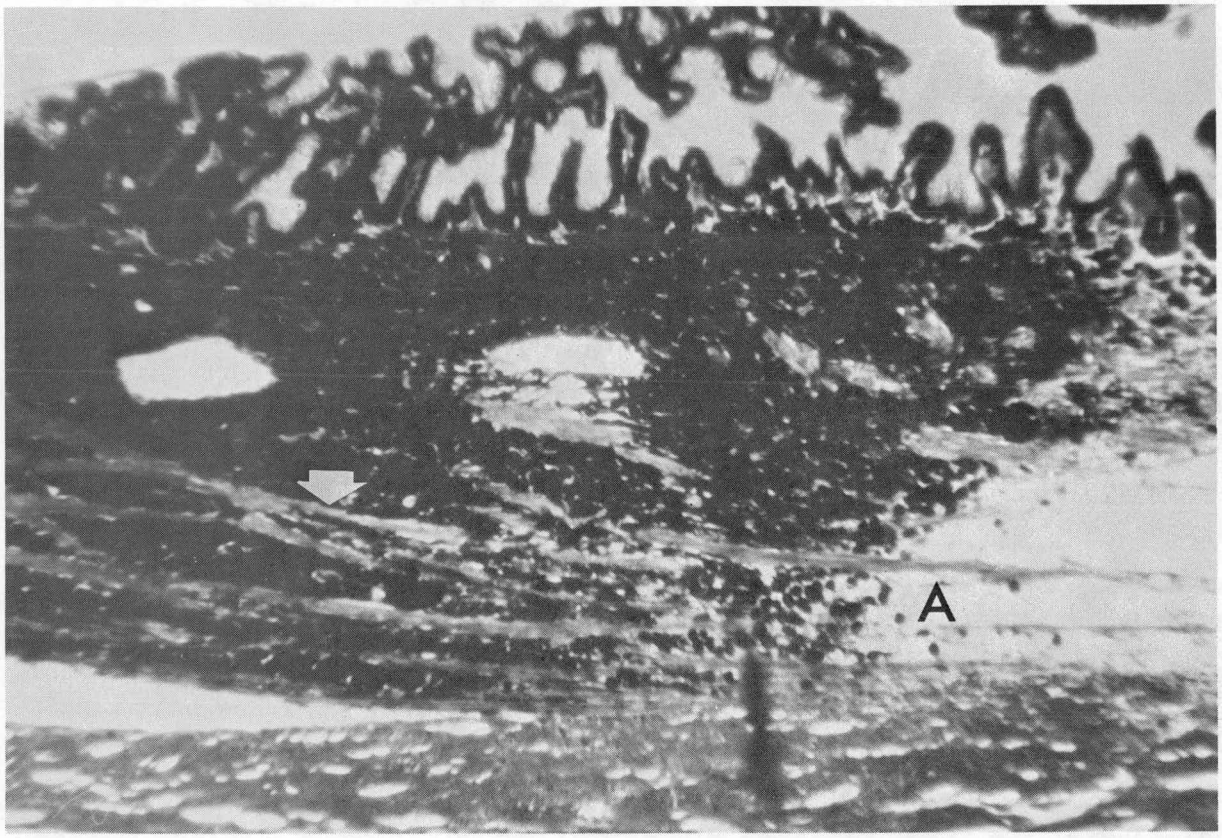

Figure 3. Meridianal section through ciliary body of beagle eye showing ^{228}Ra induced melanosis. The pigmented cells are densely packed between the fibers of the ciliary muscle (arrow) and a few cells are moving into the anterior chamber of the eye (A). 3402 days following injection of 0.0468 μCi ^{228}Ra/kg. X 93.

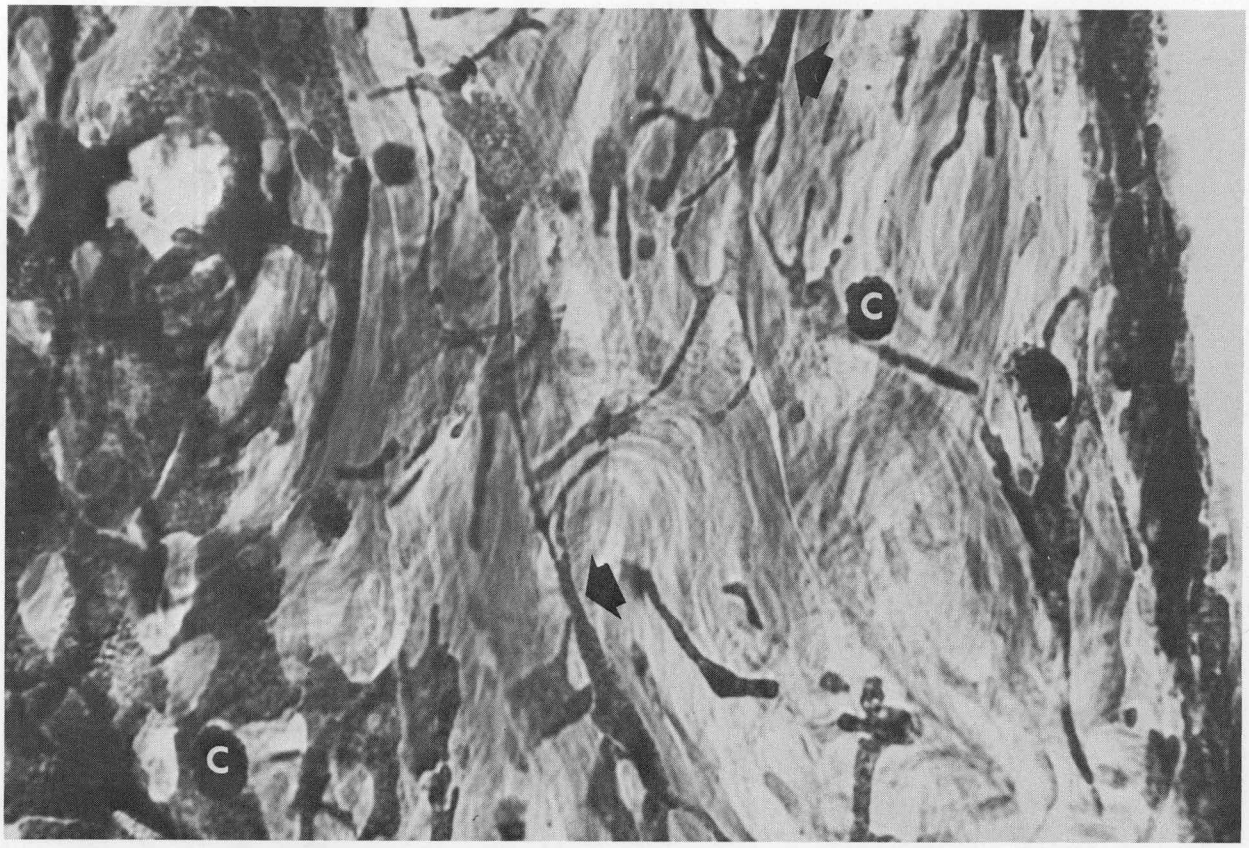

Figure 4. Cross section through anterior aspect of normal beagle iris showing chromatophore sycytium (arrow) which is normally more dense in the posterior aspect of the iris. A few "clump" cells (C) are interspersed in the stroma. The margin shown is the anterior surface. X 650.

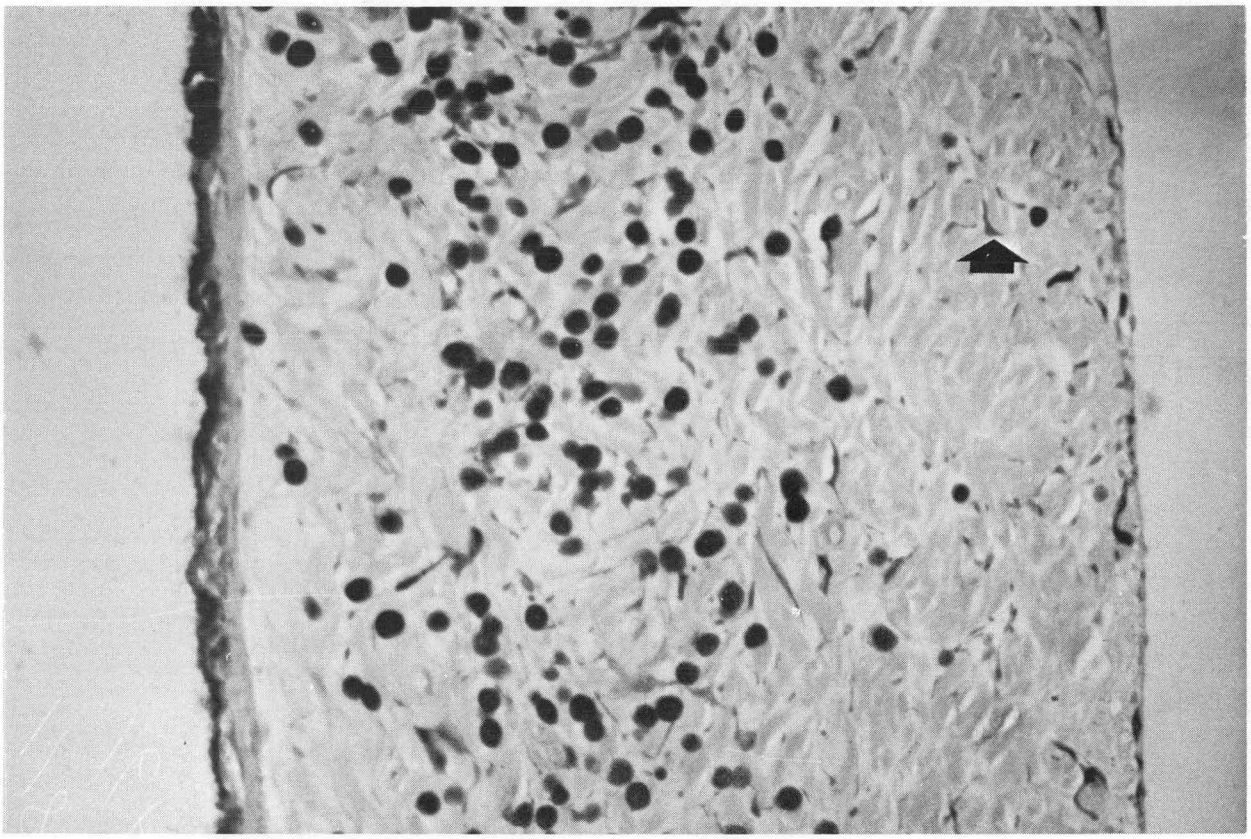

Figure 5. Microphotograph of beagle iris which shows marked loss of the chromatophore syncytium (arrow) and an increase in the "clump-like" cells. 1147 days following injection of 9.87 μCi ^{226}Ra/kg. X 266.

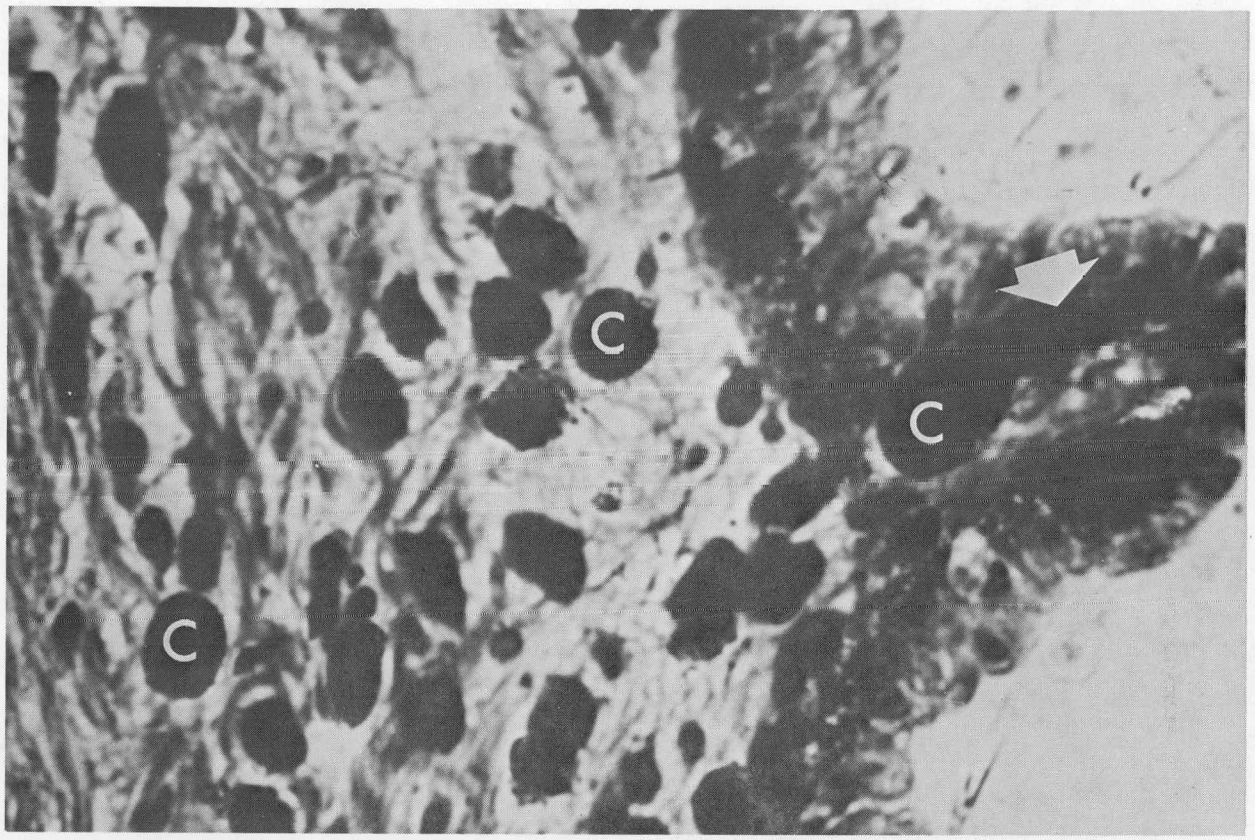

Figure 6. Meridianal section through ciliary body of a control dog, 5553 days of age, showing what appears to be a succession of "clump" cells (C) from the pigment epithelial layer (arrow). A similar pattern is seen in the irradiated dogs but usually at much earlier times. X 650.

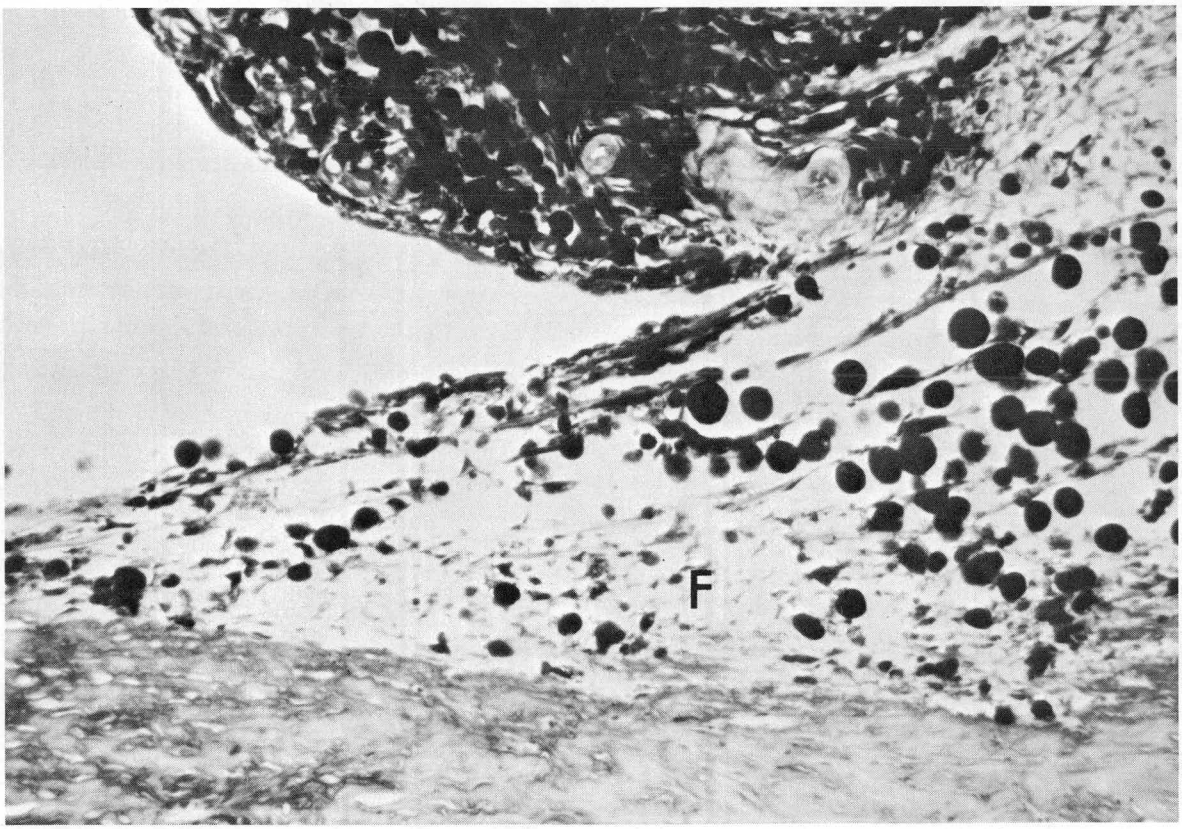

Figure 7. Meridional section showing the presence of pigment cells in the filtration angle (F) of a beagle eye 2646 days following a single I.V. injection of 0.146 μCi ^{228}Ra/kg. X 266.

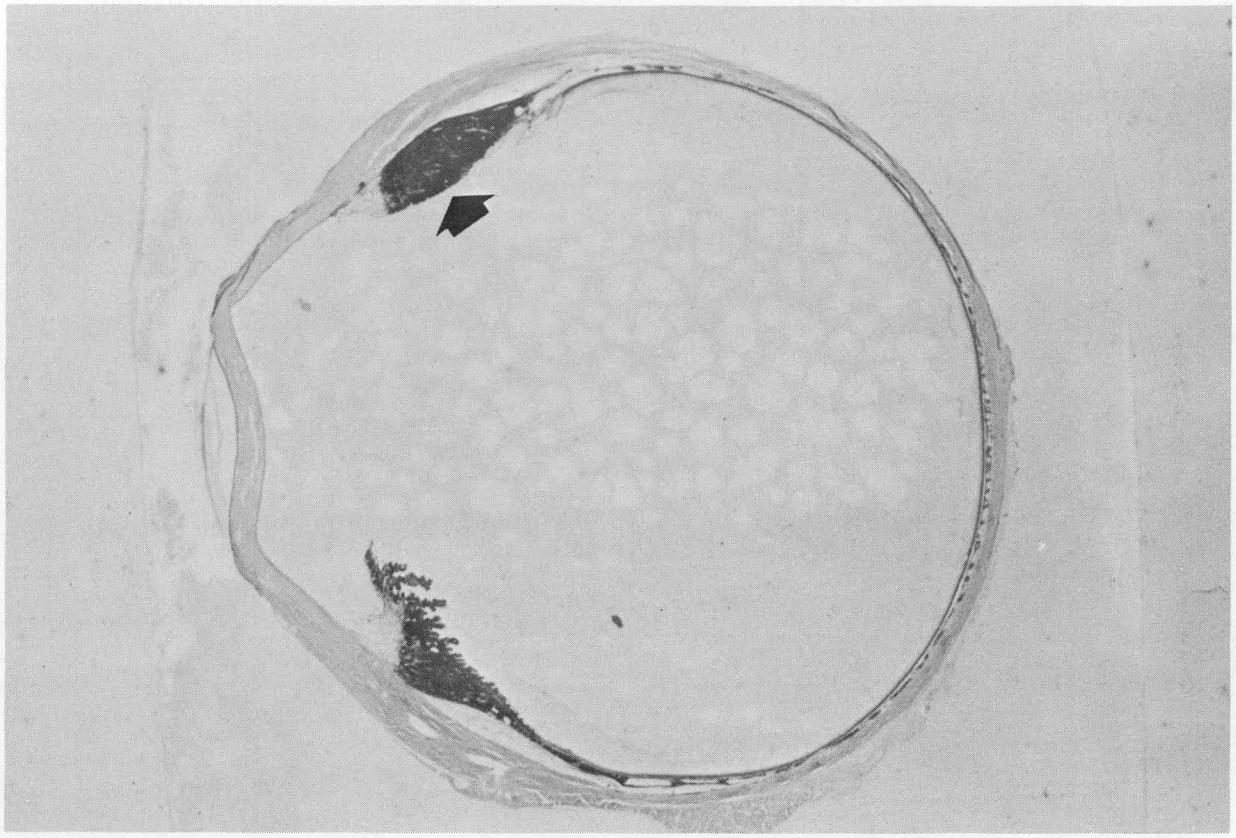

Figure 8. Meridional section of beagle eye in which an early melanoma (arrow) was found in one region of the ciliary body. This was an incidental finding at autopsy and vision was not impaired. 4703 days following injection with 0.267 μCi ^{226}Ra/kg. X 5.7.

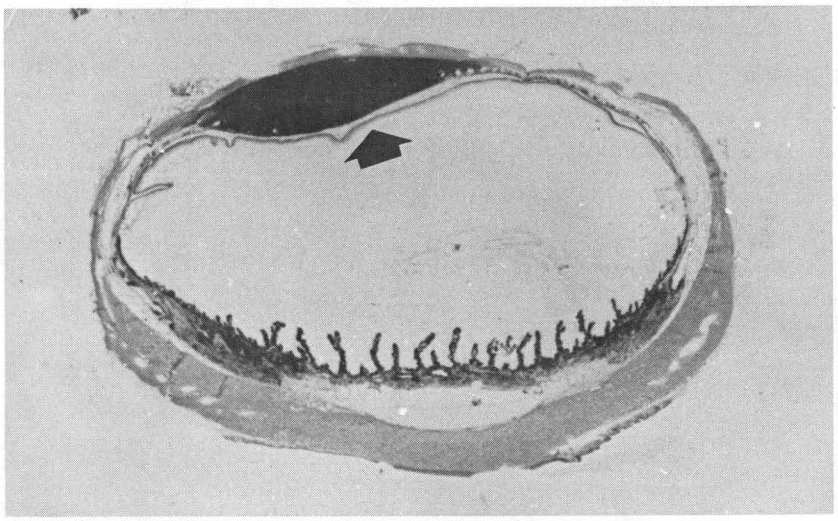

Figure 9. Section of beagle eye positioned dorsal to the horizonal meridion showing a relatively small melanoma in the choroid (arrow) underlying the tapetum. Same dog as Fig. 8. X 5.7.

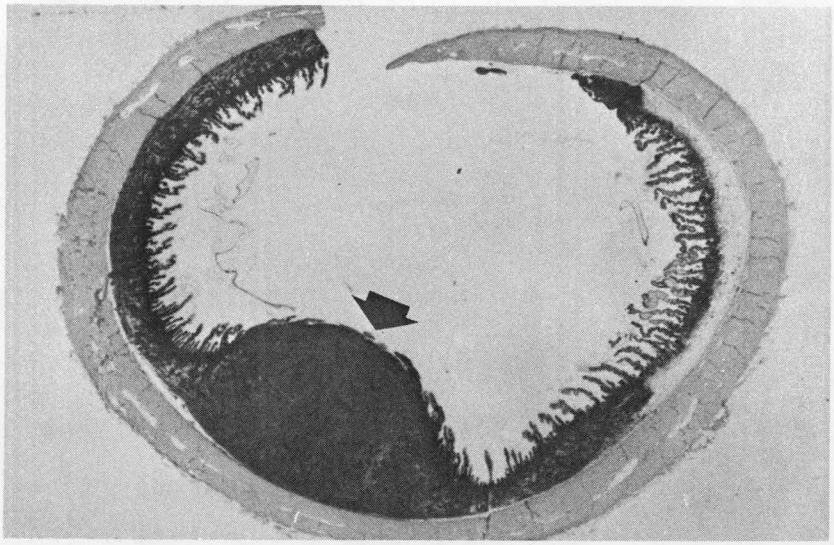

Figure 10. Section of beagle eye through the ciliary body parallel to the equatorial plane showing localized melanoma (arrow) in the ciliary body. Visual disturbances were not apparent. 2646 days following injection of 0.146 μCi ^{228}Ra/kg. X 7.3.

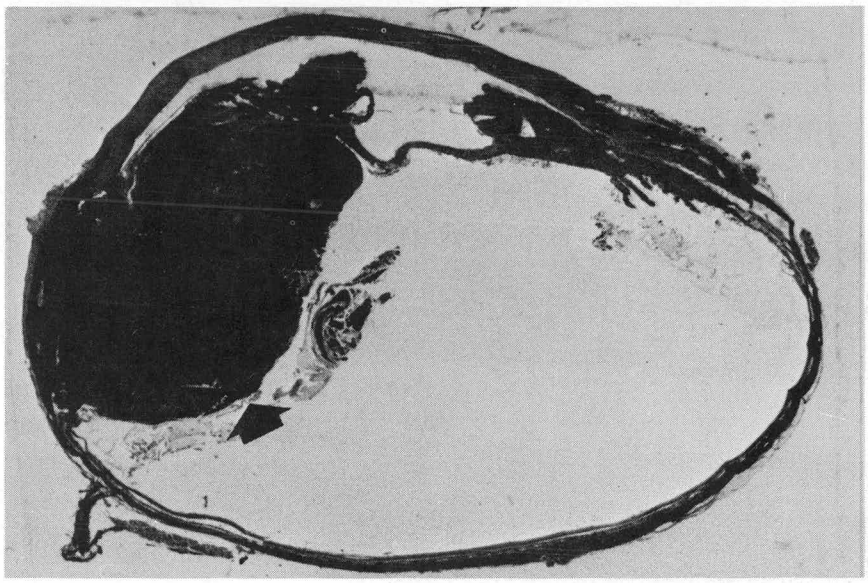

Figure 11. Meridianal section of beagle eye showing melanoma (arrow) arising from ciliary body. It was invasive and produced a secondary glaucoma and blindness. 3713 days following a single I.V. injection of 0.168 μCi ^{226}Ra/kg. X 6.

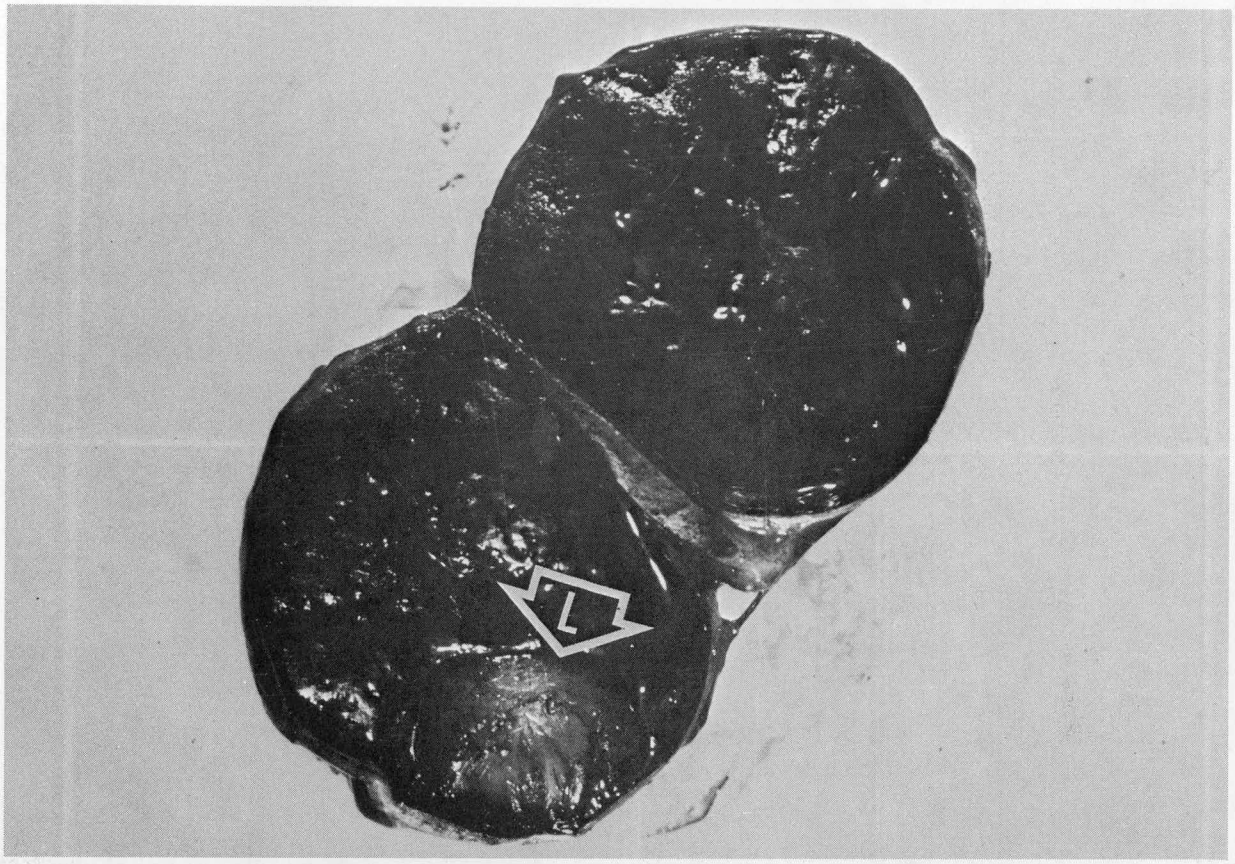

Figure 12. Beagle eye partially opened along meridional plane, showing luxated lens (L) and distention of eyeball with melanoma tissue. Periorbital invasion had not occurred and the dog is still living two years after enucleation. 4571 days following injection of 0.163 μCi ^{226}Ra/kg. X 3.

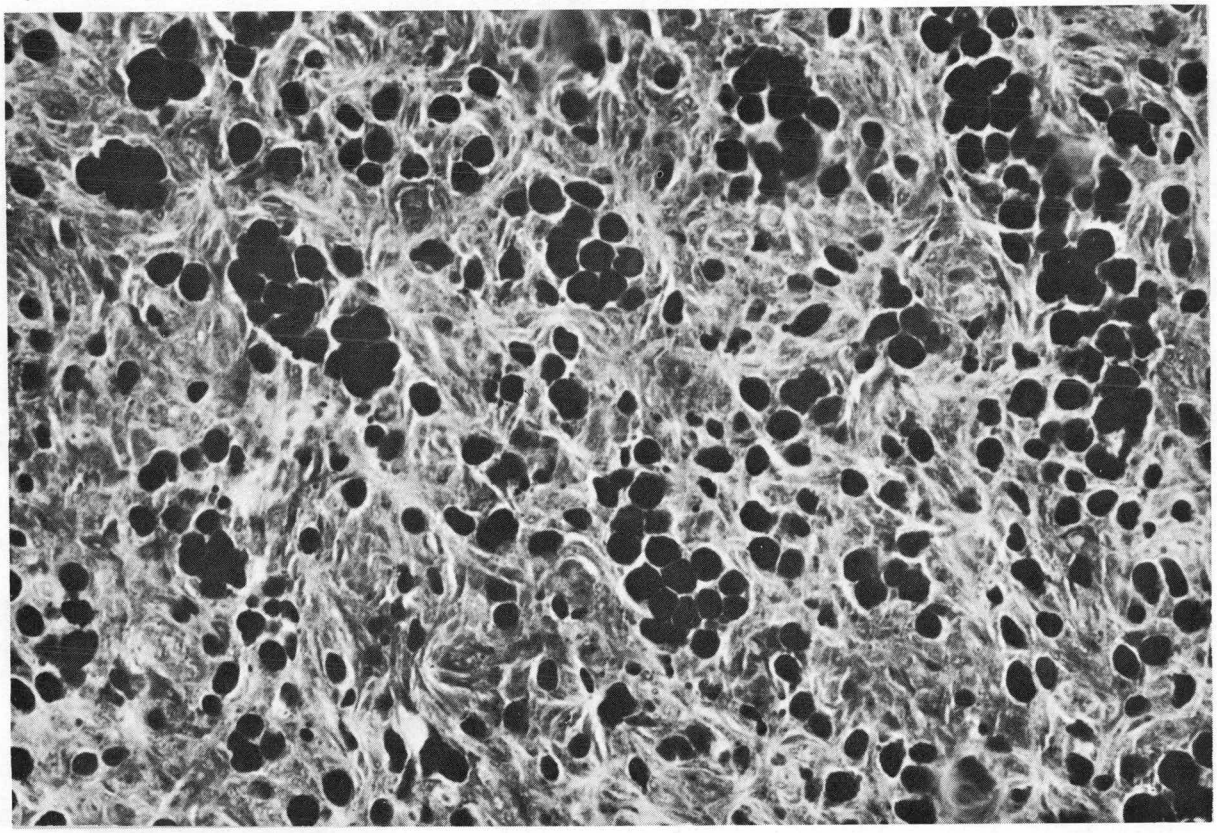

Figure 13. Microphotograph of melanoma shown in Fig. 10 indicating the large densely pigmented round cells and the lightly pigmented spindle cell types, characteristic of most of the melanomas observed in this study. X 200.

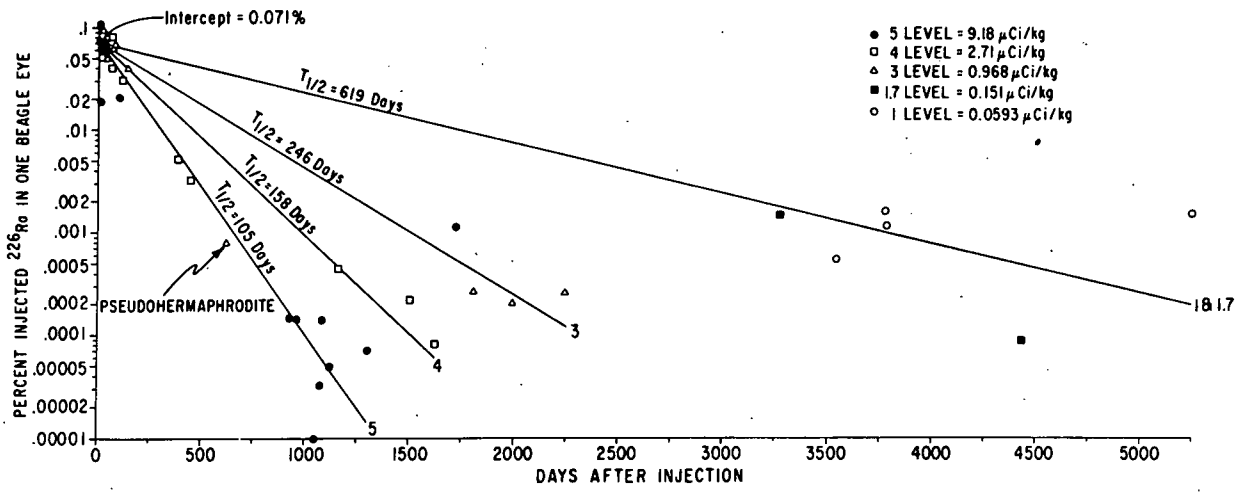

Figure 14. Relationship of ^{226}Ra retention in the beagle eye to the initial injected dose level and the post-injection time.

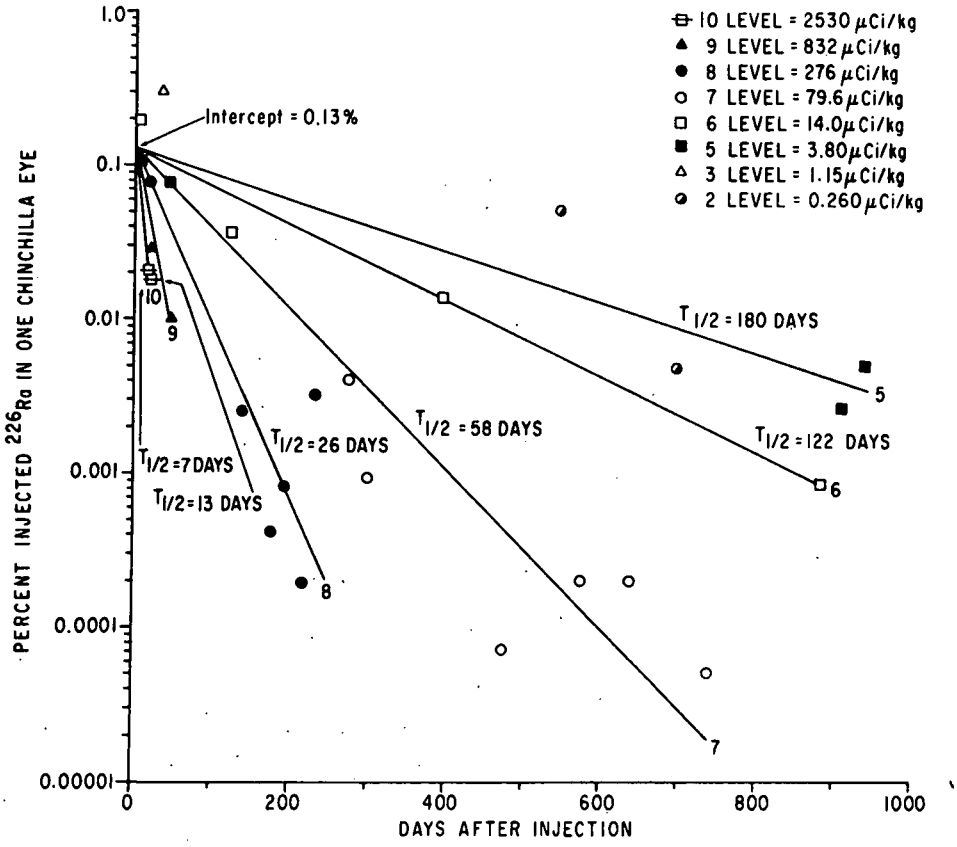

Figure 15. Relationship of ^{226}Ra retention in the chinchilla eye to the initial injected dose level and the post-injection time.

Table 1. Months post-injection when hyperpigmented plaques greater than 1 mm were first observed on the iris

	^{90}Sr			^{226}Ra			^{228}Ra			^{228}Th			^{239}Pu		
Dose* Level	Ave.	Range	Percent Incidence	Ave.	Range	Percent Incidence	Ave.	Range	Percent Incidence	Ave.	Range	Percent Incidence	Ave.	Range	Percent Incidence
5	18	19-24	18	20	16-40	22	-	-	0	(no observations)			66	-	20
4.5	38	26-51	16												
4	72	30-105	50	23	6-35	87	36	34-37	40	(no observations)			-	-	0
3	69	49-110	75	20	13-26	75	28	18-37	63	30	6-35	77	-	-	0
2	62	49-86	50	68	50-88	100	42	30-54	90	33	30-41	41	75	50-92	66
1.7	63	37-123	66	47	30-53	90	46	23-63	100				64	36-93	83
1.5										46	23-68	75			
1	76	62-114	58	56	19-83	91	56	23-103	92	60	43-100	83	85	53-137	91
0.5							54	30-70	91	58	43-74	58			
0.2				No plaques at 63 mo.						46	37-55	33			
0	70	62-79	41	104	79-133	41	82	57-125	50	109	64-132	27	93	64-139	91

* See injection tables for μCi values.

Table 2. Incidence of intraocular melanomas in dogs treated with ^{226}Ra

	Dose Level ($\mu Ci^{226}Ra/kg$)							
	control	0.0220**	0.0621	0.166****	0.339	1.07	3.21	10.4
No. of tumors	0	0	2	4	4	1	0	0
No. Dogs at risk*	33	24	21	13	12	12	12	4
Percent Incidence	0	0	9	30	33	8	0	0
Average age at neoplasia***	-	-	5217	3835	4199	1996	-	-

* Dogs surviving 5 years or beyond.

** High % of this dose level presently have short latent periods.

*** This is the age at death or biopsy.

**** Lowest dose level with osteosarcomas.

Table 3. Incidence of intraocular melanomas in dogs treated with ^{228}Ra

	Dose Level ($\mu Ci^{228}Ra/kg$)							
	Control	0.0177*	0.0505	0.148****	0.309	0.973	2.62	8.49
No. of tumors	0	1	5	2	0	0	0	0
No. dogs at risk**	13	12	13	12	10	11	1	0
Percent Incidence	0	8	38	16	0	0	0	0
Average age at neoplasia***	-	2596	3388	2656	-	-	-	-

* 3 other living dogs at this dose level presently appear to have intraocular melanomas but these are not unequivocal at this time.

** Dogs surviving 5 years or beyond.

*** This is the age at death or biopsy.

**** Lowest dose level with osteosarcoma.

(2 bilateral tumors occurred at the 0.148 dose level and 1 bilateral tumor at 0.309 dose level.)

Table 4. Incidence of intraocular melanoma in dogs treated with ^{228}Th

	Dose Level ($\mu Ci^{228}Th/kg$)							
	Control	0.00171	0.00518	0.0152***	0.0302	0.0919	0.290	0.858
No. of tumors	0	0	0	1	1	0	0	0
No dogs at risk*	12	12	12	12	12	7	0	0
Percent Incidence	0	0	0	8	8	0	0	0
Average age at neoplasia**	-	-	-	4570	2983	-	-	-

* Dogs surviving 5 years or beyong.

** This is the age at death or biopsy.

*** Lowest dose level with osteosarcoma.

Table 5. General Incidence of intraocular melanomas in Utah Beagle Colony

	Controls	^{239}Pu	^{228}Th	^{228}Ra	^{226}Ra	^{90}Sr
No. of tumors	0	0	2	8	11	0
No. of dogs at risk*	173	96	55	59	107	80
Percent Incidence	0	0	3	13	10	0
Average age at neoplasia**	-	-	3776	3106	4051	-

* Dogs surviving 5 years or beyond.

** This is the age at death or biopsy.

Table 6. Estimated initial dose rate and cumulative rad dose to the eye of beagles following a single I.V. injection of ^{226}Ra

Dose Level	Initial average dose rate (rads/day)	Cumulative average dose (rads)		Initial local dose rate* (rads/day)	Cumulative local dose (rads)	
		2000 days	3000 days		2000 days	3000 days
1 (0.0621 μCi/kg)	0.019	15	16	0.277	218	232
1.7 (0.166 μCi/kg)	0.051	41	44	0.741	595	638
2 (0.339 μCi/kg)	0.104	Insufficient Measurements				
3 (1.07 μCi/kg)	0.329	116	117	4.778	1682	1697

* Based on a non-uniformity factor of 29, zero retention of radon and absorption of one-half of the emitted α energy.

RADIUM INDUCED SUBCELLULAR TAPETAL CHANGES

G. N. Taylor, N. Anderson, K. Voigtlaender and W. Angus

Abstract: The cells of the canine tapetum lucidum were characterized by densely packed, elongated rod-like structures which were the principal sites of radium retention in the tapetum. Radiation induced changes following a single intravenous injection of ^{226}Ra or ^{228}Ra were clearly seen in these rod-shaped organelles. The lesions were dose dependent and occurred down to relatively low dose levels. Such changes were probably related, at least in part, to the rate of radium removal from this part of the eye.

Introduction

Following a single intravenous (IV) injection of approximately 10 μCi ^{226}Ra/kg into young adult beagles, one of the first clinically apparent changes occurred in the tapetum of the eye. [1] The lesions were radiation induced and were related to a relatively high uptake of radium in the pigmented intra-ocular tissues. [2,3] Such changes were seen as early as 20 days post-injection and progressed until ultimately the entire tapetum was removed. Less extreme changes occurred at lower dose levels.

It is the purpose of this report to present some of the normal anatomy of the tapetum and to indicate a few of the subcellular lesions observed with the electron microscope.

Methods

All of the dogs used in this study were purebred beagles. The radionuclide was given via a single IV injection during young adulthood according to methods described previously. [4]

The electron-micrographs are from tissues fixed in buffered

osmium tetroxide (4%) and embedded in epon 812 according to the method of Luft.[5] The embedded tissues were sectioned on an LKB Ultratome, mounted on Formvar coated grids and stained with lead citrate. The specimens were examined with an RCA EMU 3G electron microscope.

Results

The normal canine tapetum is a multilayered structure of the choroid; triangular in shape; and positioned in the dorsal fundus immediately above the optic papilla (Figure 1). It is immediately peripheral to the pigment cell layer of the retina and is separated from this layer by Bruch's membrane (Figure 2). The pigment cell layer in the region of the tapetum does not contain melanin as it does in the other areas, and the necessity of this adaptation is obvious, in view of the mirror-like function of the tapetum. Large capillaries arising from the vessel layer of the choroid penetrate the tapetum to join the choriocapillaris plexus lying between the innermost tapetal layer and Bruch's membrane (Figures 2 and 3).

The iridiocytes which comprised the tapetum were densely packed with rod-like organelles, all of which were normally oriented parallel to the retinal surface (Figure 4). However, within a given cell the rods were grouped into aggregates which frequently differed in their orientation. The direction of the rods also varied from cell to cell, but their long axes remained parallel to the retinal plane.

The individual rods, following osmium fixation and lead citrate staining, appeared as long cylinders with an electron dense wall

composed of a double membrane (Figure 5). The core of the rods was generally radiolucent, but was periodically electron dense. Thus, most of the rods in cross-section appeared hollow with a lesser percentage of the rods having a solid core. The radiolucent appearance was probably related to staining specificities and not emptiness.

The composition of the rods was not determined, but circumstantial evidence indicated a close relationship to melanin, and the iridiocytes of the canine tapetum are tentatively considered to be modified melanocytes; a similar speculation has been made relative to the cat. (6) This relationship was especially evident at the periphery of the tapetum adjacent to the choroid where an occasional melanocyte contained both typical melanin granules and organelles resembling the rods of the tapetum. Melanin granules were also seen in some of the well differentiated tapetal cells. A further similarity was the affinity of the tapetal rods for radium. (7) The significance of the extremely high zinc content or its precise location within the tapetal cells is unknown. (8)

The earliest radium induced change in the tapetum occurred in the rods. The lesions consisted of vesicle-like swellings in which the outer membrane of the rod separated from the central core (Figures 5 and 6). These sites and oftentimes the entire rod were granular and more electron dense, and the cores frequently appeared solid or their lumina markedly reduced. The increased density of the rods was possibly a direct radiation induced melanosis. (9) A relatively low number of these vesicle-like lesions were occasion-

ally found in the non-irradiated control dogs. As the degree of injury increased, a general disorganization of the spatial relationship of the rods occurred, and the parallel relationship to the retina was at least partially lost (Figure 7). Ultimately, the 10 μCi ^{226}Ra/kg dose induced a general breakdown and dissolution of the rods and finally cell death and lysis of the cytoplasmic membranes (Figure 8). The severity of the tapetal lesions was dose dependent and the changes gradually diminished down to the 1.7 level where they consisted of a small increase in the number of abnormal rods and no observable necrosis. The high incidence of lesions involving the rod organelles within isolated cells suggested that the rod degeneration may be secondary to general cell injury and not necessarily a direct effect to the rod.

Discussion

The degree of injury within the tapetum was much more easily evaluated than in the intra-ocular melanocytes. This was principally because of the more uniform and orderly arrangement of the tapetum and its organelles, whereas much of the detail in the mature melanin granules was obscured (Figure 9). Nevertheless, it is likely that significant radiation induced injury, although less obvious, also occurred in the melanin granules of melanocytes. The extent of the injury to the melanatic organelles which frequently occurred as a non-lethal change within the given cells, was probably related to the rate of radium loss from the eye. This would tend to explain the accelerated ocular excretion rates which were not entirely related to the removal of complete cells.

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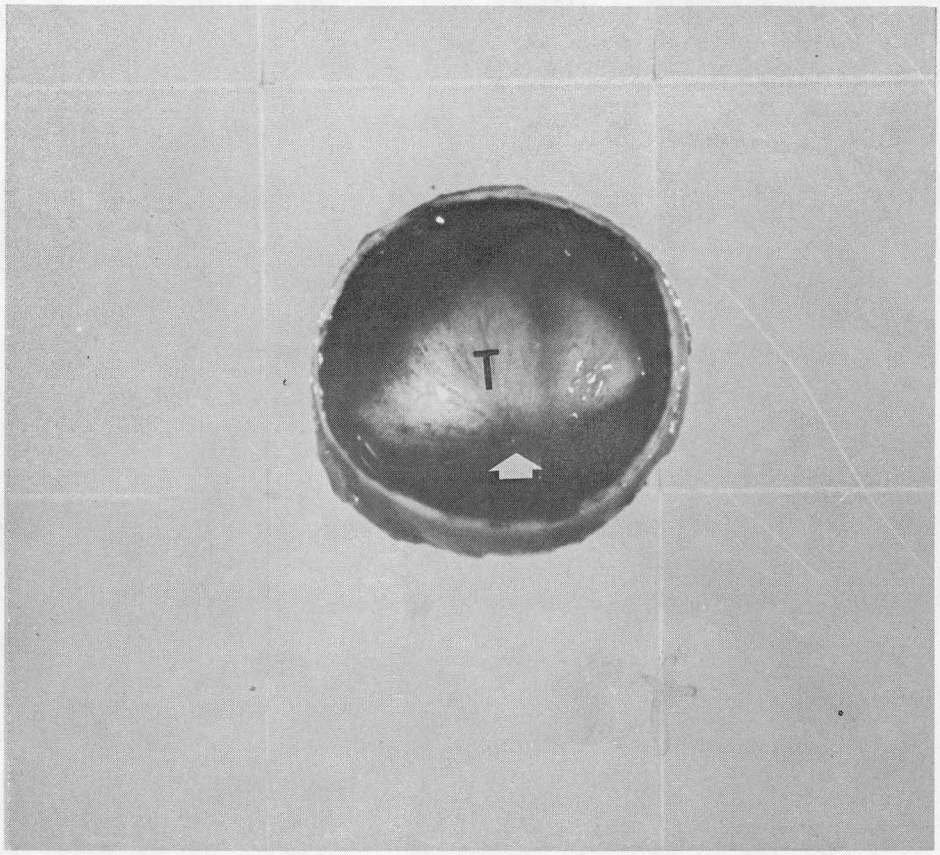

Figure 1. Fundus of beagle eye showing tapetum lucidum (T) positioned immediately dorsal to the optic papilla (→). X 2.

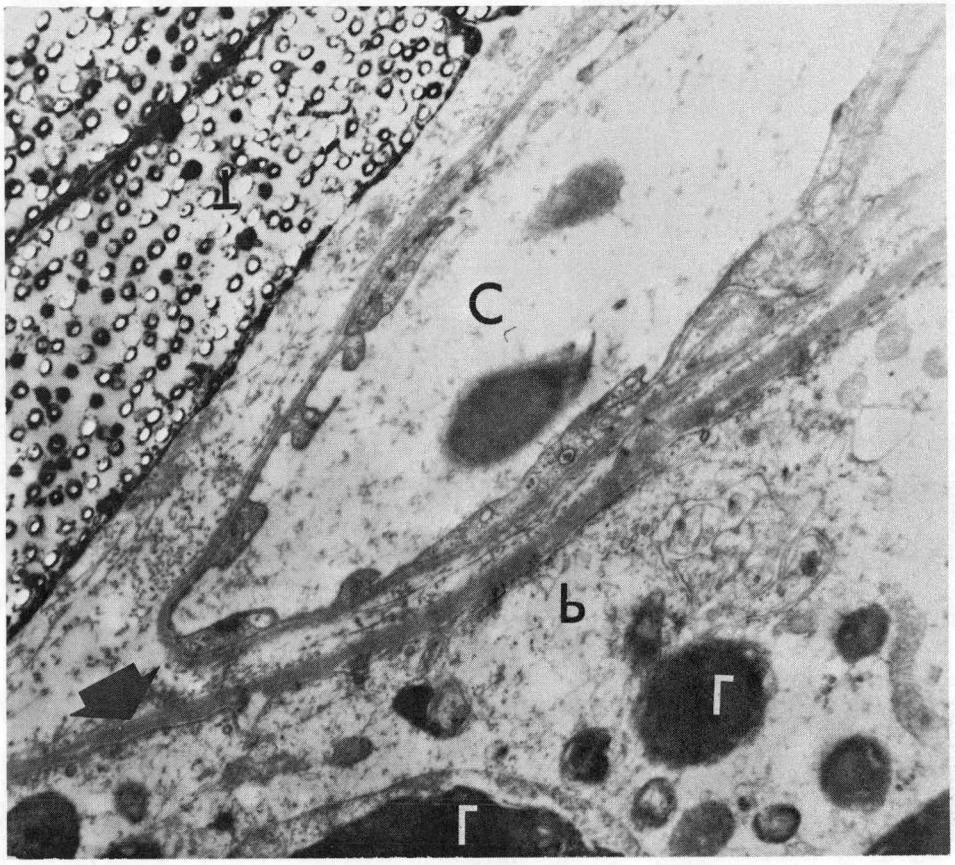

Figure 2. Electron micrograph of beagle eye showing the pigment cell layer of the retina (P), Bruch's membrane (→), a capillary of the choriocapillaris layer (C) and the tapetum lucidum (T). The pigment epithelial layer lacks melanin but contains large lipofuscin granules (L). X 8000.

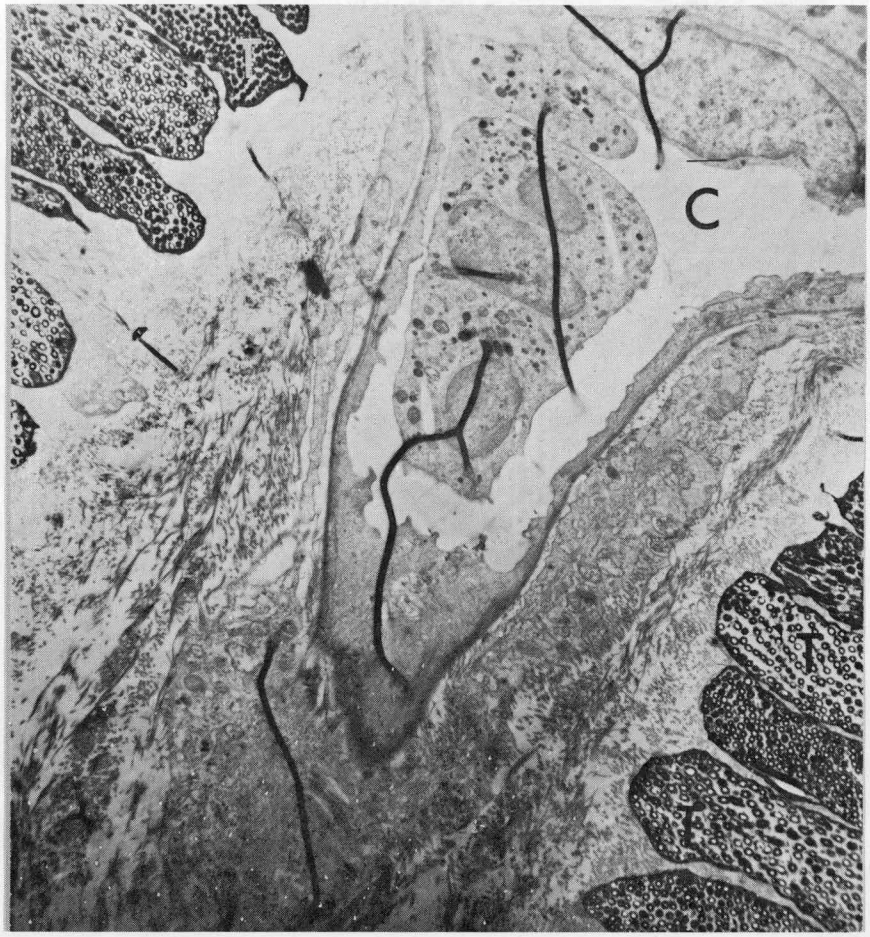

Figure 3. Electron micrograph of cross-section through beagle tapetum lucidum showing large capillary (C) penetrating the tapetum (T) to join the choriocapillaris plexus. The dark lines are artifacts. X 4800.

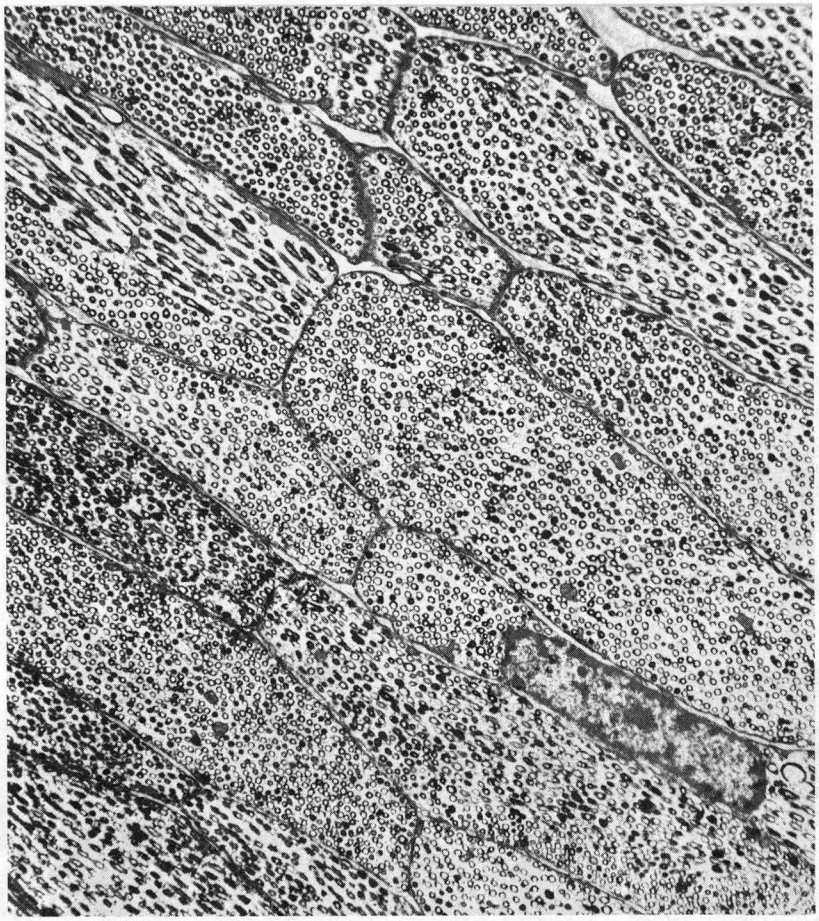

Figure 4. Electron micrograph of cross-section through normal tapetum lucidum showing the orientation of the rods. They are parallel to the retinal surface but do not necessarily lie parallel to each other. It is also noted that the cores are radiolucent in most but not all of the rods. X 4800.

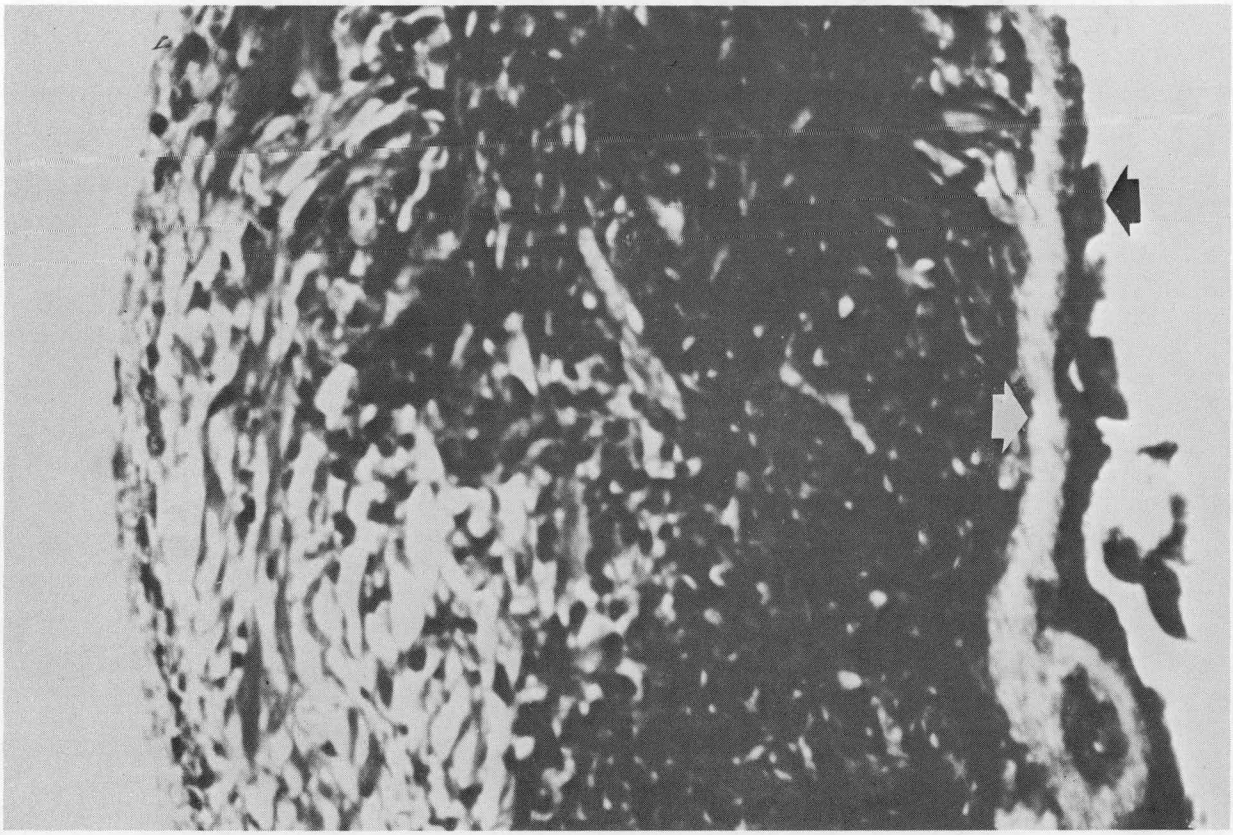

Figure 5. Radiation induced lesion in tapetal rod from beagle showing the double membranous lining (→) which is abnormally separated from the granular, pigmented core. 2948 days following injection of 0.281 μCi ^{226}Ra/kg. X 45,000.

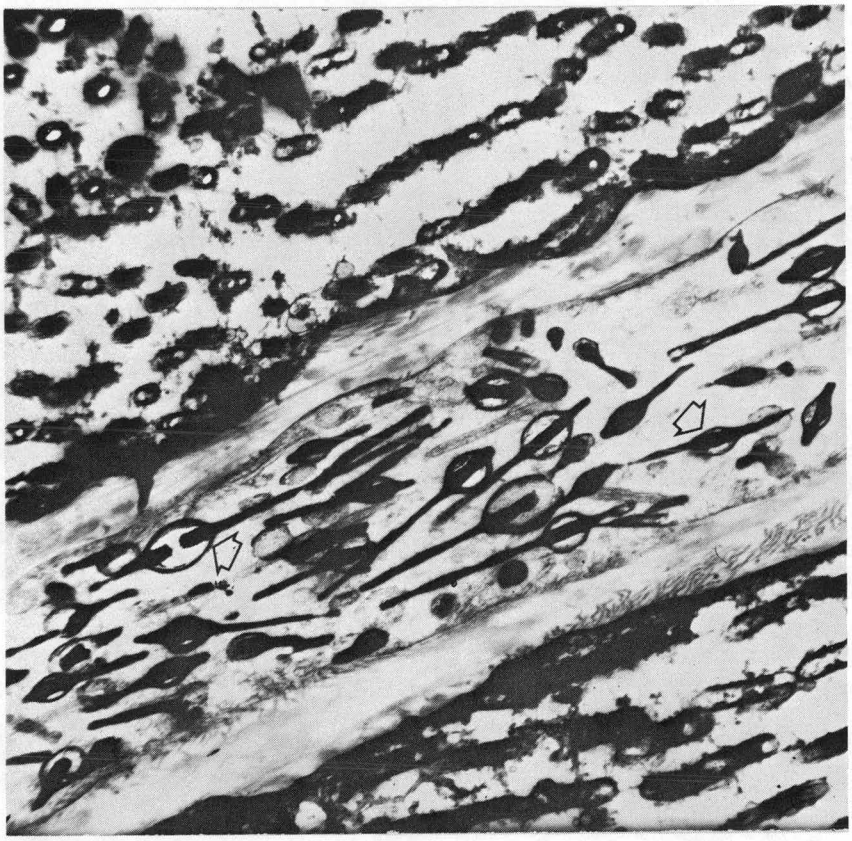

Figure 6. Degenerative tapetal cell showing numerous bullous-like expansions of the rods (→). The high incidence of abnormal rods within the isolated cell suggests a general cell injury and not a direct effect to the individual rods. 1737 days following injection of 1.12 μCi ^{226}Ra/kg. X 16,000.

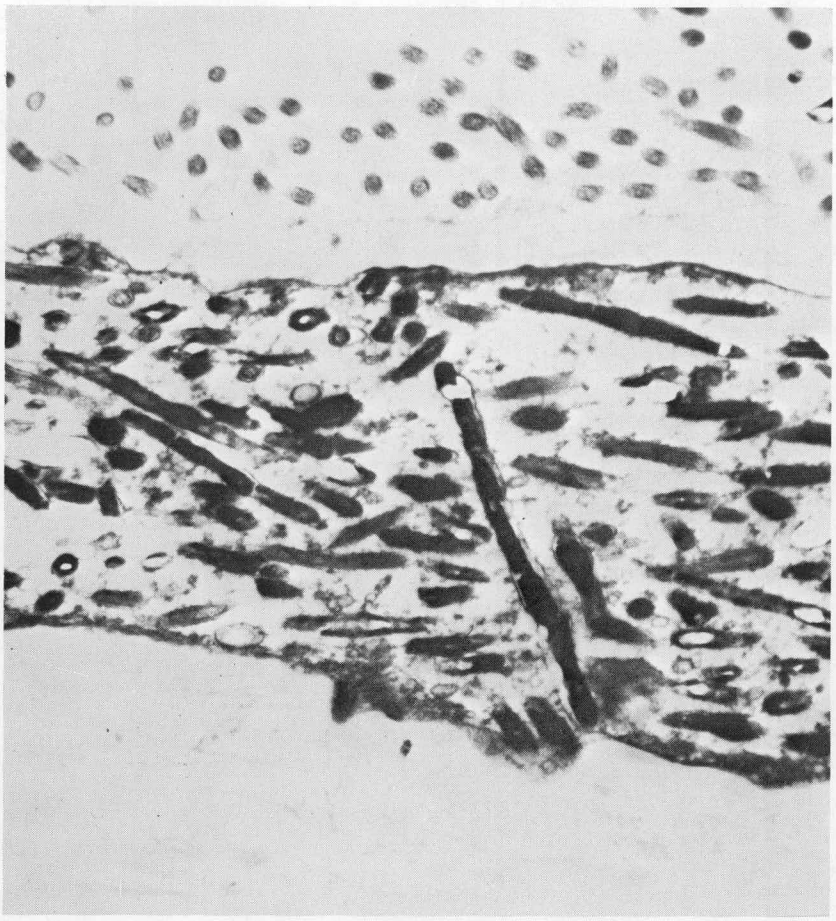

Figure 7. Tapetal cell showing general disorganization of the normally uniform arrangement of the rods. Injury to the rods is not extreme but increased density is obvious. 2399 days following injection of 0.167 μCi ^{226}Ra/kg. X 16,000.

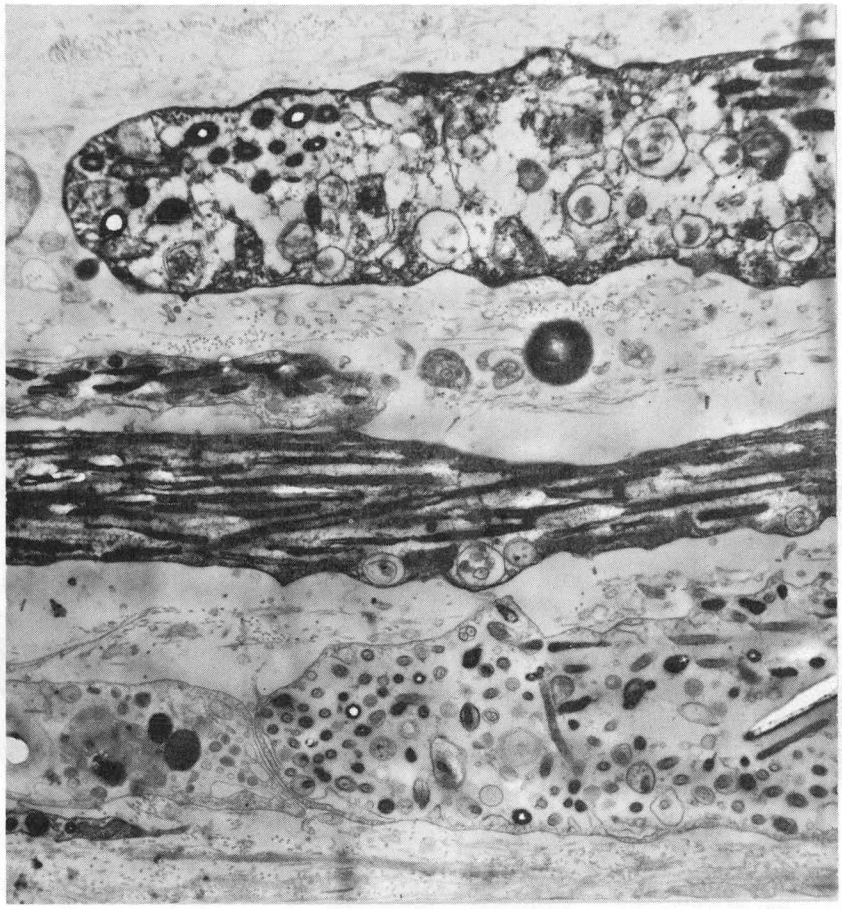

Figure 8. Cross-section through tapetum showing advanced degeneration and lysis of the organelles. 1414 days following injection of 3.34 μCi ^{226}Ra/kg. X 6,000.

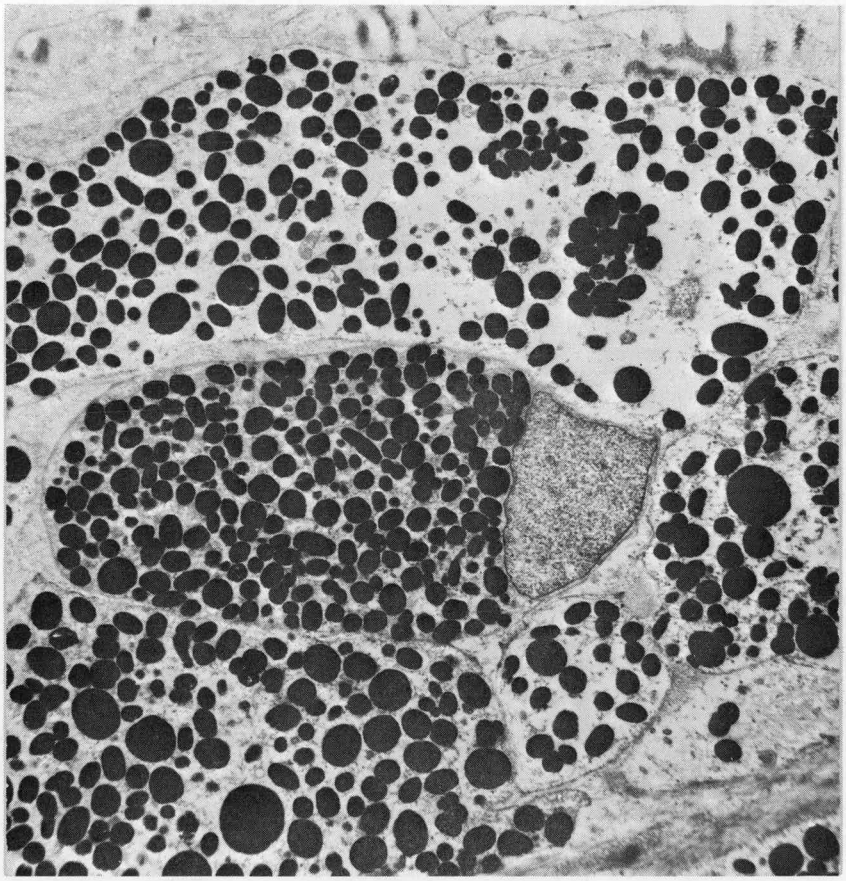

Figure 9. Melanocytes from the choroid of a beagle showing the relatively obscure detail in the mature melanin granules. 3375 days following injection of 0.387 μCi ^{226}Ra/kg. X 3,000.

AGE RELATED VARIATION IN BEAGLE ESTROUS CYCLE

L. Shabestari, G. N. Taylor and T. McClellan

Abstract - Normal female beagles experienced the first estrous cycle at about ten months of age and cycles continued at intervals of approximately seven months thereafter. Cycles became less frequent and more irregular as the animals became older but thus far have continued up until 16 years. The interval between cycles was not affected by breeding. Estrous intervals were shorter in animals which developed pyometra. Although some exceptions occurred, the dogs that survived to relatively extreme ages tended to have more regular cycles, even in senility.

Introduction

For the past several years, observations have been made and detailed records have been kept on the estrous cycles of all female beagles in the Radiobiology Laboratory. The following report summarizes data collected on our control animals.

Materials and Methods

Every female animal in the laboratory over six months of age was checked for estrus twice a week. Observations were made on genital discharge (hemorrhagic, straw, opaque), and degree of swelling of the vulva (none, slight, moderate, marked), and occasionally by vaginal smear. Observations were recorded on cards which were kept for each animal. The day on which a hemorrhagic genital discharge was first noted was taken as day one for that cycle. The interval between cycles is the number of days from day one of one cycle to day one of the succeeding cycle. Experimental number, age, and days interval between each cycle for each animal were recorded on computer cards. By means of these cards,

estrous data were summarized.

Each interval was grouped according to age class. The designated year of age includes plus or minus six months. For example, year one is from six months to 1 1/2 years of age, year two is from 1 1/2 years to 2 1/2 years of age, etc.. Each interval was recorded as having occurred in the year during which it ended. For example, if an animal was in estrus at 14 months of age and again at 21 months of age, the interval between these two cycles was included in tabulations for year two.

There are many sources of error in observing estrous cycles in this way. Several different people were involved in making observations over a period of years and thus the possibility of different interpretation. The degree of swelling of the vulva and amount of hemorrhagic discharge varied between animals and in some cases it was very slight or absent and may have been overlooked by some observers. Since neither vaginal smears nor teasing by a stud were routinely done, it was not known in most cases whether or not true estrus occurred following a hemorrhagic genital discharge. Also, some conditions, such as vaginal polyps and vaginitis , etc., might occasionally be mistaken for proestrus.

Three groups of animals were used in this study. Aging controls which are used in the breeding colony, toxicity controls which were never bred, and three animals from each group which developed pyometra. Aging controls have been bred one or more times during their life span. These animals were usually not bred after nine years of age. Pups were weaned at six weeks of age. Toxicity control dogs are those which were sham injected. Ovariohysterectomies

were performed on the animals which developed pyometra.

Results and Discussion

The mean age for onset of the first estrous cycle was 320 ± 52 days in a total of 234 uninjected animals. This age of onset of the first estrous cycle agrees with other beagle studies (1).

The estrous intervals remained fairly constant and standard deviations remained fairly small up through year 4. This corresponds to the period of greatest fertility in the female beagle as noted by Anderson (2,3).

There was little difference in estrous intervals between aging controls and toxicity controls during the years when aging controls were being bred. This indicates that pregnancy, whelping, and lactation did not delay the cyclic occurrance of ovarian activity. That is, a female beagle will have estrous cycles approximately every seven months whether or not she raises a litter.

After year 5, the intervals began to lengthen, indicating that older animals came in season less frequently. Also, the standare deviations increased and then became fairly large, reflecting the fact that beagles tend to become irregular in their estrous cycles as they become older. Irregularities were less marked and so standard deviations were smaller in bred than in non-bred animals. Also, estrous intervals tended to be smaller in the bred animals than in the non-bred as they became older. This indicates that breeding may have a beneficial effect in maintaining regular estrous cycles in the aging animals. However, the breeding animals were selected on the basis of appearance, conformation, and

breeding capability and this was probably a contributing factor.

In both bred and non-bred animals estrous intervals were the longest and standard deviations were the largest in the 11th and 12th years, although the changes were less marked in the bred animals. After year 12, values returned to near those of the 8th to 10th years. Several factors seem to have been involved in producing this pattern. First, some of the longest lived animals in the colony (whose life spans have approached 17 years), were characterized by having fairly regular estrous cycles for as long as they lived. This indicates that there may be a correlation between longevity and the ability to maintain a regular estrous interval. Some other animals, with shorter life spans, would go into prolonged periods of anestrous and then resume normal estrous cycles. These long periods of anestrous occurred most often, but not always, during the 11th and 12th years. Also, some animals developed an erratic estrous pattern during the last few years of their lives and few of these lived beyond 13 years.

It will be noted that estrous was detected in individuals as old as 16 years, indicating that these animals may have some reproductive capacity for as long as they live.

Although only six pyometra cases were available for study, some trends seem to be evident. Estrous intervals were shorter, on the average, than those for normal animals. Standard deviations were fairly small, except during years 6 through 8, the time when most of the pyometra cases became clinically evident. Animals which developed pyometra more frequently had abnormally short estrous intervals than abnormally long intervals.

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Table 1

Relationship of Estrous Interval and Standard Deviation to Age in Non-Bred Toxicity Control Beagles

Age (years)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
No. of cycles	15	57	61	51	40	27	20	14	21	14	9	6	5	2	4	1
Mean Interval (days)	191	206	211	204	241	219	264	232	272	261	360	453	232	235	286	462
Std. Deviation (days)	24	53	50	38	122	76	180	89	82	55	205	319	114	28	143	0

Table 2

Relationship of Estrous Interval and Standard Deviation to Age in Aging Control Beagles (Breeding Colony)

Age (years)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
No. of cycles	16	40	43	48	41	42	28	23	25	21	23	11	11	5	5	1
Mean interval (days)	197	204	227	211	215	224	217	238	244	274	344	300	274	273	234	279
Std. Deviation (days)	41	55	49	46	69	58	72	70	85	117	159	121	81	53	105	0

Table 3

Relationship of Estrous Interval and Standard Deviation to Age in Known Pyometra Cases, both Bred and Non-Bred

Age (years)	1	2	3	4	5	6	7	8	9	10	11	12
No. of cycles	1	4	8	7	9	7	5	4	3	2	1	0
Mean Interval (days)	196	213	170	208	155	248	215	177	151	196	172	0
Std. Deviation (days)	0	16	46	38	32	175	79	104	10	50	0	0

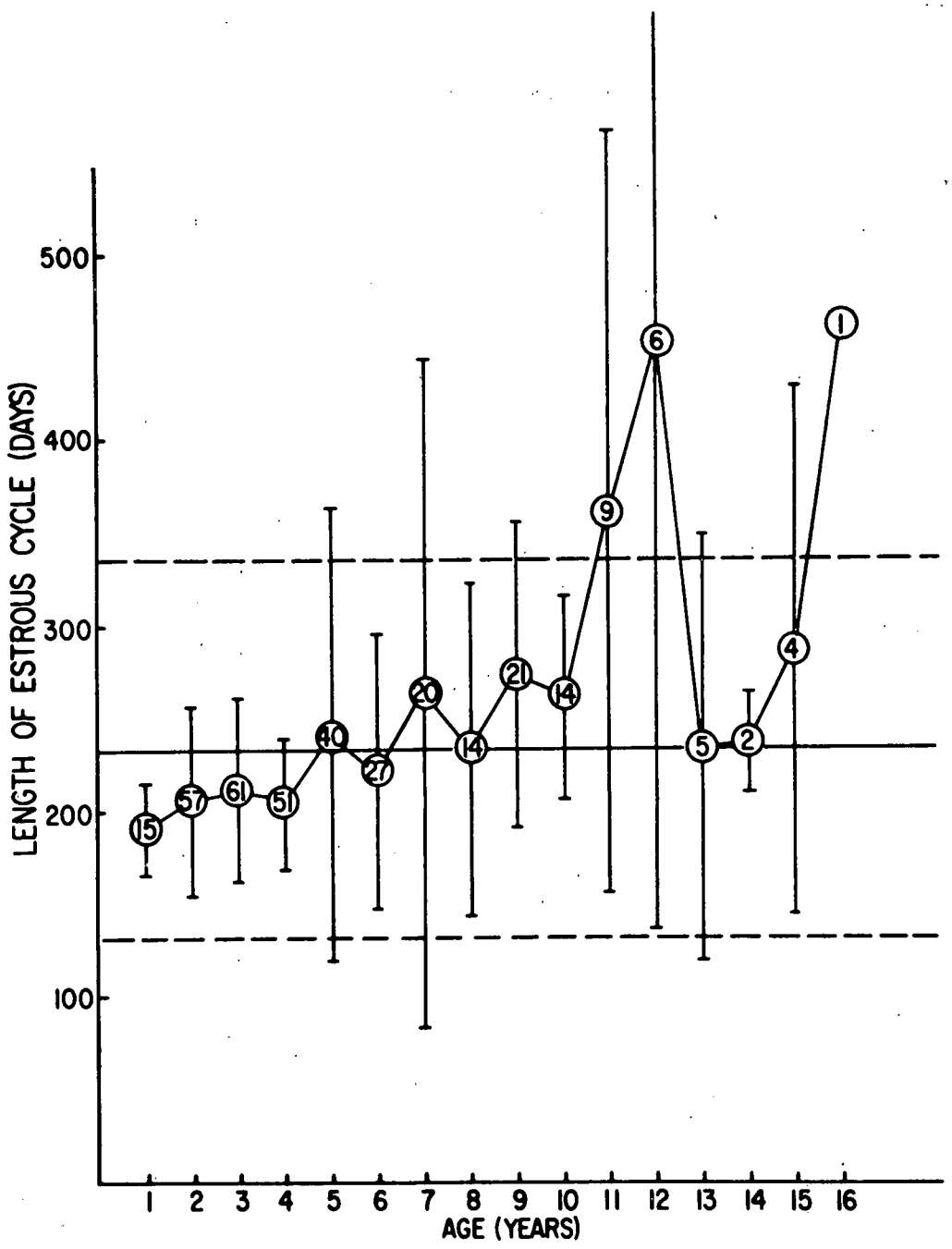

Figure 1. Relationship of estrous cycle to age in non-bred dogs. The number of cycles per age group is indicated by the encircled numbers and the standard deviation by the vertical bars. The horizontal lines indicate the mean and standard deviation of the mean.

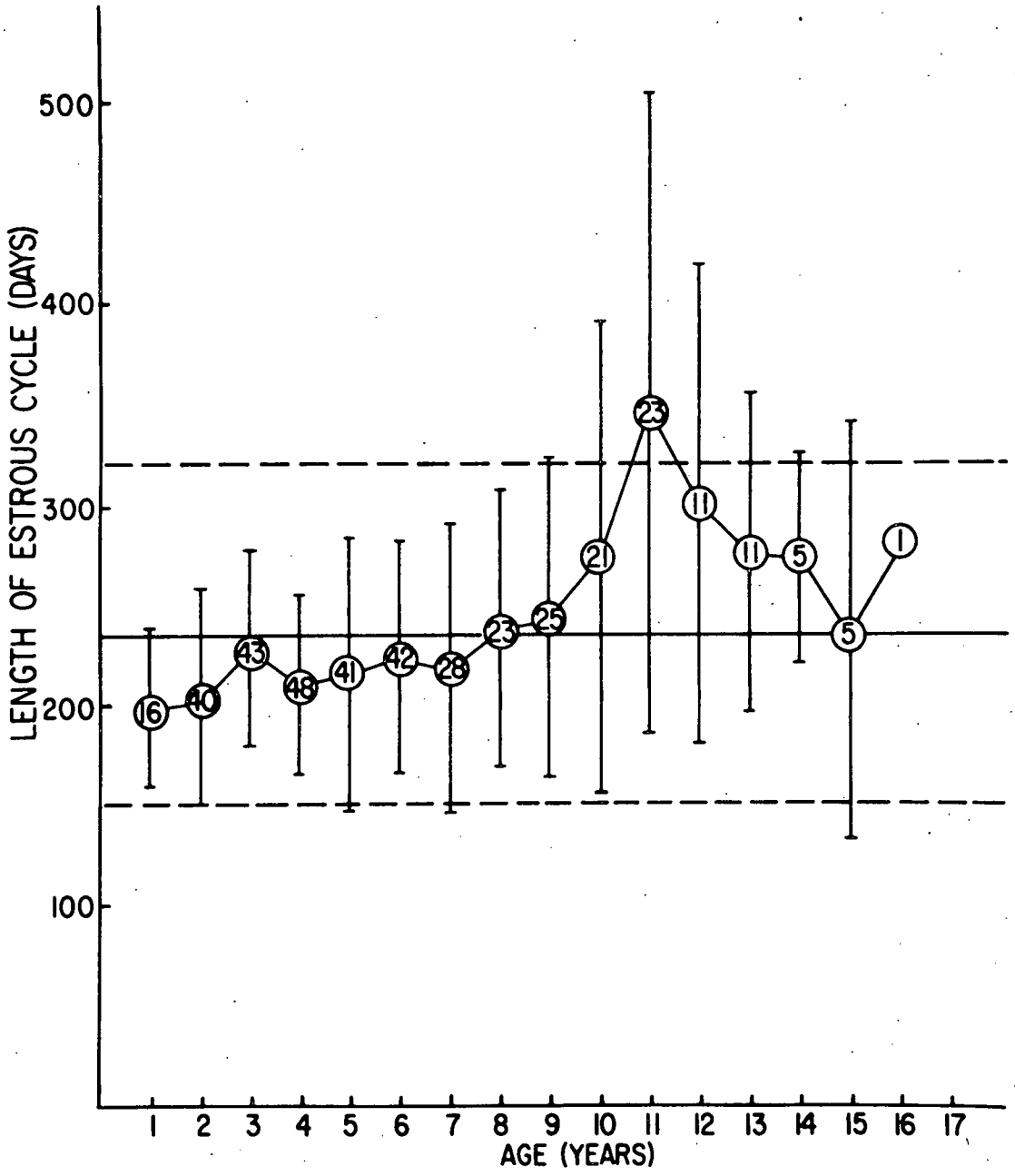

Figure 2. Relationship of estrous cycle to age in the breeding colony. The number of cycles per age group is indicated by the encircled numbers and the standard deviations by the vertical bars. The horizontal lines indicate the mean and standard deviation of the mean.

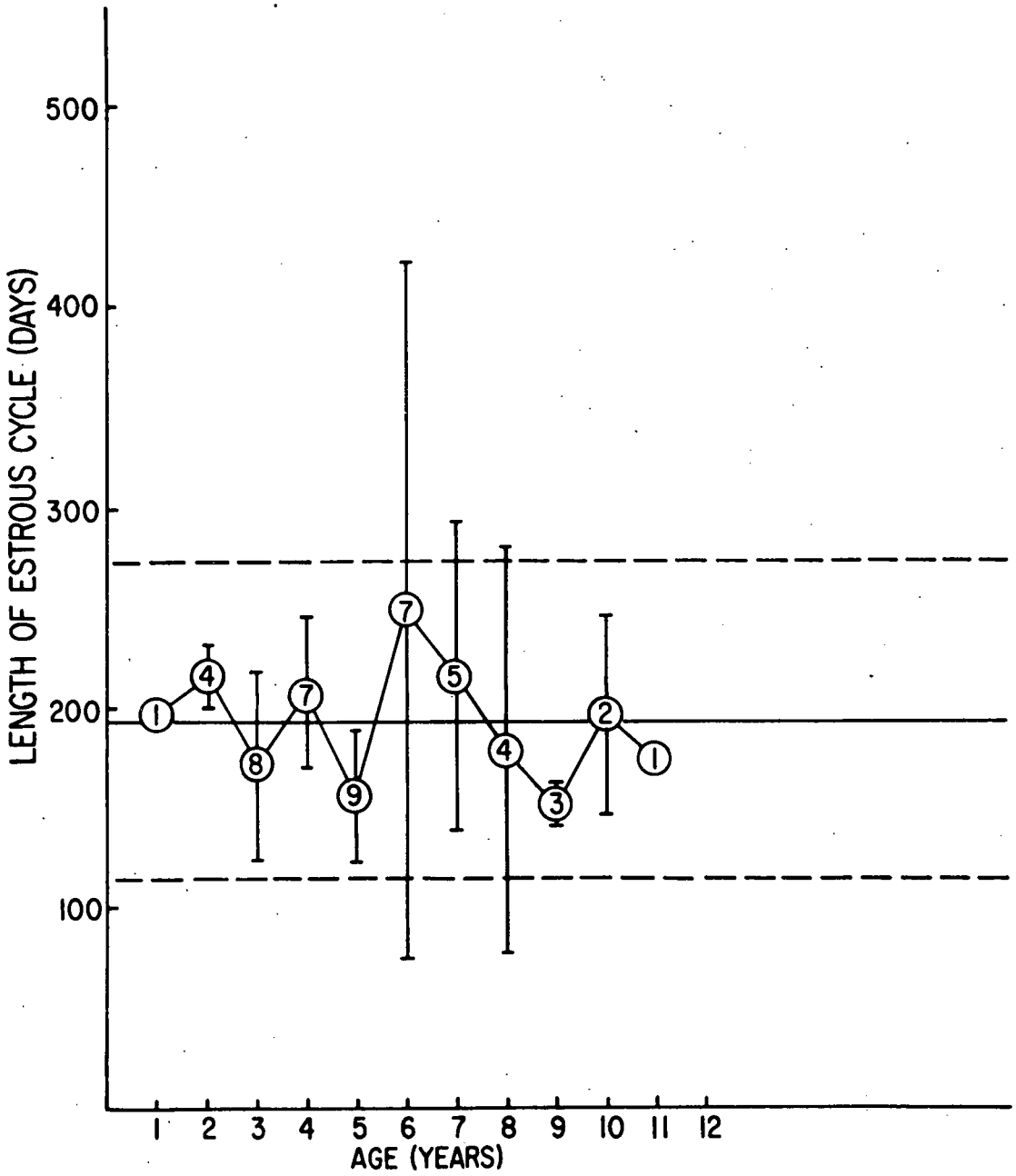

Figure 3. Relationship of estrous cycles in known pyometra cases to age. The number of cycles per age group is indicated by the encircled numbers and the standard deviation by the vertical bars. The horizontal lines indicate the mean and standard deviation of the mean.

IMPROVED METHODOLOGIES FOR DETERMINING LOCAL BONE DOSIMETRY

Webster S. S. Jee

Abstract: Improved methodologies for determining local bone dosimetry using the Becker-Johnson neutron-induced autoradiography and spark counting, the Johnson-Becker contact cellulose nitrate autoradiography and spark counting and the Jee-Miller detailed neutron-induced autoradiography are described. Lumbar vertebral bone section from a dog injected with 0.305 μCi of plutonium-239/kg and surviving for 40 days was 3.2 pCi/cm^2 and a section from a dog injected with 0.0148 μCi of plutonium-239/kg and surviving for 35 days was 0.22 pCi/cm^2.

Introduction

The conventional method of autoradiography by photographic nuclear track emulsion for determining the quantity and spatial distribution of plutonium in bone sections requires exposure times of several years (1, 2). With a combination of two techniques (3-5), (i) alpha-particle or fission fragment registration in thin polymer foils and (ii) automatic counting and magnification of the etched perforations by local evaporation of a thin metal layer with an electric spark (sparking) and automatic track counting with the quantitative television microscope, the sensitivity of the nuclear track emulsion technique for determining the distribution of plutonium can be drastically improved. Not only is valuable research time saved, but these methods eliminate the need to characterize the degree of fading or fogging of the emulsion due to long exposure intervals.

Neutron-induced autoradiography (fission fragment)

In cooperation with K. Becker and D. R. Johnson, Health Physics

Division, Oak Ridge National Laboratories (ORNL), the neutron-induced autoradiography (NIAR) techniques were utilized to determine the quantity and distribution of ^{239}Pu in lumbar vertebral bodies and distal femurs of Beagles from this project (5). A 7 micra third lumbar vertebral body section, mounted on a glass slide, was covered with a polycarbonate plastic film (Kimfol by Kimberly-Clark, Lee/Mass) 6 or 10 microns thick and exposed to thermal neutrons in the thermal column of the ORNL Bulk Sheilding Reactor. The thermal neutron fluence was $1.24 \times 10^{13} n/cm^3$. Submersion of the film in 60° C, 28% KOH for one hour etched pinholes several micra in diameter at the location of each fission fragment impact. After rinsing and drying the etched film it was placed on a circular brass electrode similar to that described in a paper to be published in Health Physics Sensitive automatic counting of alpha particle tracks in polymers and its applications in dentistry; D. R. Johnson, R. H. Boyett and K. Becker; Health Physics, in press) on the automatic counting of alpha particle tracks in polymers. The film was covered with a piece of aluminized Mylar with the aluminized side facing the etched film and making contact with an outer grounded electrode. When a positive voltage of 500 V was applied, sparks occurred through the perforation in the etched film and were coupled to a portable scaler through a quenching circuit. Each spark caused the evaporation of aluminum from the aluminized Mylar in an area several orders of magnitude larger than the original hole in the detector film. Therefore, multiple sparking occurred through individual holes and a plainly visible "replica" of the holes remained

in the aluminum layer.

Assuming an efficiency of 100% for fission track registration, bone samples from a dog injected with 0.305 μCi of plutonium-239/kg and surviving for 40 days was 3.2 pCi/cm^2, and a dog injected with 0.0148 μCi of plutonium-239 and surviving for 35 days was 0.22 pCi/cm^2.

Spark counting of tracks due to alpha particles and recoil nuclei in cellulose nitrate

In cooperation with D. R. Johnson of the Health Physics Division, Oak Ridge National Laboratory, a technique of cellulose nitrate alpha autoradiography was used to determine the quantity of plutonium-239 in bone sections (5). The procedure does not utilize exposure to a thermal neutron fluence. Bone sections are opposed to thin cellulose nitrate film. After proper exposure time, the film is etched in 5N KOH at 35-40°C, rinsed in running water, dried at 60°C and spark counted.

Detailed NIAR of Bone Sections

In cooperation with Lowell Miller, Material Testing Reactor Station (MTR), Arco, Idaho, the neutron-induced autoradiography technique was modified to produce Lexan film (polycarbonate plastics) with fission fragments from plutonium superimposed upon the bone image. The technique involved apposing thin bone sections upon the Lexan film and exposing the mounted film to the MTR neutron fluence. The etched film exhibited both a fission fragment image and a corresponding bone image, thus, permitting us to characterize

the distribution of the plutonium (Fig. 1). The quantity of the plutonium can be determined by both spark counting and by the QTM (Quantitative Television Microscope).

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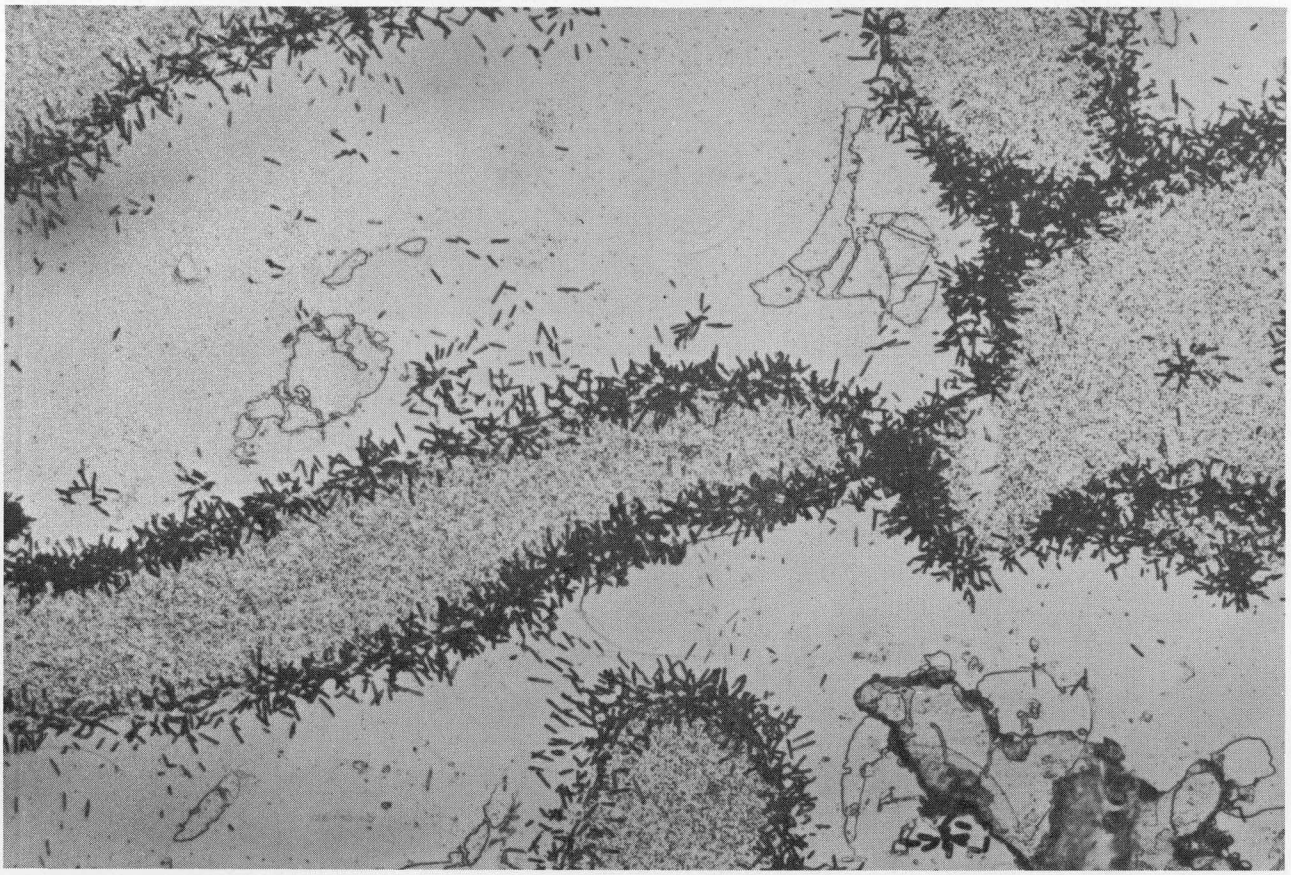

Figure 1. A detailed neutron induced autoradiograph of an area of the lumbar vertebral body from a Beagle (T52P4) injected with 2.73 μCi of ^{239}Pu/kg and surviving for 14 days. Note detailed image of the various trabeculae (dots) and the tracks of the fission fragments distributed on the bone surfaces. (X500).

PARATHYROID-CORTISOL RELATIONSHIP AS MEASURED BY PERIODONTAL LIGAMENT FIBROBLASTS LABELING INDICES

Gerald Julian, Han Z. Park, W. E. Roberts
and W. S. S. Jee

Abstract: In parathyroidectomized (PTX) rats treated with 0.5 mg of cortisol/kg the uptake of tritiated thymidine in stimulated periodontal ligament (PDL) fibroblasts was decreased by 50% as compared to those of PTX - sham injected and intact rats. The PTX rats given 250 units of parathyroid extract (PTE) exhibited labeling index (thymindine incorporation) higher than PTX rats. The stimulation of thymidine incorporation by PTE and the depression in thymidine incorporation by cortisol supports the hypothesis that the stimulation in DNA synthesis is induced by secondary hyperparathyroidism. On the other hand, cortisol possesses an action independent of the parathyroid gland in that 0.5 mg of cortisol/kg in PTX rats shortens the peak labeling time of PDL fibroblasts by 11 hours.

Introduction

It has been observed that osteoporosis develops with prolonged administration of cortisol. This has been generally thought to be caused by an anti-anabolic effect of cortisol (1). More recently, it has been proposed that the osteoporosis is caused by a secondary hyperparathyroidism triggered by cortisol (2, 3, 4). It has also been hypothesized that at low doses (0.5 mg/kg) of cortisol, the secondary hyperparathyroidism is predominant and causes the observed effect of a peak labeling index eleven hours sooner in the stimulated periodontal ligament (PDL) fibroblasts of rat molars when the rats have been treated with 0.5 mg/kg cortisol as compared to the untreated animals (5).

The present experiment was designed to test this hypothesis. By removing the parathyroid glands and subsequently administering

a low dose of cortisol, the effect of cortisol upon a rat without a parathyroid gland could be noted.

Methods and Materials

Sixty male Sprague-Dawley rats weighing 160 to 200 grams were divided into five groups of 12 each. Four of the groups were parathyroidectomized (PTX) and sustained on Purina rat chow and 2% calcium lactate water ad libitum. The operation was completed one week before the experiment. One group received five days' pre-treatment with 0.5 mg of cortisol/kg, another group was pretreated five days with sham injections of vehicle only (carboxymethylcellulose 0.5% in 9.0% saline). The third and fourth groups were pretreated one day with 250 units of parathyroid extract (PTE; Eli Lilly & Co.) and 125 units PTE/kg, respectively, each 12 hours. The last group was left intact with no PTX or injections. Injections were continued until sacrifice. To stimulate the periodontal ligament fibroblasts, Rocky Mountain Dental Products J-104 elastics were wedged between the upper right first and second molars. The contralateral side served as control. The procedure was performed between 9 and 11 a.m., and 8μCi of tritiated thymidine/g* was injected at 8, 16, 22, 27, 36 and 48 hours after the elastics were placed. The animals were sacrificed one hour after the thymidine injection. The upper jaw was removed and fixed 24 hours in buffered

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* The discussion section will explain why this particular dose was given.

2.5 glutaraldehyde and then decalcified three weeks in 10% EDTA The specimens were embedded in methylmethacrylate and sectioned at 3 to 4μ on the Jung microtome. Thirty-six sections from each side of the jaw were cut, mounted and dipped in Kodak NTB nuclear emulsion. The slides were exposed two weeks at 4°C and then developed in Kodak D-19 fine-grain developer. The slides were stained with Mayer's hematoxylin and eosin. Counts were made from the alveolar crest to the apex of the mexial buccal root of the maxillary first molar. Only cells labeled with five or more grains were counted. Each point on the graph equals two animals and about 10,000 counted cells.

Results

Fig. 1 shows the labeling indices of the molar ligament PDL cells that were mechanically stimulated by the elastic. The graph illustrates two parameters, the percentage of cells labeled and time after elastic placement at which this percentage was reached.

The PTX group receiving a supplemental dose of 250 units PTE peaked sooner and higher than the control group (PTX + sham injections). This is consistent with the reported effect of PTE (6). Although the intact animals reached a peak at the same time as the PTX + PTE groups, the response was not as high as the 250 unit PTE group but higher than the 125 unit PTE group. The endogenous secretion of parathyroid hormone (PTH) would seem to produce an effect between these doses.

PTX + PTE 250; PTX + PTE 125; and intact groups reached peak

labeling in 22 hours. The peak was not as great and was five hours quicker than in the study done by Roberts (5). The lower response may have been due to the 8μCi of tritiated thymidine/g. The usual dosage for a labeling index is 1μCi/g. Eight μCi/gm was used inadvertantly when a label for a previous shipment of tritiated thymidine was left in the box containing a new shipment that had eight times more specific activity. Dosage was computed on the basis of the old label. The possible effect would be a lowering of the response as demonstrated in other studies (7). The peaks were reached five hours sooner than reported by Roberts. Other bone cell studies showed variance due to the age of the rats (8). Liver regeneration autoradiographic results have shown variation due to cell types counted (9), and a periodontal ligament study (10) also varies from the work done by Roberts. The variance then might be due to age, cell or investigator. The main point, however, is that the relationship of one group to another is logical and consistent as related to internal controls; so this experiment would seem to be valid.

The PTX + 0.5 mg/kg cortisol group arrived at a peak labeling in 16 hours compared to the control group (PTX + sham injected) of 27 hours. It might be argued that this group's peak was merely cut off by the effect of cortisol in depressing thymidine uptake; and it would have peaked later, especially because the peak is only 1/3 to 1/2 the height of the others. This phenomenon would support the hypothesis of a secondary hyperparathyroidism particularly because the 0.5 mg/kg dose in intact animals (5) did not depress

the labeling index in comparison to the untreated animals. However, the rise in the rate of the cortisol group is abrupt in contrast to the flatter initial rates of all other groups. This would lead to the belief that a small stimulation due to cortisol itself is still present even in PTX animals.

The PTX - sham injected group was the last group to reach a maximum labeling at 27 hours after elastic placement. The lateness in comparison to the other groups is probably related to the lack of PTH and also due to intact adrenal glands secreting corticosteroids under the stress of sham injections.

Figure 2 is a graph of the contralateral side that was not subjected to mechanical stimulation. The lines are essentially straight and serve as base lines for the preceding graph.

The unstimulated side (Fig. 2) shows a relationship that supports the hypothesis of a secondary hyperparathyroidism. In all animals with either endogenous PTH or supplemental PTE, the baseline of labeling is significantly higher than the animals that received cortisol and no PTE. In intact animals, the baseline is higher for the low dose cortisol than in the untreated animals. The sham-injected PTX animals were also low in relation to the PTE groups.

Conclusions

In parathyroidectomized rats, 0.5 mg of cortisol/kg inhibits the uptake of tritiated thymidine by 1/2 as compared to PTX - sham injected animals. This result is different from what was previously

reported that an intact animal given this dose of cortisol showed a peak labeling identical to untreated rats but with an abbreviated peak labeling time (11 hours sooner). The finding supports the hypothesis that a low dose of cortisol induces a secondary hyperparathyroidism. On the other hand, a peak labeling occurs 11 hours sooner in the cortisol treated group and suggests a stimulatory effect independent of the parathyroid glands.

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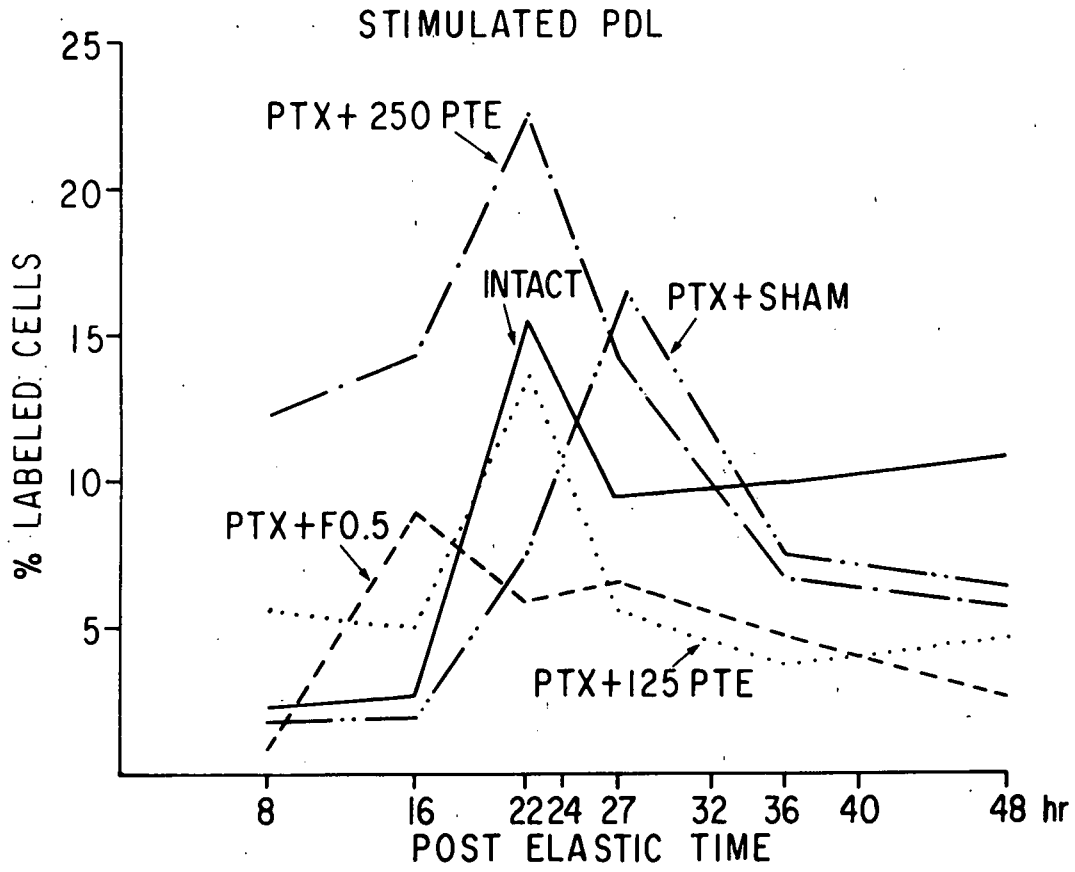

Figure 1. Curves showing the effect of parathyroid extract, cortisol and sham injections on the labeling index of rat periodontal ligament fibroblasts. The PDL was mechanically stimulated by wedging an elastic between the teeth. All animals were parathyroidectomized except for those labeled "intact".

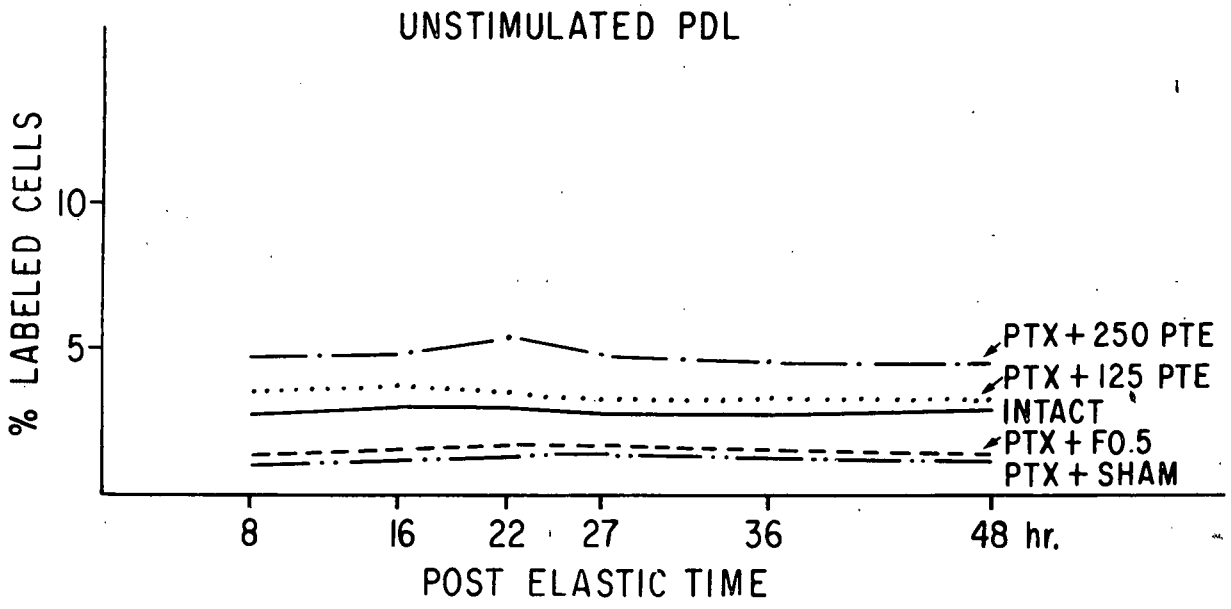

Figure 2. Curves of the contralateral side of the jaw where the periodontal ligament cells were not stimulated and are meant to serve as a baseline to Fig. 1 curves.

CORTISOL AND MINERAL TRANSPORT-EFFECT OF TIME AND GRADED DOSES UPON STRONTIUM-85 RETENTION IN YOUNG AND ADULT RATS

G. H. Kenner, W. S. S. Jee and H. Z. Park

Abstract: A whole-body counting study was done to investigate the long term effect of graded doses of cortisol on previously incorporated ^{85}Sr in the bones of 60 female Sprague-Dawley rats weighing about 200 g. The ^{85}Sr kinetics study showed statistically significant increases in the retention of radionuclide which appeared first in the group injected with 50 mg of cortisol/kg body weight/day on day 2 and gradually extended to the 20, 5 and 2 mg/kg groups by the end of the experiment (149 days). This was in contrast to the statistically significant decrease in ^{85}Sr retention which appeared at 58 days in the 0.6 mg/kg group. By 134 days this difference was non-significant and by 149 days the mean value was the same as that of the controls. Post-mortem examinations showed decreases in soft tissue weights at the 2 mg/kg level but failed to find any significant differences in either the amount of bone present in the tibial metaphysis or in the density of the femoral or humeral midshafts or the sixth lumbar vertebral body at day 150.

Introduction

In the past, most animal experiments have been done with a single high dose of cortisol and for too short a period of time. As a result, most of the data to date has been of an acute nature and contributes very little to our understanding of the chronic effects of cortisol. In an effort to correct this situation and at the same time to see if there is any relationship between the results of chronic and acute experiments, a long term dose-response study was done using rats.

A further purpose of this study was to find a simple and direct method of measuring mineral transport. The deep ^{85}Sr store model previously used by Talmage and his co-workers (31) was the method used. In this model, the animal is pretreated with ^{85}Sr which is

then used as a marker to monitor the removal of calcium from the body.

Materials and Methods

Sixty young female rats weighing about 200 gms were each given four injections of 2 μCi of high specific activity ^{85}Sr in 200 λ of 0.9% NaCl solution (International Chemical and Nuclear Corporation) over a 7 day period. The ^{85}Sr was allowed to enter the deep bone stores (diffuse component and buried hotspots) for two weeks, after which daily injections of placebo or 0.06, 0.2, 0.6, 5, 20 or 50 mg of cortisol per kilogram body weight were begun. The amount of radioactivity in the body of each animal was determined in the small iron room of the Radiobiology Division, Department of Anatomy, two hours before the first injection of cortisol. This was the day 0 count. Further whole body determinations were made at appropriate intervals until the experiment was terminated. The animals were counted between two 8 x 4 NaI crystals placed 20 cm apart, which were surrounded by 6 inch thick steel shielding. A duplicate of the ^{85}Sr injection in a 10 ml ampoule within a 1.8 cm thick lucite absorber was also counted as a standard. The analyzer was a 400 channel RIDL 34-12.

All surviving high level rats (5 mg and above) were sacrificed on day 30. The low level animals were sacrificed on day 150. An intramuscular injection of 25 mg of achromycin (Lederle Laboratories) per kilogram body weight was given four days before sacrifice to label areas of new bone formation.

At autopsy the animals were decapitated, and the blood and

right femur were collected for radioactivity determinations in the "Knothole" well counter. The right tibia was fixed in acetone, defatted, dehydrated, embedded in bioplastic and sectioned. The sections were ground to 100 microns for microradiographs and then reground to 50 microns and examined under a Leitz-Wetzlar U. V. microscope to determine the rate of endochondral bone formation. The microradiographs were examined with the Quantitative Television Microscope (Metal Products, Ltd., Cambridge, England) which gave an instantaneous direct reading of the percent bone in the metaphyseal area of the tibia. The left tibia was fixed in formalin and will be used for histology.

The densities of the midshafts of the right femur, left humerus and the 6th lumbar vertebral bodies were determined in the following manner: The ends were cut off the long bones, and the vertebral bodies were split longitudinally. Marrow and other debris was removed with a jet stream of water. The bones were placed in a beaker filled with 1% NaOH solution, allowed to stand overnight, rinsed, put in water and then placed in a vacuum dessicator for 45 minutes. They were then allowed to stand for 30 minutes to permit water to enter the spaces evacuated by air. The wet weight was determined by using a pair of forceps to place the bone on the immersed, looped end of a copper wire which was attached to the weighing arm of a Mettler balance. The bone was then jarred loose and the tare weight of the wire was determined. This procedure was repeated three times. The copper wire and forceps were kept scrupulously clean with acetone. The bones were dried at 100°F and weighed again. The density was calculated by dividing the dry weight

by the difference between the wet and dry weights.

The thymus, adrenals and uteri were collected and weighed at the end of the experiment while body weight was determined once or twice a week.

The mean standard deviation, standard error and t-values were calculated using the procedures given in Woolf (32). An Olivetti-Underwood Programma 101 computer was used for the actual calculations.

Percent whole body retention was determined with the following formula:

$$\% \text{ Retention} = \frac{W_t/S_t}{W_o/S_o}$$

where W_t = whole-body counting rate of the animal on day t
S_t = standard counting rate on day t
W_o = whole-body counting rate of the same animal on day 0
S_o = standard counting rate on day 0

Results

Treatment with cortisol resulted in a depression of mineral loss from the whole body in animals treated with high doses for a short period or low doses over an extended period of time relative to the saline treated controls. The results for the high dose level animals are summarized in Table 1 and Figure 1. Table 1 shows that possibly by 24 hours and definitely by 48 hours after the first injection of cortisol, significantly more ^{85}Sr is retained in the bodies of animals treated with 50 mg of cortisol per kg body weight than in the saline treated controls. Four and fifteen days following

the initial injection, the animals receiving 20 and 5 mg/kg also show greater retention. Table 2 and Figure 2 summarize the whole body data for the low dose level animals. For the first 39 days there was no difference in retention between the controls and any of the treated groups, but significantly more ^{85}Sr was retained by the 2 mg/kg group at 95 days.

The response of the group receiving 0.6 mg/kg merits closer scrutiny. On days 58, 95 and 112 significantly less radionuclide was retained by these animals than by saline treated controls. By day 134 the difference was not significant and by day 149 the 0.6 mg/kg animals retained the same relative amount of radionuclide as the controls. The dose of 0.6 mg/kg initially mobilized more radionuclide from the body and subsequently mobilized the same as the controls.

Strontium-85 retention by the humerus showed essentially the same trends as the day 149 whole body count but there was a difference in degree. The retention in the 0.6 mg/kg animals was still down even though the whole body values did not differ from the controls (Table 3). Also, unlike the whole body data, the 0.6 mg/kg group was significantly different while the 2 mg/kg group was not. No differences were detected in percent bone or endochondral bone growth rate in the third tibial metaphysis.

The terminal weight data of the low level animals for the body, uteri, thymi and adrenals (Table 4) show that significant responses appeared only in the 2 mg/kg group.

Table 5 shows that there was no increase in bone density in the femoral on humeral shaft or sixth lumbar vertebral body in

response to cortisol at day 150. The column for the humeral shaft is suggestive in that all treated densities are higher than those of the controls while the values for the 0.6 mg/kg group is nearly significant.

The activity in the blood was so low that it was impossible to quantify it.

Discussion

Cortisol demonstrates a time-dose relationship in its effects on mineral transport. High doses given for a short period and low doses given over a longer period of time depress the release of mineral from the body. On the other hand, low doses initially stimulate the loss of mineral.

Talmage (30) showed that the movement gradient of calcium is from the blood to the bone and postulated that an active transport mechanism is necessary to move minerals in the reverse direction. Park (24) and Belanger et al. (3) have demonstrated the importance of the osteocyte in mineral metabolism and theorized that it is the dominant cell of bone. A review of the literature shows that cortisol depresses active transport. Dougherty and co-workers (5,12,13,14,15, 28) have shown that cortisol causes a decrease in pinocytosis. Other workers have demonstrated decreases in calcium packaging and the synthesis of mucopolysaccharides, proteins, RNA, DNA and ATP (7,8,9,10,11,17,18,19,20,21,29,31). Furthermore, cortisol stimulates glycogen production (22,23).

The effect on the active transport of the osteocyte is enough to depress the rate at which mineral is removed from the body. In

addition, cortisol decreases the availability of ^{85}Sr for removal from bone. There is decreased recrystallization, increased pericanalicular changes and increased polymerization of the calcified matrix (2). Taken together, the changes result in a decrease in the rate at which mineral can move from the deeper regions of the bone to the surface.

The above result can be called the pharmacological response while the early reaction to 0.6 mg cortisol/kg is the physiological response. Man produces about 0.4 mg of cortisol/kg body weight/day (16). If this figure is extrapolated to rats, which are primarily corticosterone secretors, and if it is assumed that a dose of 0.6 mg/kg is enough to shut off most of the endogenous corticosteriod production, then we are studying a condition where the animal's body is continuously subjected to slightly elevated levels of drug. Any changes which occur should provide a clue to the action of cortisol on the normally functioning body. Under these conditions cortisol stimulates the release of previously incorporated mineral.

Even low doses can eventually decrease mineral release. This is probably compensated for at first by the body's capacity to adapt to a changing internal environment and by its ability to repair itself. Berliner (4) has shown that cells have the ability to adapt to the presence of corticosteroids by increasing the rate of biotransformation of the hormones to relatively innocuous metabolites. He hypothesizes that there is also a change in membrane permeability which impedes the ability of corticosteroid molecules to get to active sites. Whatever the system, like all such

mechanisms, it is possible to overload it. The result, which is initially a physiological situation, becomes pharmacological. This is precisely what has happened in this study. By day 150, the rate of loss of mineral from the body of an animal treated with 0.6 mg/kg, has been impeded to such a high degree that the total retention value for the treated animal is the same as that of the controls.

Arnold et al. (1) have shown that there is increased mineral density in the lumbar vertebrae of osteoporotic and Cushingoid patients. A possible explanation of this increase is due to the decreased release of mineral which is caused by cortisol. In this respect, the trend seen in the humerus at the 0.6 mg/kg level is interesting.

Graph 2 shows a break which occurs in strontium released at 59 days. A possible explanation is that due to the centrifugal cortical growth, the inside diameter of the long bones at 59 days is greater than their outside diameter at the time the last ^{85}Sr injection was given. This would eliminate a major reservoir of radionuclide since the only radionuclide found in the cortex of the long bones after 59 days would be that which has redeposited from the blood at some time following the final strontium injection. This may account for why the ^{85}Sr retention by the humerus does not completely reflect the whole body values.

Bhatti et al. (6) pretreated a group of young beagles with ^{45}Ca, waited two months and then showed that a dose of 2 mg of 6-methylprednisolone/kg/day depressed the loss of mineral from the terminal phalanges. Schafer et al. (27) showed that cortisol has a direct effect on newborn mouse bones. When treated in a culture

system, there was a depression of release of previously incorporated ^{45}Ca from the bone to the media. Talmage, Park and Jee (31) have shown that 5 mg of cortisol/kg depresses the levels of ^{45}Ca found in lavage fluids of rats which had been pretreated two weeks previously with ^{45}Ca.

Park et al. (25) have shown in an experiment using hypophysectomized rats that cortisol depresses bone resorption at moderate dose levels. Conversely, Roberts (26) has shown that 0.5 mg of cortisol/kg/day shortened the peak labeling time of stimulated periodontal ligament cells and caused a shift towards osteoclasts on the unstimulated periodontal ligament cells. High doses lengthened the time and reduced the number of cells which were capable of becoming osteoclasts.

Conclusions

Cortisol demonstrates a time-dose relationship in its effects on mineral transport. High doses for a short period and low doses given over a long period of time depress the release of mineral from bone. On the other hand, low doses show a biphasic effect. Initially, there is a stimulation of mineral loss which is followed eventually by a depression.

Acknowledgements

The counting system used for measuring whole-body ^{85}Sr retention in rats was designed with the help of R. D. Lloyd, C. W. Mays, and W. W. Wagner who also provided guidance in acquisition and analysis of counting data.

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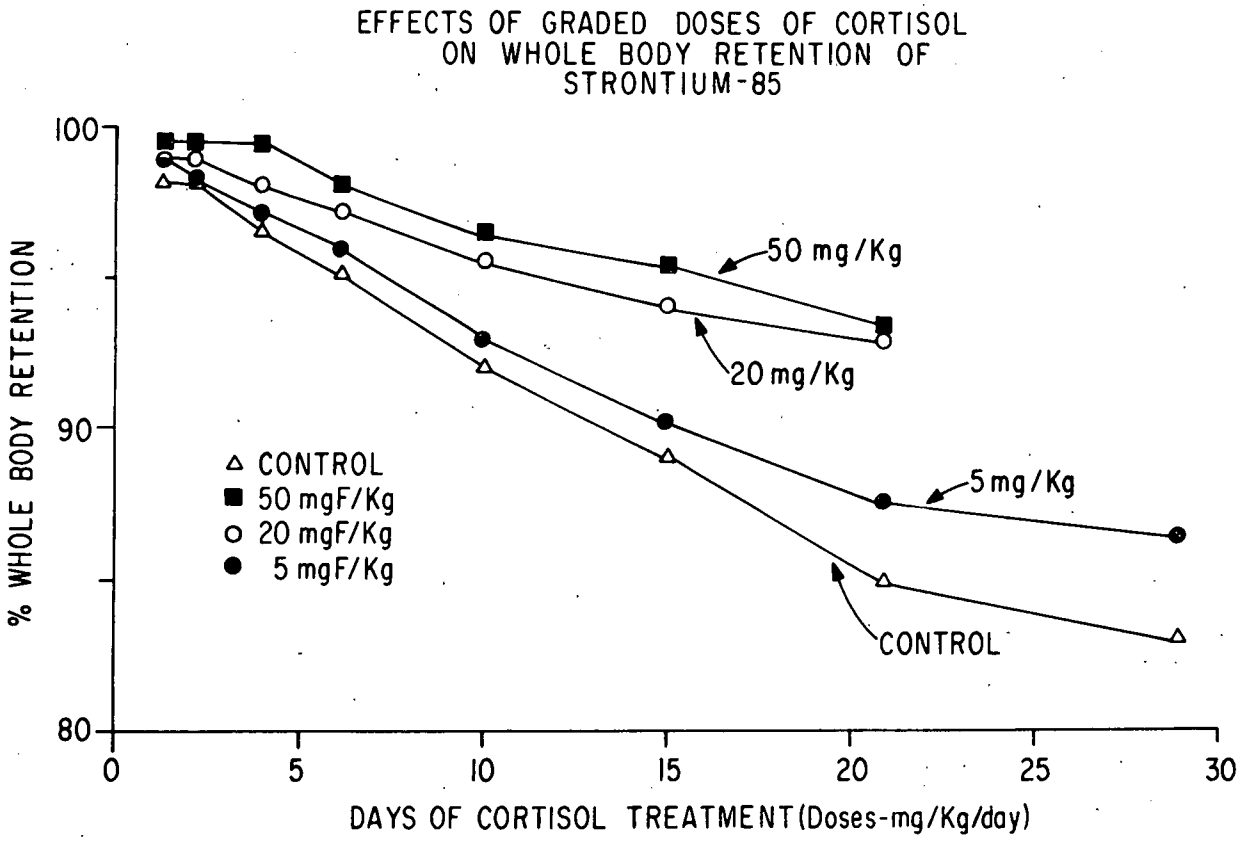

Figure 1. Cortisol depresses the release of mineral from the body of the rat. Animals treated with 50, 20 and 5 mg F/kg/day, retain more ^{85}Sr than the controls. Notice the effect of time.

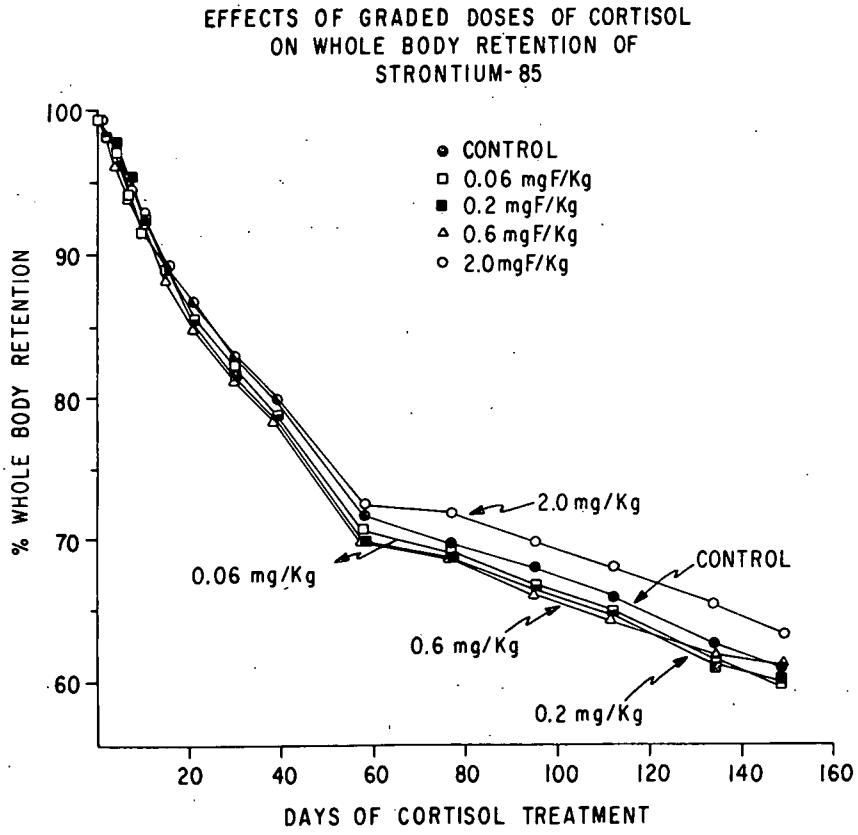

Figure 2. By day 95, animals treated with 2 mg F/kg/day also retain more. Notice the break in the slope of the line which occurs at 39 days.

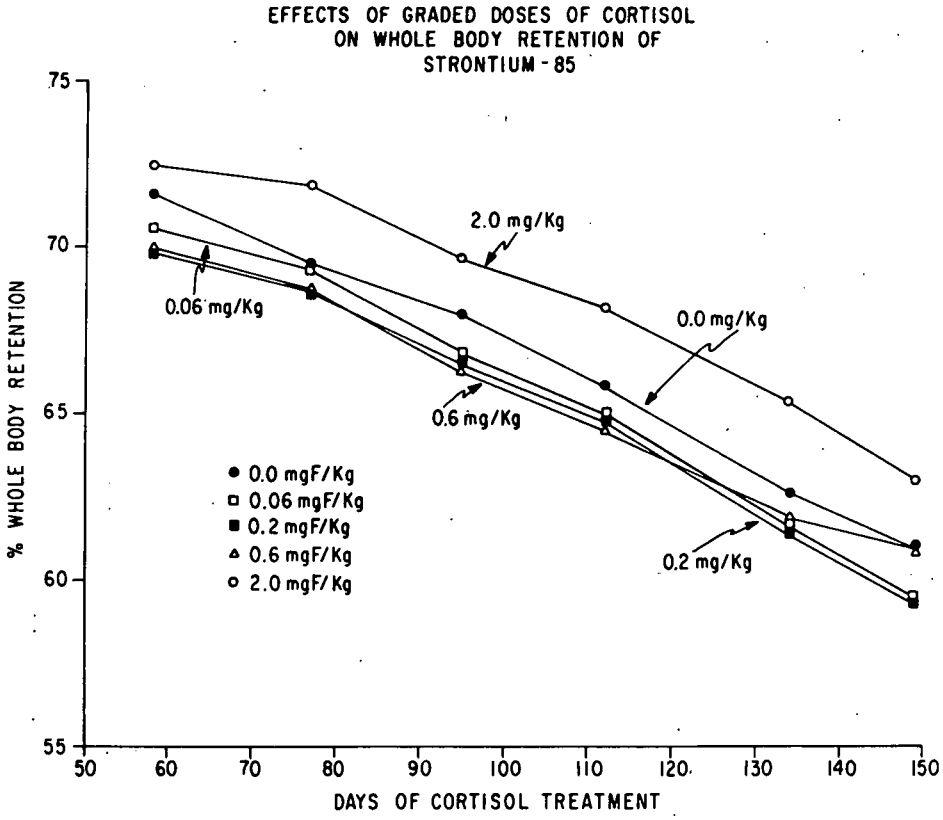

Figure 3. An enlargement of the right half of Figure 2. Note that at 59 days, animals treated with 0.6 mg F/kg/day retain less ^{85}Sr than the controls while at day 149, the values are the same.

Table 1

Whole Body Retention of Strontium-85 in Young Rats Treated with Graded Doses of Cortisol

Time	Controls $\bar{x}$ ± S.D.	5 mg F/kg $\bar{x}$ ± S.D.	20 mg F/kg $\bar{x}$ ± S.D.	50 mg F/kg $\bar{x}$ ± S.D.
Day 1	98.2 ± 0.7	99.5 ± 0.8	99.5 ± 1.0	99.5 ± 1.0
t-value		(3.70)*	(2.96)*	(3.28)*
Day 2	98.4 ± 1.4	98.4 ± 0.7	99.3 ± 1.2	99.6 ± 0.4
t-value		(0.00)	(1.27)	(2.17)*
Day 4	96.6 ± 0.8	97.1 ± 1.1	98.1 1.4	99.6 ± 1.2
t-value		(1.04)	(2.72)*	(6.24)*
Day 6	95.1 ± 1.3	95.8 ± 1.0	97.4 ± 0.9	98.6 ± 0.7
t-value		(1.17)	(4.06)*	(6.65)*
Day 10	91.6 ± 1.0	92.5 ± 1.2	95.5 ± 1.2	96.3 ± 1.1
t-value		(1.62)	(6.86)*	(8.82)*
Day 15	88.9 ± 1.0	90.2 ± 0.7	94.0 ± 1.1	95.7 ± 1.7
t-value		(2.88)*	(9.70)*	(9.30)*
Day 21	84.8 ± 0.6	87.4 ± 1.3	92.7 ± 1.0	93.4 ± 1.5
t-value		(5.29)*	(18.77)*	(14.28)*
n	9	7	7	7

Values given are mean and standard deviation.

*Significant at 0.05 level

Table 1. Data on which Figure 1 is based. Notice the time dose relationship in the response of rats to cortisol. The 50 mg F/kg group retains significantly more by day 1, the 20 mg F/kg group by day 4 and the 5 mg F/kg group by day 15.

Table 2

Whole Body Retention of Strontium-85 in Young Rats Treated with Graded Doses of Cortisol

Time	Controls $\bar{x}$ ±S.D.	0.06 mg F/kg $\bar{x}$± S.D.	0.2 mg F/kg $\bar{x}$ ± S.S.	0.6 mg F/kg $\bar{x}$ ± S.D.	2 mg F/kg $\bar{x}$ ± S.D.
Day 1	99.7±0.4	99.1±0.9	99.3±0.7	98.9±1.1	98.9±0.4
t-value		(1.29)	(1.16)	(1.38)	(3.22)*
Day 4	97.9±1.0	97.2±0.6	98.0±1.0	96.1±2.4	98.0±0.7
t-value		(1.47)	(0.08)	(1.59)	(0.09)
Day 10	92.9±0.7	91.7±0.8	92.6±1.2	91.9±1.4	92.8±1.2
t-value		(2.08)	(0.80)	(1.81)	(0.50)
Day 21	86.8±1.8	85.6±1.2	85.1±0.8	84.8±1.5	86.5±1.5
t-value		(1.34)	(1.85)	(1.88)	(0.16)
Day 39	79.6±0.8	78.7±0.9	87.6±1.4	78.1±1.6	80.0±1.4
t-value		(1.64)	(1.41)	(1.77)	(0.72)
Day 58	71.6±1.0	70.6±1.2	69.8±1.8	69.9±1.2	72.5±1.6
t-value		(1.48)	(1.94)	(2.40)*	(-1.14)
Day 95	68.0±0.5	66.8±1.3	66.5±2.1	66.3±1.3	69.7±1.4
t-value		(1.82)	(1.50)	(2.73)*	(-2.53)*
Day 112	65.9±0.6	65.0±1.2	64.8±1.7	64.5±1.1	68.2±1.6
t-value		(1.61)	(1.43)	(2.51)*	(-3.02)*
Day 134	62.7±1.4	61.2±1.3	61.4±1.3	61.9±1.1	65.4±0.9
t-value		(0.53)	(1.73)	(1.14)	(-3.96)*
Day 149	60.8±1.7	59.4±1.7	59.7±1.8	60.9±3.6	63.2±0.9
t-value		(1.44)	(1.11)	(-0.06)	(-3.07)*
n	6	6	6	6	6

Values given are mean and standard deviation

* Significant at 0.05 level

Table 2. Data on which Figs. 2 and 3 are based. The 2 mg F/kg group is significant by day 95. At day 58, the 0.6 mg F/kg animals retained significantly less than the controls. By day

149, this result has been reversed to the point that the 0.6 mg/kg group and the controls retained similar amounts of strontium-85.

Table 3

Effect of 150 Days of Cortisol Treatment Upon the Terminal Body Weight, Percent Bone and Endochondral Growth Rate in the Tibial Metaphysis and the Percent Retention of Strontium-85 by the Humerus in Young Rats

Treatment	n	Percent Bone	Endochondral Growth (mμ/day)	Humerus Retention
Control	6	24.9 ± 4.9	4.4 ±0.33	0.15 ± 0.003
0.06 mg/kg	6	23.5 ± 4.1	4.2 ± 0.17	0.14 ± 0.004
t-value		(0.221)	(0.775)	(2.460)*
0.2 mg/kg	6	29.5 ± 4.1	4.3 ± 0.11	0.14 ± 0.008
t-value		(-0.736)	(0.326)	(0.866)
0.6 mg/kg	6	20.3 ± 3.8	4.3 ± 0.11	0.13 ± 0.003
t-value		(0.737)	(0.602)	(6.128)*
2.0 mg/kg	6	27.9 ± 5.1	4.2 ± 0.17	0.15 ± 0.004
t-value		(-0.432)	(0.775)	(-1.773)

All values are mean and standard error

* Significant at 0.05 level

Table 3. Some terminal data. Note that the humerus retention approximates but does not duplicate the day 149 whole body retention data.

Table 4

Soft Tissue Weights of Young Rats after 150 Days of Cortisol Treatment

Treatment	Uterus	Thymus	Adrenals	Terminal Weight
Controls	0.81 ± 0.13	0.32 ± 0.01	0.066 ± 0.004	289 ± 6
0.06 mg/kg	0.76 ± 0.11	0.29 ± 0.04	0.066 ± 0.004	296 ± 8
t-value	(0.316)	(0.726)	(-0.158)	(-0.665)
0.2 mg/kg	0.64 ± 0.04	0.28 ± 0.03	0.068 ± 0.006	281 ± 9
t-value	(1.240)	(1.288)	(-0.317)	(0.733)
0.6 mg/kg	0.69 ± 0.04	0.29 ± 0.04	0.067 ± 0.004	285 ± 12
t-value	(0.875)	(0.923)	(-0.230)	(0.288)
2.0 mg/kg	0.54 ± 0.03	0.19 ± 0.01	0.052 ± 0.003	261 ± 5
t-value	(2.023)	(7.517)*	(3.165)*	(3.730)*

All values are mean and standard error in grams

* Significant at 0.05 level

Table 4. Only the 2 mg F/kg group differs significantly from the controls.

Table 5

Density of Air-Dried Young Rat Bones After 150 Days of Cortisol Treatment

Treatment	Femur	Humerus	Vertebrae
Controls	2.49 ± 0.02	2.52 ± 0.01	2.24 ± 0.06
0.06 mg/kg	2.50 ± 0.01	2.61 ± 0.06	2.19 ± 0.10
t-value	(-0.371)	(-1.889)	(0.523)
0.2 mg/kg	2.50 ± 0.03	2.55 ± 0.02	2.15 ± 0.09
t-value	(-.065)	(-1.205)	(0.975)
0.6 mg/kg	2.48 ± 0.01	2.61 ± 0.04	2.24 ± 0.09
t-value	(0.620)	(-2.080)	(.057)
2.0 mg/kg	2.49 ± 0.02	2.54 ± 0.02	2.26 ± 0.08
t-value	(-0.039)	(-1.064)	(-0.142)

All values are mean and standard error in grans air-dried weight per cubic centimeter.

Table 5. Cortisol did not cause any changes in density. Note the value 0.6 mg F/kg humerus.

DIURNAL RHYTHM IN LABELING INDICES OF RAT PERIODONTIUM LIGAMENT FIBROBLAST

W. E. Roberts, L. I. McKay, H. Z. Park and W. S. S. Jee

Abstract: Incorporation of tritiated thymidine (labeling index) in unstimulated and stimulated rat periodontal ligament (PDL) fibroblasts varied as a function of time of day. Highest levels of thymidine labeling of unstimulated PDL fibroblasts were found at 9 a. m. (2.19%) and 3 p. m. (1.85%). Lower values were observed at 3 a. m. (1.41%) and 9 p. m. (1.02%). For stimulated PDL fibroblasts, highest peak labeling was observed when the rubber bands were inserted at 9 a. m. Lower peak labeling occurred at 3 p. m. and 3 a. m. When the rubber bands were inserted at 9 p. m., not only was the incorporation of thymidine depressed but the peak labeling time post elastic was delayed somewhat. These data supply ample evidence that the diurnal variation affects the incorporation of thymidine in PDL fibroblasts, and that possibly this is mediated by the adrenal cortex.

Introduction

A diurnal variation in activity of the adrenal cortex has been shown to occur in rats (1, 2). Recently it has been shown that cortisol influences the thymidine incorporation (labeling index) in rat periodontal ligament (PDL) fibroblasts (3, 4). It was deemed advisable in conjunction with our studies on the effects of corticoid upon bone cells to examine the possibility of such a diurnal variation in the responses of the PDL fibroblasts. Furthermore, we were interested whether the endogenous secretion of corticosteroid influenced the capacity of PDL fibroblasts to incorporate thymidine.

Materials and Methods

The influence of diurnal variation upon both the unstimulated and stimulated rat PDL fibroblasts was studied. All animals were preconditioned for three days in a minimum stress environment with

the lights on at 6 a. m. and off at 6 p. m. For the unstimulated PDL fibroblast studies, 32 male 200 g Sprague-Dawley rats were used. Two animals were sacrificed at 3 a. m., 9 a. m., 3 p. m. or 9 p. m. and each animal at one hour prior to sacrifice was injected with 1μCi of tritiated thymidine/g of rat. At sacrifice the maxilla was removed, separated at the mid-palatal suture and fixed in 2.5% glutaraldehyde buffered at pH 7.4. Following a 24 hour fixation, tissues were decalcified in 10% Versene buffered to pH 7 with glacial acetic acid. The decalcified specimens were embedded according to a modified methyl methacrylate technique of Cathey (5), sectioned at 3 micra on a Jung microtome, and mounted on 0.5% gelatinized slides. Autoradiographs were prepared with Kodak's NTB liquid emulsion using the technique described by Arnold (6) and Fabrikant (7).

The labeling index of the unstimulated PDL fibroblasts (% thymidine labeled fibroblasts) were determined from the fibroblasts located in the periodontal ligament from the alveolar crest to the apex of the mesial root of the maxillary first molar. Three sections were counted for each animal for a sample size exceeding 2,000 fibroblasts.

For the stimulated PDL studies, 24 rats were divided into four groups. Each group had light elastic placed between the maxillary first and second molars at 3 a. m., 9 a. m., 3 p. m. or 9 p. m. Two rats per group were sacrificed at 16, 27 or 36 hours post elastics. One hour prior to sacrifice, each rat received 1μCi of tritiated thymidine/g. The same procedures were used to determine

the labeling indices of the stimulated PDL fibroblasts.

Results

Table 1 shows the labeling indices of unstimulated PDL fibroblasts at various time intervals. At 9 p. m. and 3 a. m. the labeling indices are only 1.02 ± 0.30% and 1.41 ± 0.04% respectively. These values are far below the labeling indices of 2.19 ± 0.13% at 9 a. m. and 1.85 ± 0.07% at 3 p. m.

Table 1

Labeling Indices of Unstimulated PDL Fibroblasts in Male Rats at Various Time Intervals

	3 a. m.	9 a. m.	3 p. m.	9 p. m.
Rat 1-R	1.38%	2.36%	1.64%	0.70%
L	1.32	2.39	2.00	1.93
Rat 2-R	1.46	1.82	1.89	0.73
L	1.50	2.22	1.88	0.74
Mean ± S.E.	1.41±0.04	2.19±0.13	1.85±0.07	1.02±0.30

Figure 1 summarizes the results of the stimulated PDL fibroblasts studies. Placing the elastics in at 9 a. m. to stimulate the PDL fibroblasts yields a peak labeling index of 25% at 27 hours post elastics. When the elastics are placed at 3 p. m. and 3. a. m. the labeling indices rose to peak labeling of approximately 15% at 27 hours post elastics. In the group where the elastics are placed at 9 p. m., the peak labeling index is 15% at 36 hours post elastics

and the peak labeling response occurring some 9 hours later.

Discussion

Corticosterone was well known as the glucocorticoid of physiological significance in the rat (2). Recently, Bartley et. al., demonstrated that this corticoid was 1/4 to 1/2 times as effective as cortisol in suppressing bone accretion in the rabbit (8). It was assumed in this study that corticosterone is as effective in the rat. With the findings that the highest level of plasma corticosterone was detected between 9 and 10 p. m., the next highest between 2 and 3 p. m., followed by the levels between 9 and 10 a. m. and between 2 and 3 a. m. (2) and that low doses of cortisol stimulated and high doses of cortisol depressed thymidine incorporation in rat PDL fibroblast (3, 4), it was not too surprising to note in the data reported here the highest level of thymidine incorporation occurred in the unstimulated PDL fibroblasts at 9 a. m. and 3 p. m. and much lower values at 9 p. m. and 3 a. m. These values were almost mirror image of the level of plasma corticosterone in male rats (Fig. 2). In other words, high plasma corticosterone suppressed thymidine incorporation and vice versa. Unfortunately, the labeling indices were about 6 hours out of synchrony with the reported levels of plasma corticosterone.

Quite surprisingly, the endogenous secretions at times other than 9 a. m. were sufficient to depress the peak labeling indices of stimulated PDL fibroblasts by 40%. Previously, an identical depression of thymidine incorporation in stimulated PDL fibroblasts was obtained after 2.5 mg of cortisol/kg (3). Moreover, the group

of rats stimulated at 9 p. m. showed a delayed peak labeling time of some 9 hours. This was identical to the response reported by Roberts for 5 mg of cortisol/kg (3).

It is most difficult to explain the lower thymidine incorporation level at 3 a. m. when it corresponds to the time of the lowest level of plasma corticosteroid. Possibly at 9 a. m. the plasma corticosteroid is optimal for thymidine incorporation, while at 3 a. m. the plasma corticosteroid level is insufficient to trigger the same response. On the other hand, it may be that at 3 a. m. the fibroblasts were still recovering from the after effects (depression of thymidine incorporation) of the high 9 p. m. dose of plasma corticoid. The PDL fibroblasts conceivably did not recover until 9 a.m., and at 3 p.m. the fibroblasts were again subjected to high plasma corticoid levels. This can also explain why the unstimulated PDL fibroblasts labeling indices levels do not exactly fit the plasma corticoid secretion levels. Even though the plasma level is low at 3 a.m., the fibroblast may be first getting over the effects of the high 9 p.m. secretion and did not fully recover until 9 a.m. At 3 p.m. and 9 p.m., they were again subjected to high plasma corticoid levels.

Other factors such as the diurnal effects upon parathyroid and androgen secretion may play an important role upon thymidine incorporation in PDL fibroblasts. Recently, parathyroid extract and parathyroid hormone have been shown to stimulate thymidine uptake in these same cells (9, 19).

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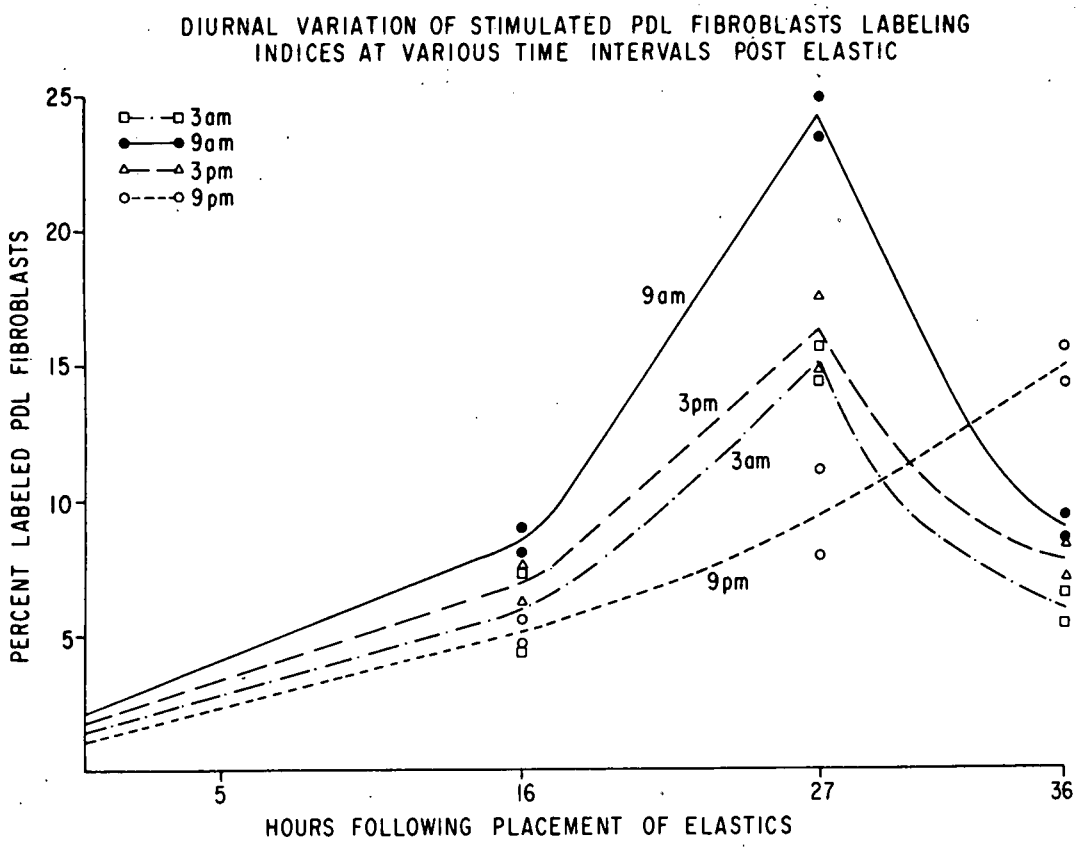

Figure 1. Diurnal variation of stimulated PDL fibroblasts labeling indices at various time intervals post elastic.

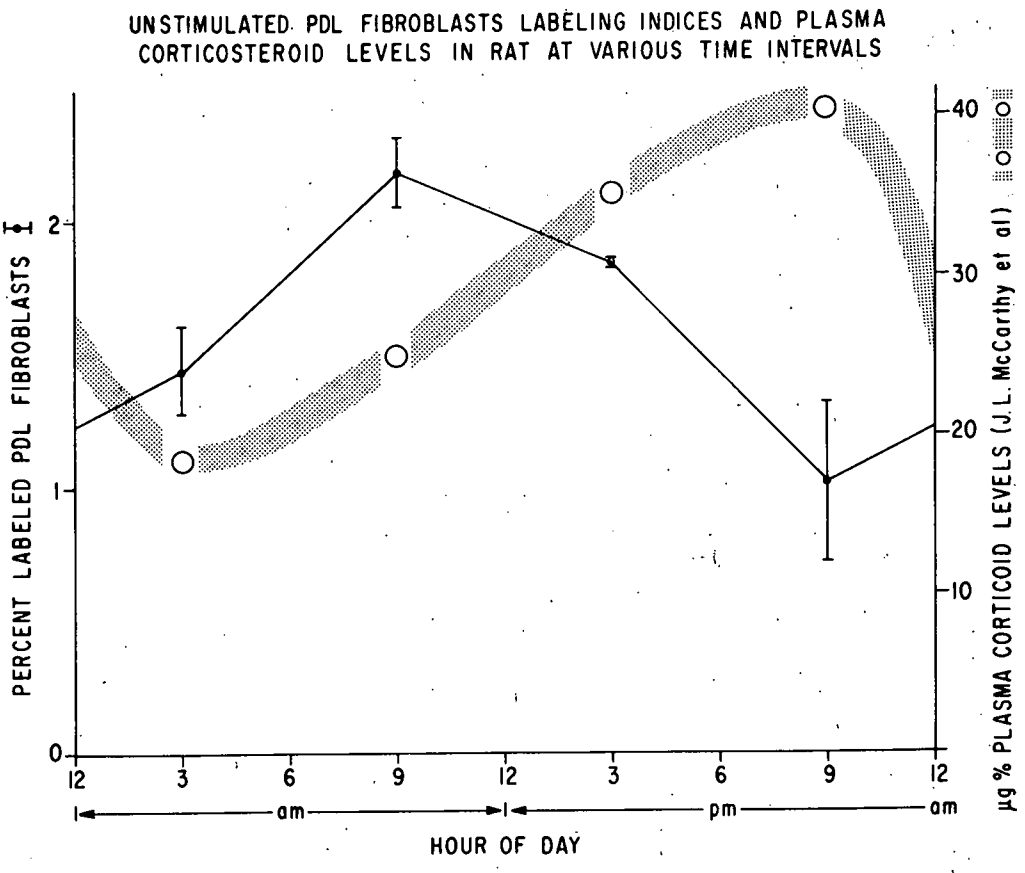

Figure 2. Comparison of the unstimulated PDL fibroblasts labeling indices and plasma corticosteroid in rats at various time intervals as reported by McCarthy et. al.

(2) Horizontal bars signify standard error of means.

CORTICOSTEROID AND BONES

Webster S. S. Jee, Han Z. Park, Wilbur E. Roberts,
Edward L. Blackwood and Gerry H. Kenner

Abstract: Cortisol administration enhanced the reduction in bone volume by: (1) increasing progenitor cell proliferation at low dose levels, (2) decreasing progenitor cell proliferation at high dose levels, (3) shortening the time interval to mobilize cells to enter the cell cycle at low dose levels, (4) delaying the time interval to mobilize cells to enter the cell cycle at high dose levels, (5) inhibiting osteoblastic differentiation and (6) enhancing osteoclastic differentiation. Low doses were more effective in reducing bone volume than high doses by making more progenitor cells available for differentiation and speeding up the mobilization rate of cells to go into the cell cycle.

Cortisol administration altered the ^{85}Sr kinetics in bone and teeth by: (1) enhancing the surface ^{85}Sr store exchange, (2) decreasing the efflux of deep ^{85}Sr store in bone and (3) enhancing the efflux of deep ^{85}Sr store in teeth. The decreased efflux of the deep mineral store of bone by cortisol coupled with the well established observation that cortisol inhibits bone formation and thus the formation of new crystals may partially contribute to the observed increase in bone density after cortisol.

Introduction

The main purpose of the present review is to discuss those events occurring after the administration of various dose levels of corticosteroid (cortisol) which are relevant to the decay of osseous tissues (i.e, reduction in bone volume and increases in bone density). Bone volume is constant if the rates of apposition and resorption are equal. Bone density is constant (mass of Ca/unit volume) if the influx and efflux of calcium are equal. Thus, one of the objectives of this review is to clarify how corticosteroid reduces bone volume and increases bone density.

Often there is a dichotomy of effects of cortisol which can be attributed to dose and time. Thus, another objective of this review is to emphasize the necessity to characterize the dose responses of hard tissues to cortisol.

Experimental Models

Surprisingly, we found that the experimental models yielding the most meaningful data were often the most simple. These models were mostly chronic studies in which dose and treatment intervals varied. The models include: (1) The Rat Unstimulated Periodontal Ligament (PDL) Model: A bone resorption model to study the responses of the alveolar bone and the PDL cell of the mesial surface of the mesial root of the maxillary first molar (Roberts, '68, '69; Roberts and Jee, '70). Bone resorption, labeling index of PDL fibroblasts, PDL cell populations and bone cell differentiation are measured in rats pretreated for five days with cortisol. (2) The Rat Stimulated Periodontal Ligament Model: A bone formation model to study the responses of the alveolar bone and the PDL cells of the mesial surface of the mesial root of the first maxillary molar after an orthodontic elastic is placed between the first and second maxillary molars of rats pretreated for five days with cortisol (Roberts, '68; Roberts, Chase and Jee, '68; Roberts, '69; Roberts, Chase and Jee, '69). The measurements of bone accretion, labeling index of PDL fibroblast, PDL cell population and bone cell differentiation were studied after appropriate post elastic intervals. (3) Intact Rat-Tibial Metaphyseal Resorption Model: Intact rats of both sexes were treated for seven days with graded doses of cortisol, and

the osteopenic effect of cortisol upon the tibial metaphysis was measured on the Quantimet Television Microscope (QTM; Park et. al., '70). (4) Hypophysectomized (Hypox) Rat-Tibial Metaphyseal Resorption Model: The rate of metaphyseal bone resorption is studied in hypox rats, devoid of endochondral bone formation. The hypox rat loses its ability to elongate bone and lay down metaphyseal bone after removal of its pituitary. The response to various doses of cortisol in males and females for seven days was used to study the influence of cortisol upon bone resorption (Park et. al., '70) (5) The Intact Rabbit Bone Bioassay Models: Weanling and adult (1 year old) New Zealand white rabbits were treated for 30 days with daily subcutaneous doses of steroid to study the relative potencies of corticosteroids upon growing and adult bones using morphometric techniques (Jee et. al., '67; Bartley, '68a, '68b; Bartley et. al., '68; Bartley and Jee, '68a; Young et. al., '68; Bartley et. al., '69; Bartley et. al., '70; Blackwood, '69; Berliner et. al., '70). These parameters include: (a) periosteal accretion (transverse growth), (b) endosteal accretion (trabecular bone formation), (c) endochondral bone formation, (d) quantity of metaphyseal trabeculae, (e) osteoblast population, (f) osteoclast population in the tibial and lumbar vertebral body metaphyses. (6) Rat-Surface Store - ^{85}Sr Kinetic Model: The initial uptake and serial retention up to seven days of ^{85}Sr in young and adult rats pretreated for five to seven days with various dose levels of cortisol were determined by whole body counting and counting of serum, femur, incisor and molar (Kenner et. al., '70a, '70b). These studies were designed

to study the influence of cortisol and/or surgical ablation of pituitary and adrenal upon the early movement (influx and efflux) of ^{85}Sr in hard tissue. (7) Rat-Deep Store - ^{85}Sr Kinetic Model: The model was designed to study the influence of cortisol upon the efflux of ^{85}Sr located within hard tissue (Kenner et. al., '69; Schafer et. al., '69; Talmage, Park and Jee, '70; Kenner et. al., '70a, '70b). The ^{85}Sr was administered for five days, followed by a two week interval before seven days' and 150 days' treatment with cortisol. The two week interval before treatment allowed sufficient time for the ^{85}Sr to become buried in hard tissue before the introduction of the exogenous corticosteroid.

Influence of Low Dose of Cortisol Upon Bone Remodeling

Very little is known about the influence of low doses of cortisol upon the skeleton. Clinical studies are our best sources of information; but unfortunately, such studies lack depth (usually inadequate controls). Thus, any attempts to derive a definitive answer from the literature is almost impossible. Furthermore, bench investigators shy away from low dose-long term studies for fear of having little to show for their time consuming efforts. Therefore, much of the literature is based on large doses of corticosteroid.

All our studies indicated low doses of cortisol resulted in the reduction of bone volume.

Bone loss has been shown in two species. In rats, resorption of alveolar bone produced wider periodontal ligaments after 0.5 to 5 mg of cortisol/kg for five days in the unstimulated PDL studies

(Roberts, '69; Roberts and Jee, '70; Fig. 1) and significant reduction of tibial metaphyseal bone occurred after 1 and 5 mg of cortisol/kg for 7 days (Park et. al., '70; Fig. 2). In rabbits, a reduction in tibial metaphyseal bone occurred after 0.1 to 2.5 mg/kg/day for 30 days (Blackwood, '70).

The responses of bone cells to low doses of cortisol include (Table I-III): (1) enhanced progenitor cell proliferation, (2) shortened time of maximum labeling of progenitor cells, (3) decreased osteoblasts, (4) increased osteoclasts, (5) decreased bone formation and (6) increased resorption.

Roberts, '69, and Roberts and Jee, '70, showed a 50 percent increase in the labeling index of unstimulated rat PDL fibroblasts after 0.5 and 2.5 mg of cortisol/kg for five days (Fig. 3). Roberts, '69, shortened by 11 hours the time of peak labeling of stimulated rat PDL fibroblasts after 0.5 mg of cortisol/kg for five days (Fig. 4).

Roberts, '69, and Roberts and Jee, '70, showed the decreased osteoblasts and bone formation to be attributed to a block in osteoblastic differentiation in their unstimulated rat PDL model studies. Furthermore, Blackwood, '69, reported a reduction in osteoblasts (Fig. 5) and osteoblasts transforming into inactive or resting osteoblasts and reduced accretion in rabbits treated with cortisol.

Roberts, '69, and Roberts and Jee, '70, also observed a preferential differentiation to osteoclasts and increased osteoclasts in the unstimulated rat PDL model (Fig. 6). Moreover, Blackwood and Hashimoto, '68, and Blackwood, '69, detected increased osteoblasts after 0.05, 0.1 and 0.5 mg of cortisol/kg of rabbits for 30 days

(Fig. 7).

In the rat, the reduction in bone volume is attributed to an enhanced osteoclasia alone, while in the rabbits the osteopenia is due to a combination of subnormal bone formation coupled with enhanced osteoclasia.

Influence of High Dose of Corticosterone Upon Bone Volume - The "Sledge Hammer" Syndrome

Briefly, huge doses of cortisol put cells to sleep ("round up"; Dougherty et. al., '56) and massive doses are cytotoxic (Dougherty et. al., '56). Thus, the response of bone tissue to high doses of cortisol is similar to low doses but at a reduced rate--a reduction in bone volume at a slower rate.

Bone tissue responses to high doses of cortisol include (Table I-III): (1) decreased progenitor cell proliferation (Simmons and Kunin, '67; Roberts, '69; Roberts and Jee, '70; Fig. 3), (2) delay in peak labeling time of progenitor cells (Roberts, '69; McKay et. al., '70; Fig. 4), (3) blocked differentiation to osteoblasts (Roberts, '69; Roberts and Jee, '70), (4) decreased osteoblasts (Jee et. al., '67; Simmons and Kunin, '67; Bartley, '68a, '68b; Bartley et. al., '68; Bartley and Jee, '68; Young et. al., '68; Bartley et. al., '69; Blackwood, '69; Jee et. al., '69; Berliner et. al., '70; Bartley et. al., '70), (5) enhanced differentiation to osteoclasts (Roberts, '69; Roberts and Jee, '70), (6) increased or decreased osteoclasts (Jee et. al., '67; Roberts, '67; Roberts and Jee, '70; Blackwood, '70), (7) decreased accretion (Blackwood, '69) and (8) decreased resorption (Follis, '51; Park et. al., '70).

Unstimulated and stimulated PDL fibroblast studies by Roberts, '69, and Roberts and Jee, '70, showed the depression in labeling indices of rat PDL fibroblasts and the delay in peak labeling times of stimulated PDL fibroblasts after doses greater than 5 mg of cortisol/day for 5 days (Figs. 3 and 4). Even though there was an apparent preferential differentiation to osteoclasts, the number of osteoclasts was only slightly increased and more often reduced (Figs. 6 and 7). The production of osteoclasts was limited by the poor supply of stem cells available for differentiation. The stem cells in turn were a victim of the antimitotic influence of cortisol and the usual numbers of cells available to differentiate into osteoclasts are insufficient to maintain the normal population of this cell.

Not only can osteoclast numbers be diminished, but they can be completely eliminated. Park et. al., '70, have shown complete inhibition of resorption in the tibial metaphysis of hypox rats with 75 mg of cortisol/kg for 7 days (Fig. 8).

An Overview of the Effects of Cortisol on Bone Volume

A unified concept on the influences of cortisol on bone remodeling can be best summarized as (Fig. 9; Table III): (1) increased progenitor cell proliferation with low doses, (2) decreased progenitor cell proliferation with high doses, (3) inhibition of osteoblast differentiation, and (4) enhanced osteoclast differentiation. Thus, a poor supply of stem cells available for cellular differentiation after high doses of cortisol can result in a subnormal popu-

lation of osteoclasts and a more subnormal population of osteoblasts. A hardy supply of progenitor cells will trigger an abrupt increase in osteoclast population. Regardless of the supply of stem cells, the net response to cortisol is a reduction in bone volume.

Probable explanations of the stimulated cellular proliferation at low doses of cortisol may be attributed to the biotransformation of cortisone from cortisol (Berliner and Dougherty, '58; Berliner et. al., '58) and/or to secondary hyperparathyroidism (Storey, '60; Stoerk and Arison, '61; Myers, '62; Storey, '63; Stoerk et. al., '63; Gordan et. al., '67; Rasmussen, '68).

Cortisone has been reported to stimulate cellular proliferation (Berliner and Ruhmann, '66). At lower concentrations of cortisol, transformation to cortisone is essentially complete (Berliner and Dougherty, '58; Berliner et. al., '58) and the ratio of cortisol to cortisone is in favor of cortisone. High doses of cortisol saturate the 11β-hydroxydehydrogenase system; thus, the ratio of cortisol to cortisone is heavily in favor of cortisol at high doses.

Currently we have some experiments in progress to clarify the role of the parathyroid hormone in the action of cortisol upon bone. It is well known that parathyroid hormone (Talmage, '65; Talmage, '66) and parathyroid extract stimulated progenitor cell proliferation and shortened the peak labeling time of stimulated PDL fibroblasts (Chase et. al., '69).

The high dose response can be characterized by the "sledge hammer" effect in which all cellular activity is retarded. It is well known that high doses of cortisol inhibit pinocytosis (Dougherty

et. al., '56; Schneebeli and Dougherty, '63; Dougherty et. al., '66; Berliner et. al., '67; Dougherty and Berliner, '68), glucose transport (Bartlett et. al., '62; Morita and Munck, '64), RNA (Makman et. al., '66, Peck et. al., '67), DNA (Makman et. al., '66; Stevens et. al., '66) and protein syntheses (Clark, '53; Daughaday and Mariz, '62). Thus, the availability of raw material (Peck, '69) for synthesis and of the assembly line to keep the cell cycle in motion are not available. Furthermore, the high influx of calcium can decrease osteoblastic activity directly (Cooper and Talmage, '65).

Influence of Cortisol on ^{85}Sr Kinetics

The studies on the influx and efflux of ^{85}Sr in the whole body, femur, incisor, molar and serum during cortisol treatment was initiated to explore if the steroid regulates bone density (mass of Ca/unit volume). In one model, the cortisol was given for 7 days followed by an injection of ^{85}Sr and 7 more days of cortisol. This was called the surface store model in which the radioactivity in hard tissues of young rats was the sum of the ^{85}Sr being incorporated into new crystals as a result of new bone apposition ("hotspots"), rapid short term exchange on bone surfaces, diffusion of radionuclide in canaliculi, and exchange and radial diffusion into the canalicular territory (Marshall, '69). In the case of adult rats, the "hot-spot's" (appositional) contribution to the total skeletal uptake was minimized.

In our deep store model, the radioactivity was given two weeks

prior to the daily administration of cortisol. Thus, the radioactivity was entrenched within the bone by the burial of the "hotspots" and the diffusion into the canalicular territory to form the "diffuse" component (Arnold, Jee and Johnson, '56). The surface store influx value at day one is the sum of the uptake of ^{85}Sr by apposition, short term exchange, canclicular diffusion and exchange. The surface efflux is the loss of radioactivity from hard tissues between day one and seven.

The deep store efflux value is a measurement of the ability of osseous or dental tissues to mobilize the buried radioactivity from "hotspots" and the "diffuse" component. We were unable to study deep store influx with the above techniques.

Both bone and tooth were studied to compare the influence of the osteoblast, osteoclast, osteocyte and odontoblast on ^{85}Sr kinetics. The role of the osteocyte in ^{85}Sr kinetics was deduced from the comparable studies of dental and osseous tissues. Dental tissue is without a comparable cell to the osteocytes. Furthermore, there is much to learn about mineral transport in dental tissue, a model normally devoid of the resorption.

Influence of Low Doses of Cortisol on ^{85}Sr Kinetics

Very little is known of the influence of low doses upon mineral kinetics. Thus far, we can report a significant increase in the uptake of ^{85}Sr at 0.6 and 2.0 mg of cortisol/day and a decreased long term retention in females at 0.2, 0.6 and 0.06 mg/kg/day (Table IV). Currently we believe that low doses require longer treatment

periods to produce changes observed with high dose. The whole body counting data from 0.6 gm/kg/day dose level showed changes between 60 and 110 days post ^{85}Sr injection. The long term retention was significantly reduced, and between day 110 and 150 the efflux of ^{85}Sr was reduced sufficiently so that the retention equals that of the controls at day 150 (Fig. 10). Thus, the 0.2 and 0.06 mg of cortisol/kg/day should at longer treatment periods behave in the same manner.

Influence of High Doses of Cortisol on ^{85}Sr Kinetics

High doses of cortisol decreased the 0 - 24 hour whole body uptake of ^{85}Sr in rats. The seven day retention was down and the long term retention was up from controls (Table IV). The bone influx (24 hour uptake) in the young was down and equal in the adults. The surface (seven day retention) efflux was up and the deep store efflux was down. The bones were latching on to less ^{85}Sr which explains why the whole body 24 hour uptake is depressed. The bones between one and seven days were giving up more ^{85}Sr which explains the decreased seven day retention; and the deep bone store efflux was down, which explains the long term whole body retention being up. In a nutshell, the whole body counting values go along with the skeletal values.

In the teeth (Kenner, '70a), the effects of high doses of cortisol were most surprising (Table IV). The surface influx and efflux and the deep store efflux of ^{85}Sr were elevated. Thus, dental tissue differs from skeletal tissues in its ability to elevate the surface influx and the deep store efflux of ^{85}Sr.

Measurements of influx and efflux performed on the molars (to eliminate the complication of continuous eruption found in incisors) were similar (Kenner, personal communication).

An Overview of the Effects of Cortisol on ^{85}Sr Kinetics

A unified concept of the action of cortisol on ^{85}Sr kinetics of hard tissues can be summarized as follows (Table V): (1) surface influx was increased, (2) surface efflux was increased, (3) deep store efflux in bone was decreased (Kenner et. al., '69; Talmage, Park and Jee, '70) and (4) deep store efflux in teeth was elevated. The depressed influx in bone was principally due to the depression in osteoblastic activity which in turn inhibited the formation of "hotspots". The reduction in "hotspots" canceled out the increase in influx due to cortisol; so in reality, there was an increased influx of ^{85}Sr. Furthermore, in adult rats where "hotspot" formation is minimized, the surface influx is found to be identical to controls.

The depressed deep store efflux seen in bone was the consequence of the reduced metabolic activity of the osteocyte. The osteocyte must be involved in the active transport of calcium (Talmage, '69; Talmage, Park and Jee, '70), since the same phenomenon was not observed in dental tissues.

In summary, cortisol increased overall bone density by inhibiting the formation of new bone and decreasing the efflux of deep mineral store. Its influence upon the density of teeth is not known and needs further investigations.

A probable explanation of the increased surface influx and efflux and deep store efflux of ^{85}Sr by cortisol is that cortisol inhibits active calcium transport, increases passive calcium influx and efflux (diffusion and exchange) and increases the availability of ^{85}Sr for exchange (Fig. 11).

The following effects of cortisol described by previous investigators suggest a decrease or inhibition of active calcium transport (Table VI): (1) inhibition of pinocytosis (Dougherty et. al., '56, '61; Schneebeli and Dougherty, '63; Dougherty et. al., '66; Berliner et. al., '67; Dougherty and Berliner, '68), (2) decrease of packaging of calcium (Matthews et. al., '68; Talmage et. al., '70), (3) decrease in mucopolysaccharide synthesis (Layton, '51; Clark and Umbreit, '54; Kowalewski, '58), (4) decreased protein (Clark, '53; Daughaday and Mariz, '62), RNA (Makman et. al., '66; Peck et. al., '67), DNA (Makman et. al., '66; Stevens et. al., '66) and ATP syntheses (Clark and Pesch, '56; Gallagher, '59; Bottoms and Goetsch, '68) and (5) increased glycogen storage (Pabst et. al., '47; Matschinsky et. al., '61). These findings suggest that cortisol blocks the active transport of calcium.

Enhanced passive influx and efflux (diffusion and exchange) of calcium after cortisol is supported by the following observations: (1) massive accumulation of calcium in mitochondria (Matthews et. al., '68), (2) increased vascular surface (Zweifach et. al., '53; Cahn and Thoma, '55; Anneroth and Bloom, '66), (3) increased bare calcified matrix surface (Jee, unpublished observation), and (4) stabilization of membranes (Weissman, '64; Fell, '69). The accumulation

of calcium in mitochondria (reduction in storage sites) could be interpreted as an indication of enhanced diffusion of calcium through the cell while the increase in bare surface due to the rounding up or loss of bone lining cells (Fig. 11), the retraction of odontoblastic tubules and the increased vascular volume due to hyperemia increases the chance for exchange.

Another reason for the improved influx and efflux of ^{85}Sr after cortisol is because the ^{85}Sr is more accessible for exchange as a consequence of the reduced recrystallization and accelerated polymerization of calcified matrix (Asadi et. al., '56). Fewer rapidly forming new crystals means less buried Sr atoms and more Sr atoms on crystal surfaces available for exchange. The enhanced polymerization of calcified matrix retards the diffusion of ^{85}Sr into the canalicular territory and allows more ^{85}Sr to be exposed to canalicular exchange.

The dichotomy in responses of the deep store efflux in teeth must be attributed to the fact that dental tissue lacks a cell homologous to the osteocytes; thus, the deposition of ^{85}Sr in dentin is similar to the surface store in bone and teeth in that the osteocyte is not regulating its distribution. Therefore, the efflux of deep ^{85}Sr store should behave just like the surface store efflux (Fig. 12).

Concluding Remarks

Cortisol reduced bone volume and increased bone density. Low doses of cortisol were more effective in stimulating resorption

than high. The exact role of corticosteroids in regulating the state of health of hard tissues needs further investigation. Some have speculated that cortisol may be why we become osteoporotic with age. As we secrete less corticosteroid coupled with its prolonged half life with age (West et. al., '61; Dorfman and Ungar, '65), the reduced steroid level may stimulate bone resorption. The stimulated resorption coupled with an increased sensitivity of osteoblasts to cortisol (depression of bone accretion) in adults (Blackwood, '69) will result in decreased bone volume. Also, increasing bone density may make bone more brittle.

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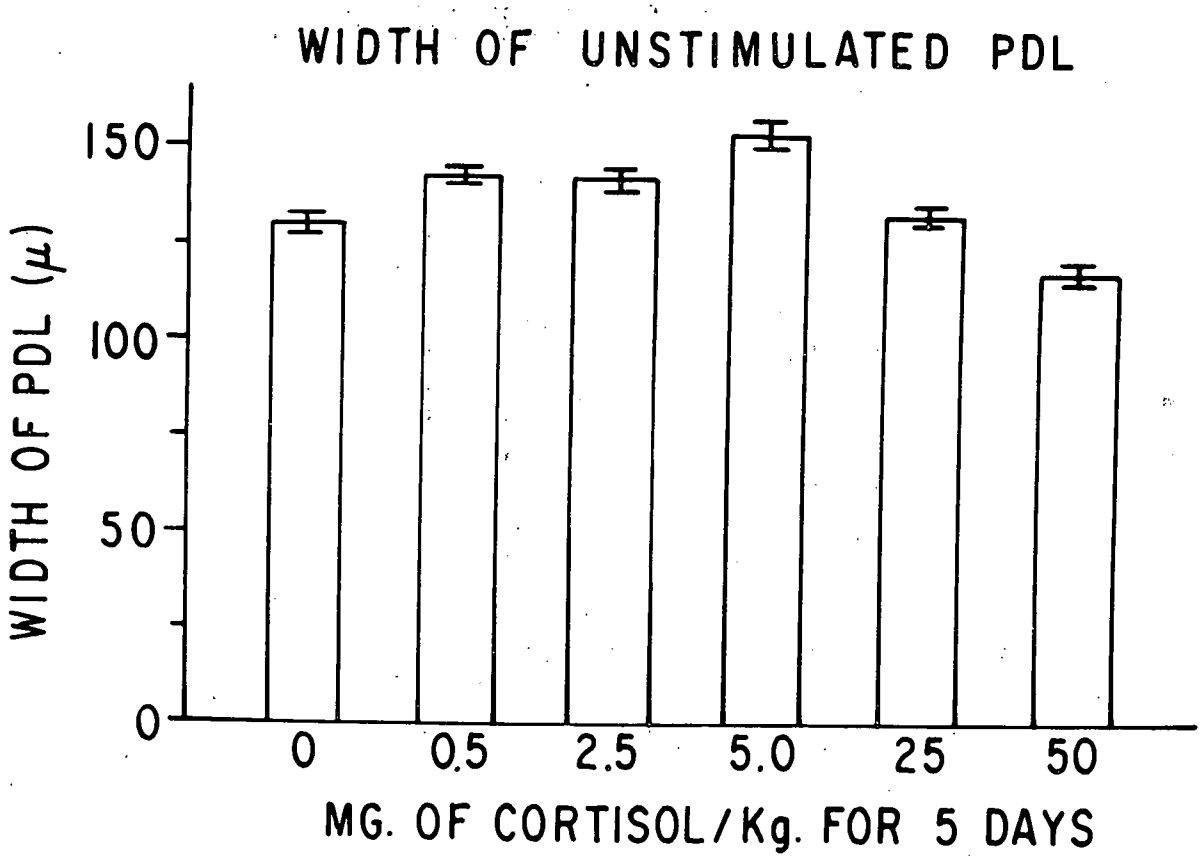

Figure 1. Changes in the width of periodontal ligament (PDL) after five daily injections of cortisol. The measurements were taken at 200μ below the alveolar crest of the maxillary first molar of six week old Sprague-Dawley Rats. Note the enlarged PDL after 0.5, 2.5 and 5.0 mg of cortisol/kg resulting from the resorption of alveolar bone. Vertical bar=standard deviation of means.

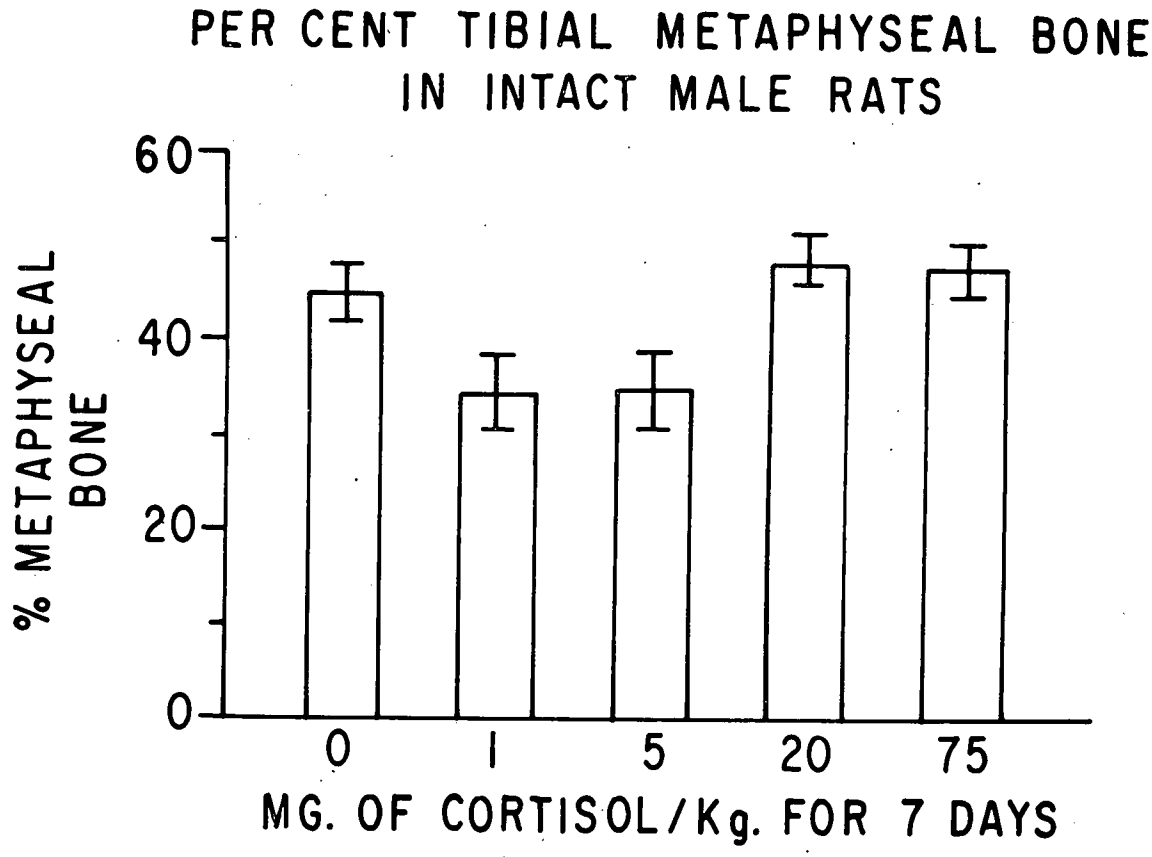

Figure 2. Percent of tibial metaphyseal bone in intact male Sprague-Dawley rats after seven daily injections of cortisol. The measurements were taken by a QTM (Quantitative Television Microscope, Metals Research, Cambridge, England) of micro-radiographs of the proximal tibia. Note the reduction in metaphyseal bone after 1 and 5 mg of cortisol/kg. Vertical bar = standard deviation of means.

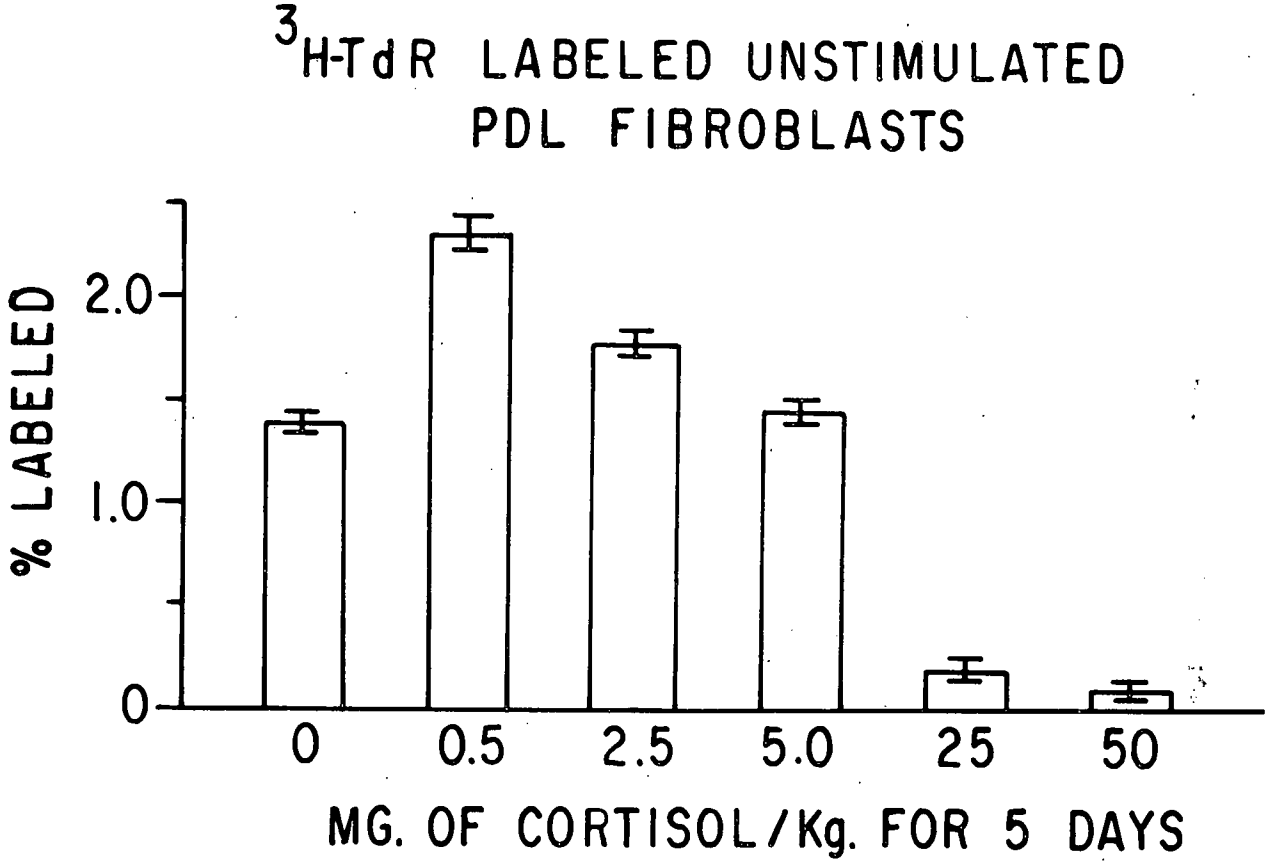

Figure 3. Labeling indices of periodontal ligament fibroblast of six week old Sprague-Dawley rats after five days of cortisol treatment. Note the increased labeling after low doses (0.5 and 2.5 mg/kg) and decreased labeling after high doses (25 and 50 mg/kg). Vertical bar = standard deviation of means.

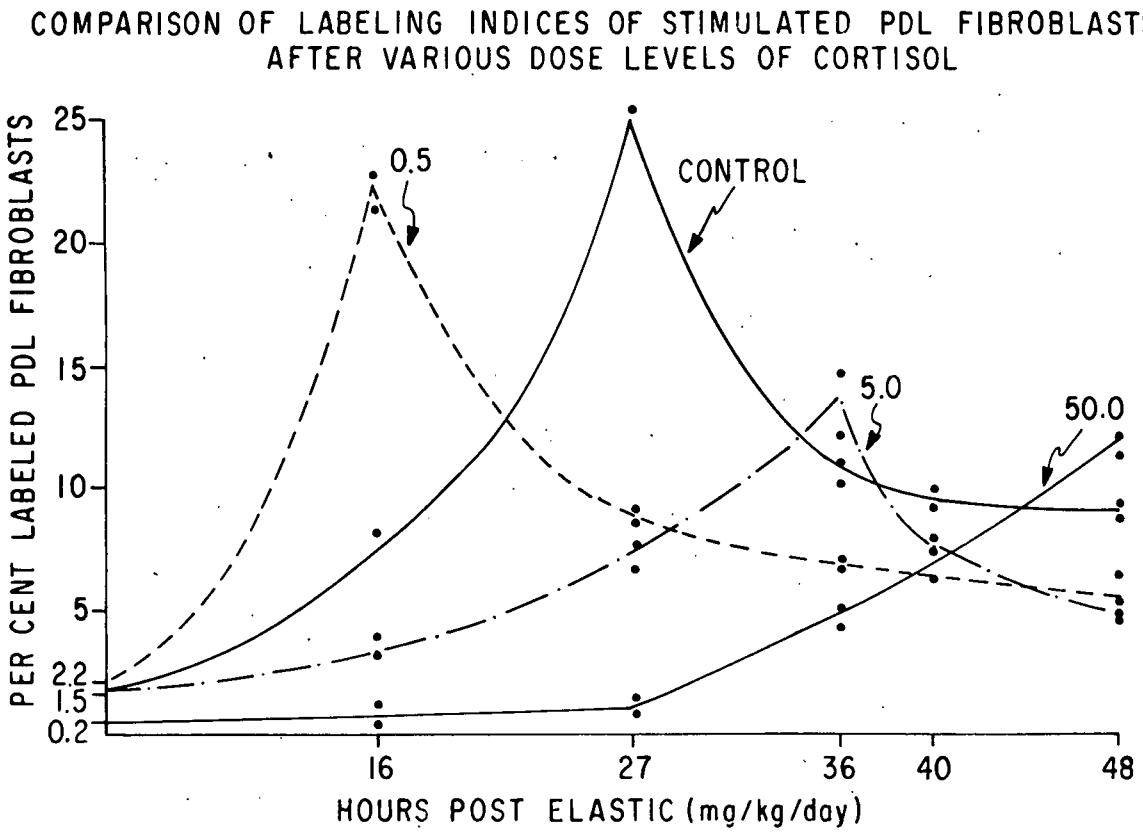

Figure 4. Comparison of labeling indices of stimulated periodontal ligament fibroblasts of six week old Sprague-Dawley rats after insertion of orthodontic elastics between maxillary first and second molars following five days of pretreatment with cortisol. Note the accelerated peak labeling of rats treated with 0.5 mg of cortisol/kg at 16 hours as compared to that of the control at 27 hours. High doses (5 and 50 mg/kg) delayed the peak labeling response (9 and 21 hours).

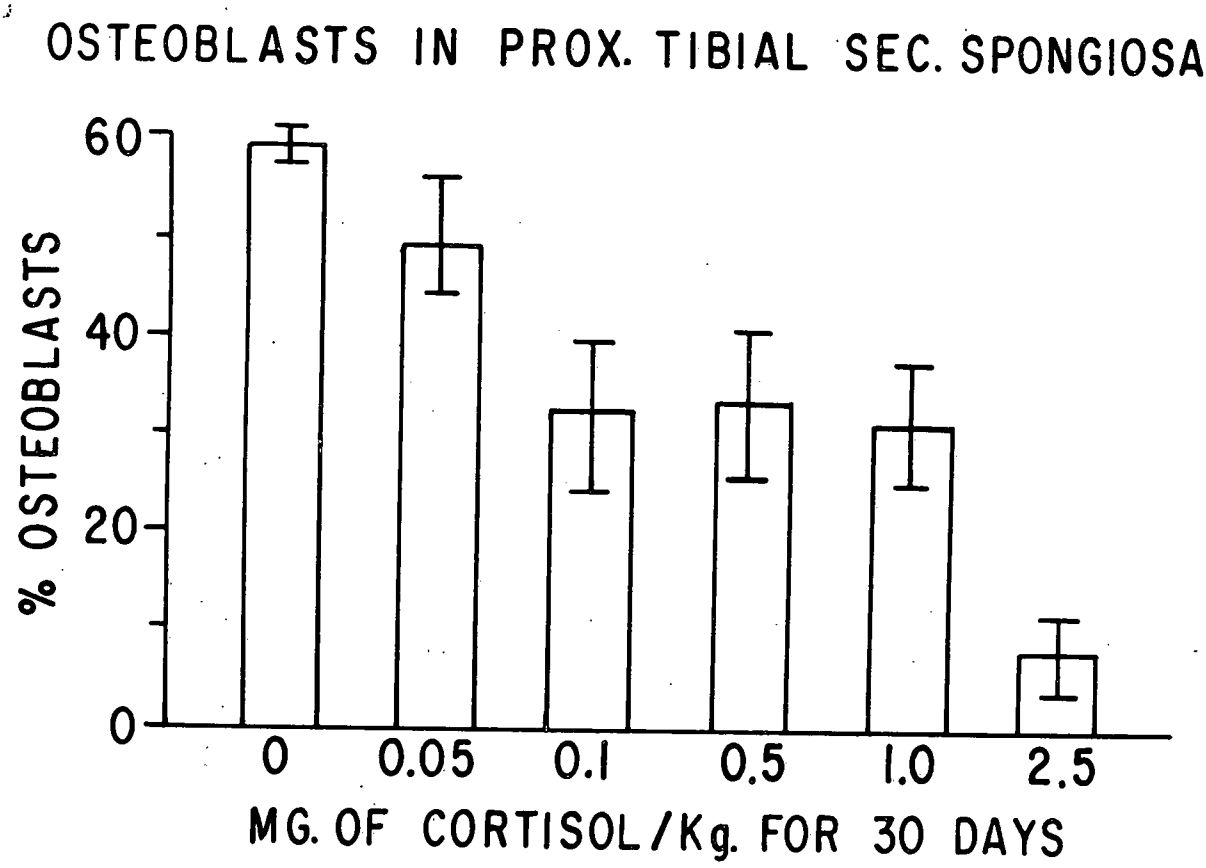

Figure 5. Decrease in percent osteoblasts in the proximal tibial secondary spongiosa of 8 week old female New Zealand white rabbits after 30 days of treatment with cortisol. Vertical bar = standard deviation of means.

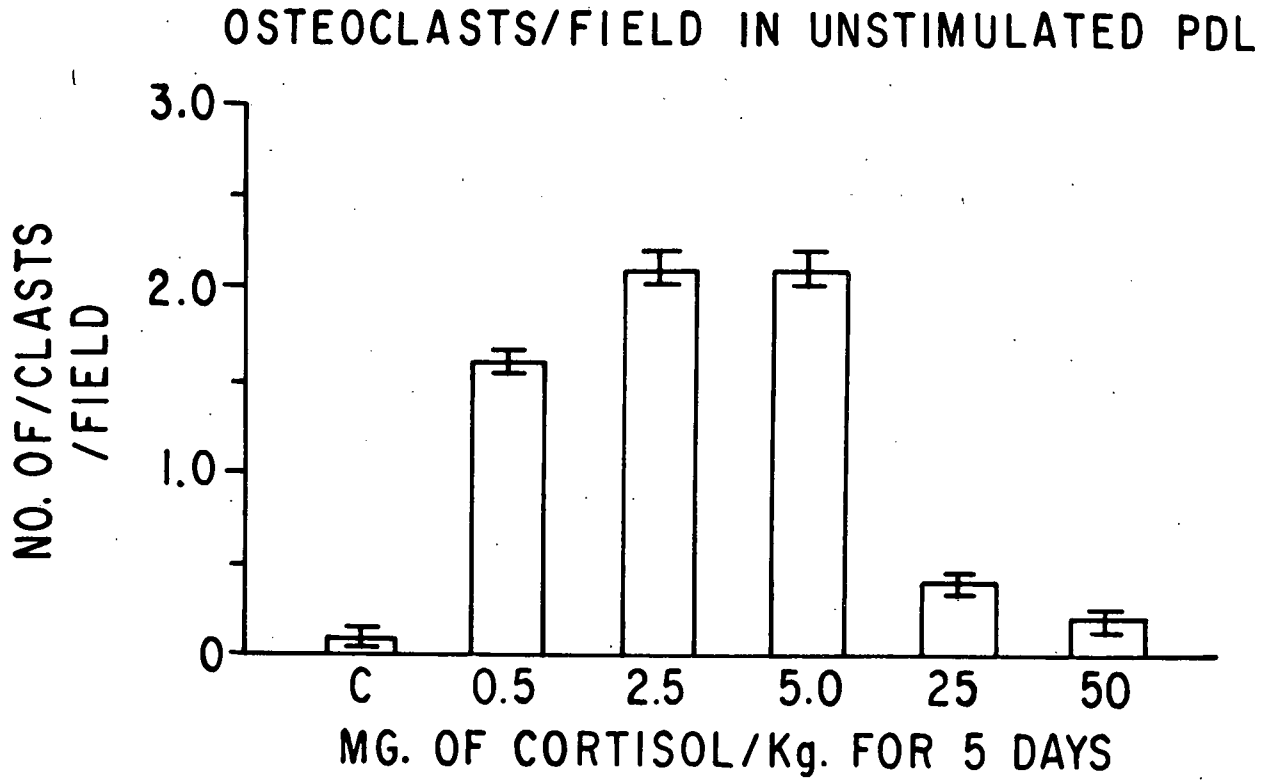

Figure 6. Changes in number of osteoclasts per field (40,000μ^2) in the periodontal ligament from the first maxillary molar of 6 week old Sprague-Dawley rats after 5 days of cortisol treatment. Note the marked increase in osteoclasts after 0.5, 2.5 and 5.0 mg of cortisol/kg as contrasted to the slight increase after 25 and 50 mg of cortisol/kg. Vertical bars = standard errors of means.

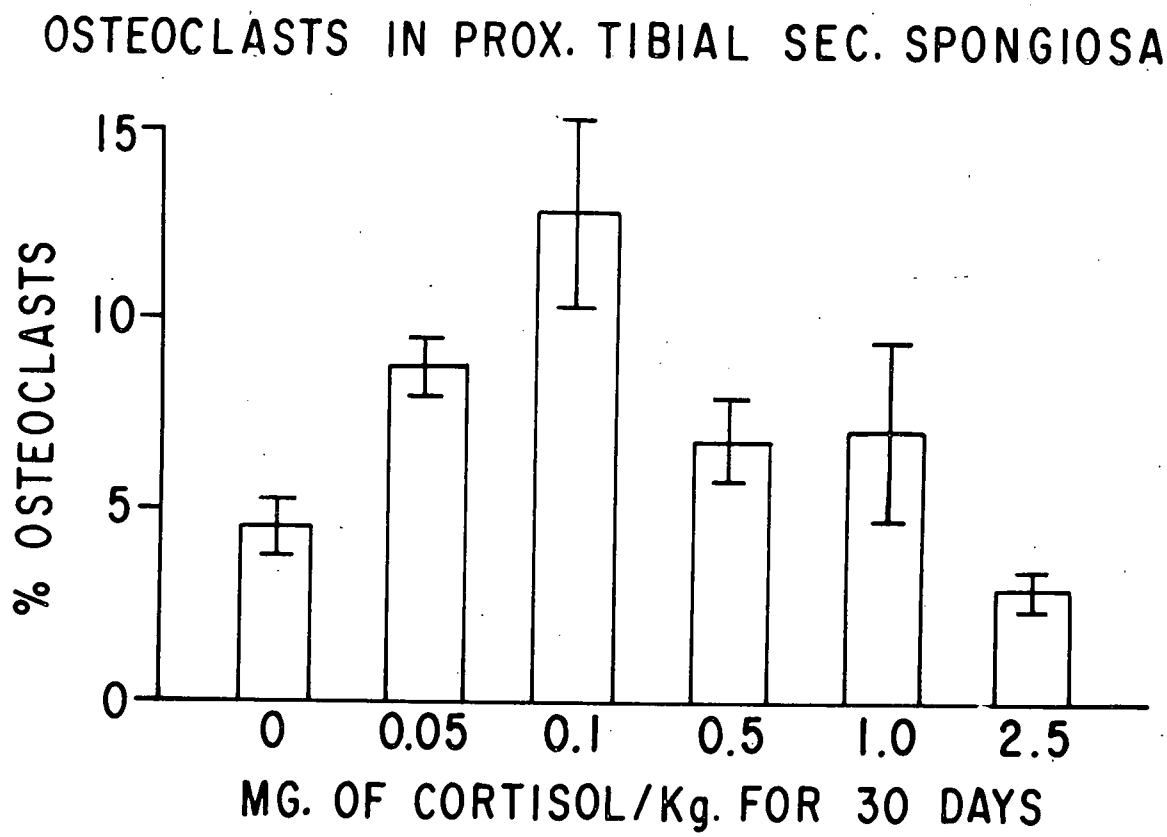

Figure 7. Changes in the percent of osteoclasts in the proximal tibial secondary spongiosa of 8 week old female New Zealand white rabbits after 30 days of treatment with cortisol. Note the increase in osteoclasts after 0.05 mg, 0.1 mg and 0.5 mg of cortisol/kg and the reduction in osteoclasts after 2.5 mg of cortisol/kg. Vertical bars = standard deviation of means.

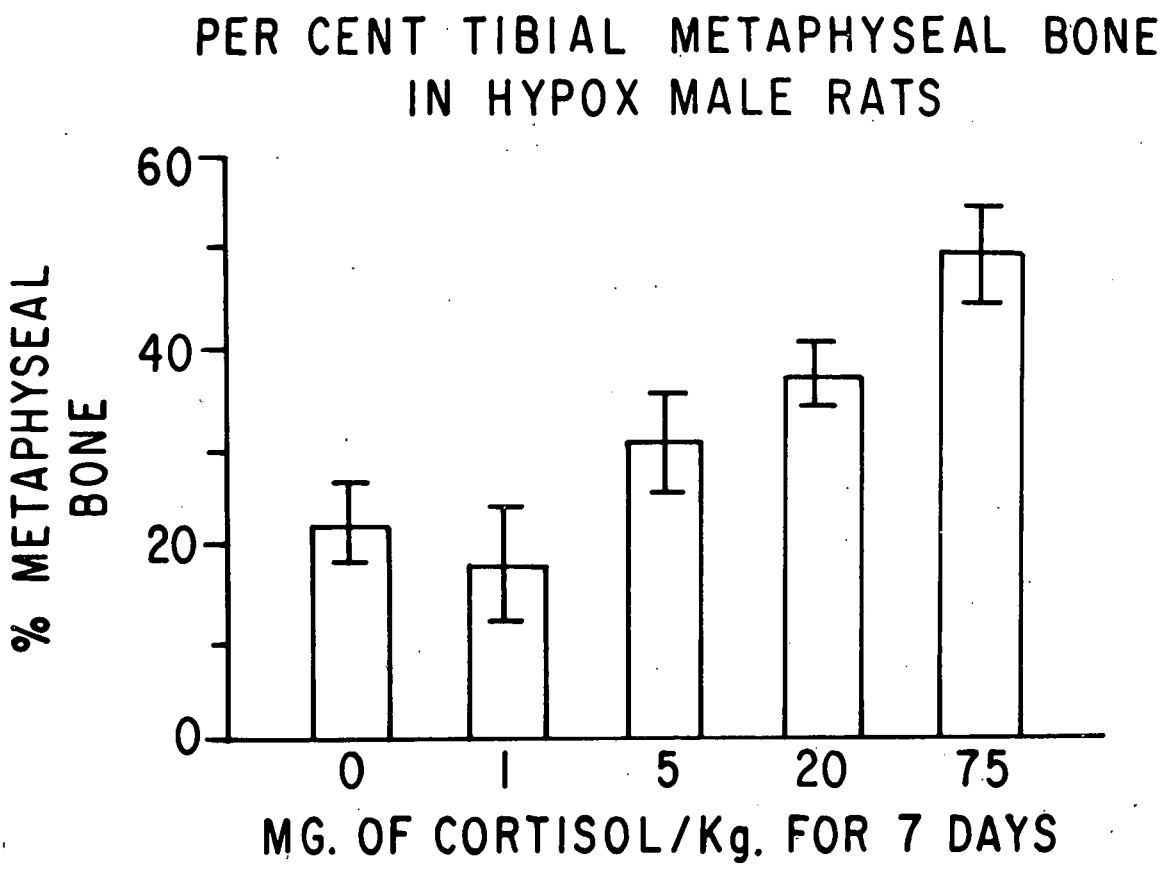

Figure 8. Changes in percent of tibial metaphyseal bone of hypophysectomized (hypox) male Sprague-Dawley rats after 7 days of cortisol. Percent metaphyseal bone measured from microradiogradiographs of proximal tibia with a QTM. Note the persistence of the metaphyseal bone (inhibition of resorption) after 75 mg of cortisol/kg (50 percent) as contrasted to the reduction of metaphyseal bone to 20 percent by bone resorption in the untreated hypox rats. Vertical bar = standard errors of means.

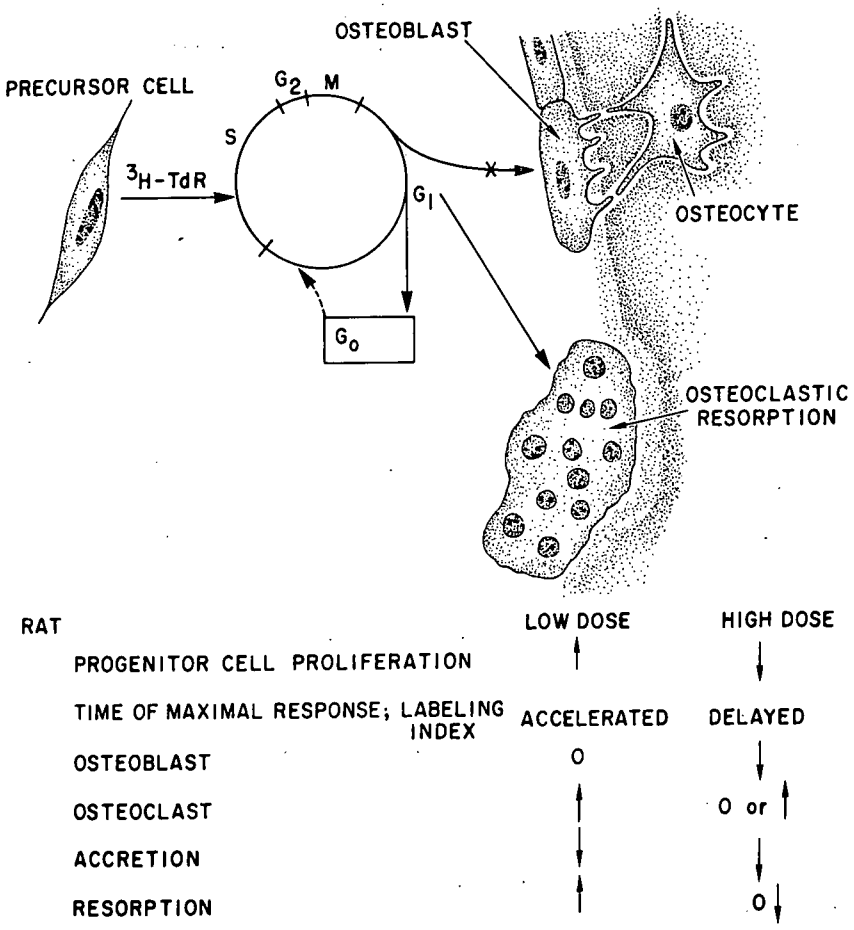

Figure 9. Comparison of the effects of low and high doses of cortisol upon bone cell population kinetics, accretion and resorption. Note the preferential differentiation to osteoclasts regardless of dose. ↓ = decrease; ↑ = increase; 0 = no change.

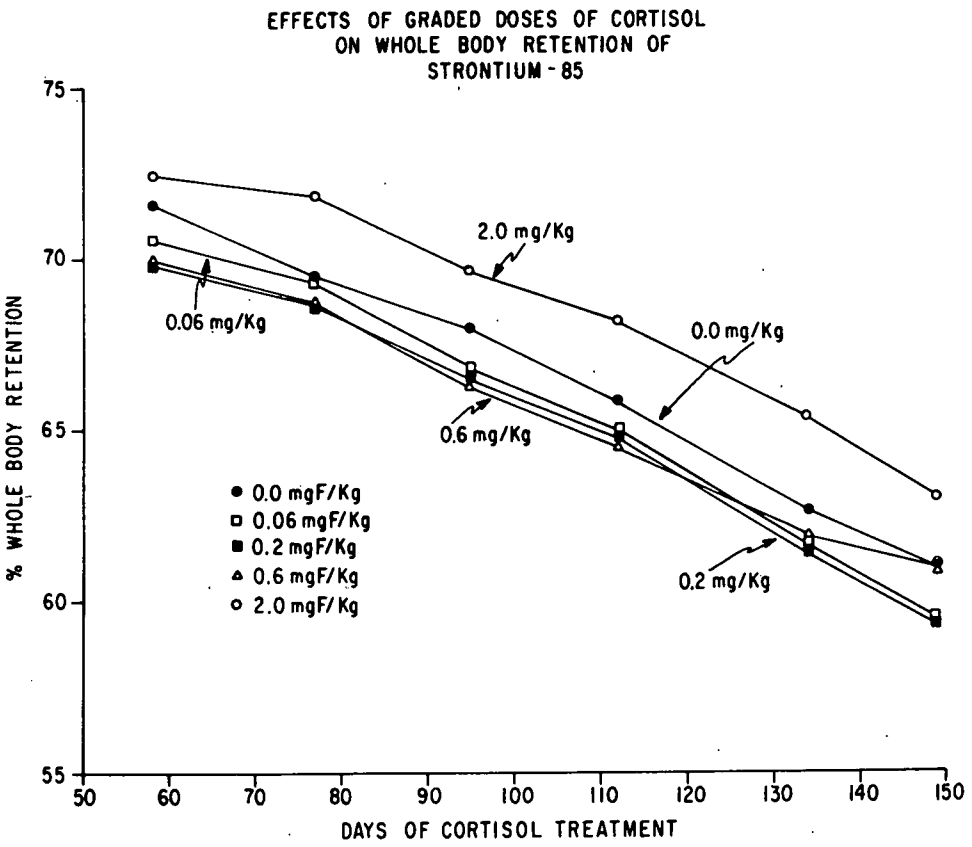

Figure 10. Effects of graded dose of cortisol on whole body retention of ^{85}Sr in 200 gm female Sprague-Dawley rats. The rats were treated initially with 4 injections of 2μCi of ^{85}Sr over a 7 day period, followed by 2 weeks' rest, followed by daily injections of cortisol. The whole body count was expressed as 100 at day 0 of cortisol injection. Note at 2 mg/kg the increased retention of ^{85}Sr and the decreased retention with 0.2 and 0.06 mg/kg. Initially at 0.6 mg/kg, there was an increased retention, but after 150 days of treatment the retention was identical to the controls.

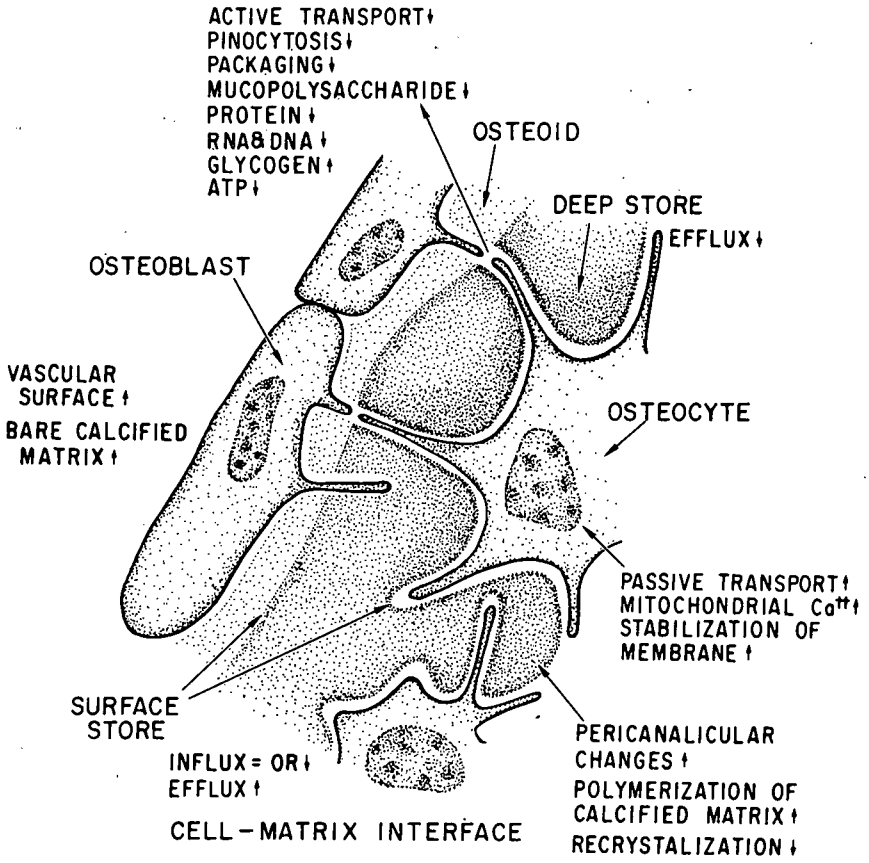

Figure 11. Summary of the possible actions of cortisol on mineral fluxes of bone (see text).

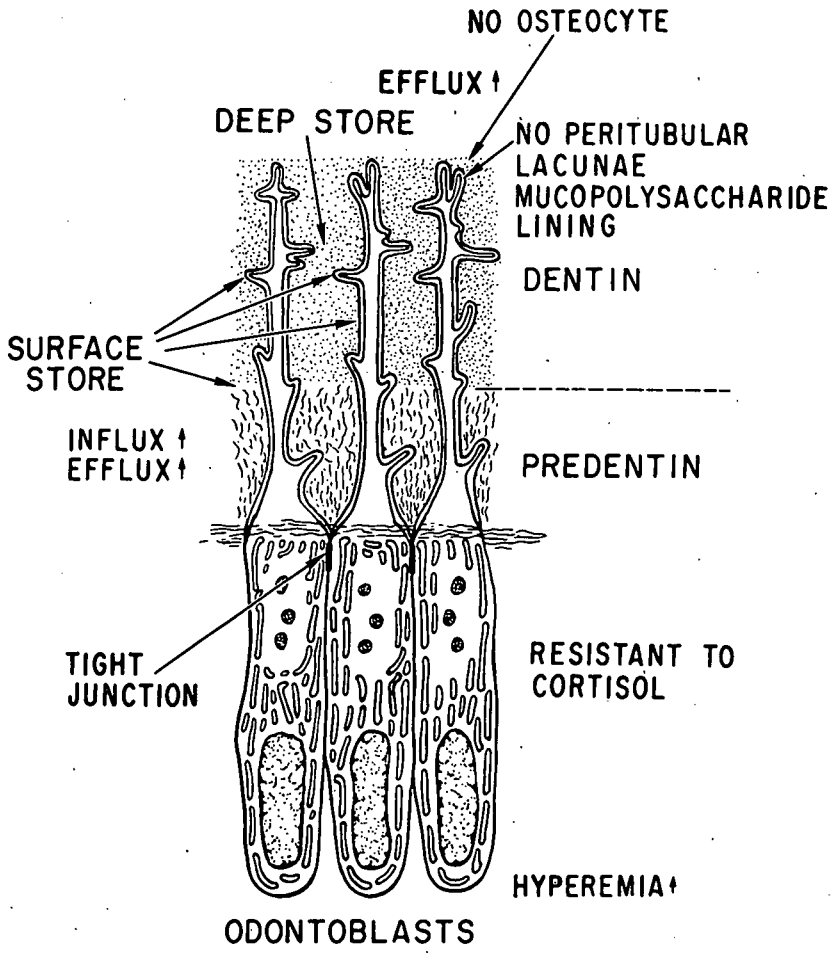

Figure 12. Summary of possible actions of cortisol on mineral fluxes of teeth. Note the absences of a cell homologous to the osteocytes and the tight junction between odontoblasts (see text).

Table I. Summary of the actions of low and high doses of cortisol upon rat bones. ↑, increase; ↓, decrease; 0, no change. Treatment time = 7 days.

CORTICOSTEROID AND RAT BONES

PARAMETERS	LOW DOSE	HIGH DOSE
PROGENITOR CELL PROLIFERATION	↑	↓
P.C. TIME OF MAX. RESPONSE	ACCELERATED	DELAYED
OSTEOBLAST	0	↓
OSTEOCLAST	↑	0, ↑
ACCRETION	↓	↓
RESORPTION	↑	0, ↓

LOW DOSE = 0.5, 1.0 AND 5 mg/kg/DAY FOR 5 DAYS
HIGH DOSE= 25 & 50 mg/kg/DAY FOR 5 DAYS

Table II. Summary of the actions of low and high doses of cortisol upon rabbits' bones. ↑, increase; ↓, decrease; 0, no change. The dose levels were much lower than those for rats, but the treatment interval was more prolonged (30 days).

CORTICOSTEROID AND RABBIT BONES

PARAMETERS	LOW DOSE	HIGH DOSE
OSTEOBLAST	↓	↓
RESTING OSTEOBLAST	↑	↑
OSTEOCLASTS	↑*, 0**	↓
ACCRETION	↓	↓
RESORPTION	↑	↓

LOW DOSE = 0.1 TO 2.5 mg/kg/DAY FOR 30 DAYS

HIGH DOSE= >5 mg/kg/DAY FOR 15 DAYS

* 0.05 & 0.1 mg/kg/DAY FOR 30 DAYS

**0.5 TO 2.5 " " " " " "

Table III. A unified concept of the actions of low and high doses of cortisol upon bone cells. The obvious difference due to dose is that high dose depresses progenitor cell proliferation.

CORTISOL AND BONE REMODELING

	LOW DOSE	HIGH DOSE
PROGENITOR CELL PROLIFERATION	↑	↓
OSTEOBLAST DIFFERENTIATION	↓	↓
OSTEOCLAST DIFFERENTIATION	↑	↑

LOW DOSE RESPONSES:

1. BIOTRANSFORMATION TO CORTISONE
2. SECONDARY HYPERPARATHYROIDISM

HIGH DOSE RESPONSES:

1. SLEDGE HAMMER EFFECT (CELL ACTIVITY ↓)
   a. PINOCYTOSIS ↓ (PRECURSORS)
   b. DNA & RNA SYNTHESIS ↓
   c. PROTEIN SYNTHESIS ↓
2. OSTEOBLASTIC ACTIVITY WITH HIGH Ca^{++} ↓

Table IV. Summary of the actions of high and low doses of cortisol upon short and long term whole body surface and deep bone stores and surfaces and deep teeth stores. ↑, increase; ↓, decrease and 0, no change.

CORTISOL AND ^{85}Sr KINETICS IN HARD TISSUES

FINDINGS:	BONE	TEETH
SURFACE INFLUX	=, ↓ *	↑
SURFACE EFFLUX	↑	↑
DEEP EFFLUX	↓	↑

* DEPRESSED INFLUX DUE TO INHIBITION OF BONE APPOSITION

THUS:	HARD TISSUE
SURFACE INFLUX	↑
SURFACE EFFLUX	↑
DEEP EFFLUX	↓BONE**, ↑TEETH

** DEPRESSED EFFLUX DUE TO SUPPRESSION OF OSTEOCYTE ACTIVITY INVOLVING ACTIVE CALCIUM TRANSPORT

THEREFORE:	BONE	TEETH
NET FLUX	↓	=

Table V. A unified concept of the actions of cortisol on mineral fluxes of hard tissues. Decrease net flux indicates suppression of efflux of mineral. ↑, increase; ↓, decrease and =, no change.

EFFECT OF CORTISOL ON ^{85}Sr AND ^{45}Ca KINETICS

			LOW DOSE	HIGH DOSE
WHOLE BODY	SHORT TERM	UPTAKE (0-24Hr)	♀↑, ♂ O	↓
		RETENTION (7 DAY)	O	↓
	LONG TERM	RETENTION	↓	↑
BONE; FEMUR	SURFACE	INFLUX	O	↓
		EFFLUX	O	↑
	DEEP STORE	EFFLUX	–	↓
TEETH; INCISOR	SURFACE	INFLUX	O	↑
		EFFLUX	O	↑
	DEEP STORE	EFFLUX	–	↑
TEETH; MOLAR	SURFACE	INFLUX	O	↑
		EFFLUX	O	↑
	DEEP STORE	EFFLUX	–	↑
SERUM	DEEP STORE		O	↓
TIBIA IN VITRO CULTURE	DEEP STORE	EFFLUX	↑ OR O	↓

Table VI. Action of cortisol which influences mineral kinetics. ↑, increase; ↓, decrease.

INFLUENCE OF CORTISOL ON ^{85}Sr KINETICS

1. ACTIVE TRANSPORT ↓
   a. PINOCYTOSIS ↓
   b. PACKAGING ↓
   c. MUCOPOLYSACCHARIDE SYNTHESIS ↓
   d. PROTEIN SYNTHESIS ↓
   e. RNA & DNA SYNTHESIS ↓
   f. GLYCOGEN STORAGE ↑
   g. ATP SYNTHESIS ↓ (OXIDATIVE PHOSPHORYLATION)
2. PASSIVE FLUX ↑ (DIFFUSION & EXCHANGE)
   a. ACCUMULATION OF CA^{++} IN MITOCHONDRIA ↑
   b. VASCULAR SURFACE ↑ (HYPEREMIA)
   c. BARE CALCIFIED MATRIX SURFACE ↑
   d. STABILIZATION OF MEMBRANE ↑
3. AVAILABILITY OF ^{85}Sr ↑
   a. RECRYSTALLIZATION ↓
   b. POLYMERIZATION OF CALCIFIED MATRIX ↑
   c. PERICANALICULAR CHANGES ↑

PRELIMINARY REPORT ON HEMATOLOGICAL EFFECTS OF ^{241}Am IN THE BEAGLE

Jean H. Dougherty

Abstract: The hematological changes following intravenous injection of a wide range of doses (from 0.0018 to 2.8 μCi/kg) of ^{241}Am into young adult beagles are reported for 18 months post-injection. There is a dose dependent depression of white cells at the four highest dose levels (2.8, 0.9, 0.3, 0.1 μCi/kg injected activity) which for granular leukocytes and monocytes is maximal by one month after injection. Lymphocytes decrease more slowly with minimal values observed about one year or later. Depression of red cells is seen only after injection of 0.9 and 2.8 μCi/kg. The hematological response in general is similar to that seen after injection of comparable amounts of ^{239}Pu.

Introduction

Approximately one-half of the proposed number of test dogs have been injected with ^{241}Am, beginning in 1966. It is felt that there are enough data to make a preliminary analysis of the hematological changes at this time. This report will summarize changes in blood cells up to 18 months post-injection. It should be emphasized that these findings are based on relatively few dogs (at most 6 at each dose level) and the data will be reanalyzed in the future when the experiment has been completed (with 12 dogs at each level) and the results will be extended over a longer time after injection.

Methods

Three hematological examinations are performed on each dog prior to injection. Since there were no 0-level ^{241}Am dogs injected concurrently with the experimental animals, the changes noted at

each dose level with time have been analyzed in two ways: (1) to the means of the dogs' own pre-injection counts and (2) to mean values of 0-level ^{239}Pu and ^{226}Ra dogs which were sham injected over the same calendar years (1966-67). Thus, the mean counts on 14 0-level dogs for the first 18 months post-injection serve as an additional base line to detect changes in the dogs injected with americium.

The red cell picture is evaluated by determination of volume of packed red cells (VPRC), hemoglobin (Hgb), reticulocyte counts and occasional red cell counts. It is felt that the VPRC is the most accurate determination of fluctuation in red cells so emphasis will be placed on this measurement.

White cell values determined are total leukocyte count (WBC) and absolute numbers of polymorphonuclear leukocytes (pmns), stab or immature pmns, lymphocytes, monocytes and eosinophils. These cells are counted differentially from at least 400 cells per May-Grünwald-Giemsa stained blood films. Blood platelets and sedimentation rates (mm/hr, uncorrected) are also determined.

The frequency of the counts was as follows: the 4- and 5-levels of ^{241}Am were sampled weekly for the first month and then monthly. The lower levels (0.2, 0.5, 1, 1.7, 2, 3) and the 0-level ^{239}Pu and ^{226}Ra dogs were counted every three months with some of the 3-level ^{241}Am dogs bled monthly. The exact injected activity of ^{241}Am for each dog may be found in the injection tables.

The data on all dogs were put on computer cards and the analyses were made at the University of Utah Computer Center. The

mean values for each hematological trait for each dose level for each time interval after injection were compared to the pre-injection mean of the same dogs at each dose level by means of a t-test. Only those means based on at least 4 dogs were analyzed statistically. Most post-injection data include 6 dogs at each time interval. The same statistical analyses were also made comparing the experimental dogs to the 0-level controls at 3, 6, 9, 12, 15 and 18 months post-injection. There were only two dogs at the 5-level (T1W5, T2W5) which were on experiment beyond three weeks so that no statistical analyses could be made at this level.

Results

Acute and Subacute Changes at the Highest Dose Levels

These are shown in Fig. 1 for the first three months post-injection for dogs T1W5, T2W5 (2.8 μCi/kg) and dogs T3W4 and T4W4 (.85 μCi/kg).

The pmns (and consequently the WBC which are made up of 55-65% pmns) fall to minimal values at three weeks to one month followed by a slight recovery by two months post-injection. The lymphocytes, particularly at the higher dose level, fall to one-half of pre-injection values at one month and remain depressed over the three month period. Dog T4W4 shows little early effect on the lymphocytes. Monocytes and eosinophils (not graphed) followed the pattern of pmns..

The red cells (as represented in Fig. 1 by VPRC) decrease during the first three months in the two 5-level dogs. This is

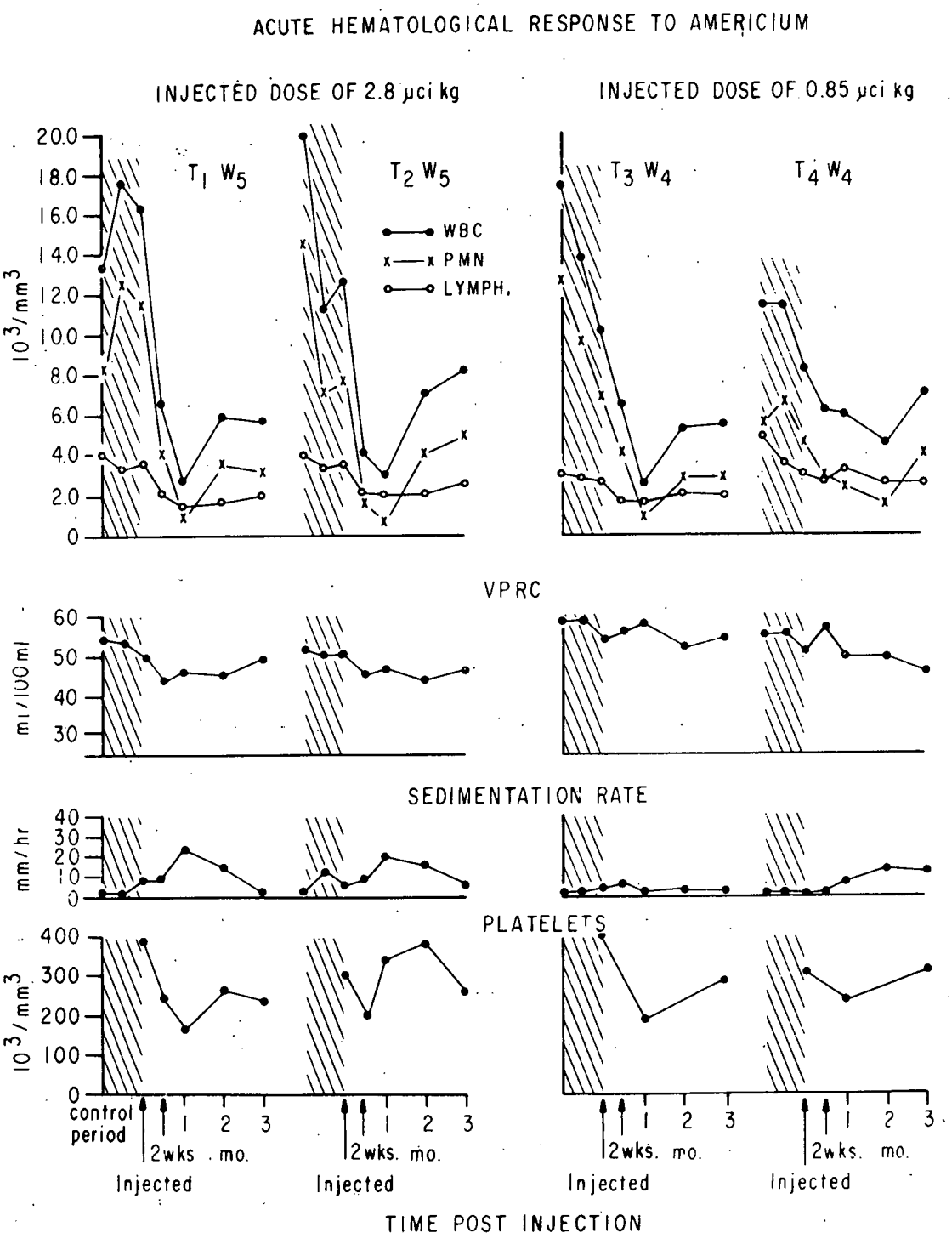

Figure 1. Acute hematological response of four dogs (T1W5, T2W5, T3W4, T4W4) to ^{241}Am.

accompanied by an elevation in sedimentation rate. There is no appreciable change in the VPRC or sedimentation rate of T3W4. However, there is a fall of VPRC in T4W4 and rise in sedimentation rate. The platelets fall after injection with lowest values at one month. These also show a subsequent rise toward normal values.

The two 5-level dogs survived thirteen and fifteen months, respectively. During this time all types of white cells remained depressed. Beginning at ten months, a moderate anemia and thrombocytopenia developed and was progressive until death. Another terminal change was an increase in immature or stab pmns which rose from pre-injection values of less than 1% to between 15-20% of the total WBC. There were also atypical pmns in the blood terminally.

Chronic Hematological Changes at the 0.2, 0.5, 1, 1.7, 2, 3, and 4-level.

There have been no significant changes in blood cells in dogs receiving the two lower dose levels (0.2 and 0.5 level) over the period studied. In order to simplify the graphs, those dose levels which did not show any significant changes from either pre-injection values of that particular level or from 0-level controls were omitted from Figs. 2 and 3.

Fig. 2 relates the various dose levels to their pre-injection mean values and Fig. 3 compares the different dose levels of americium to 0-level control dogs. A large solid dot at a particular time interval indicates the mean value at that time is significantly different from the particular standard used ($P < 0.05$).

A depression in red cells (as shown by a decrease in VPRC)

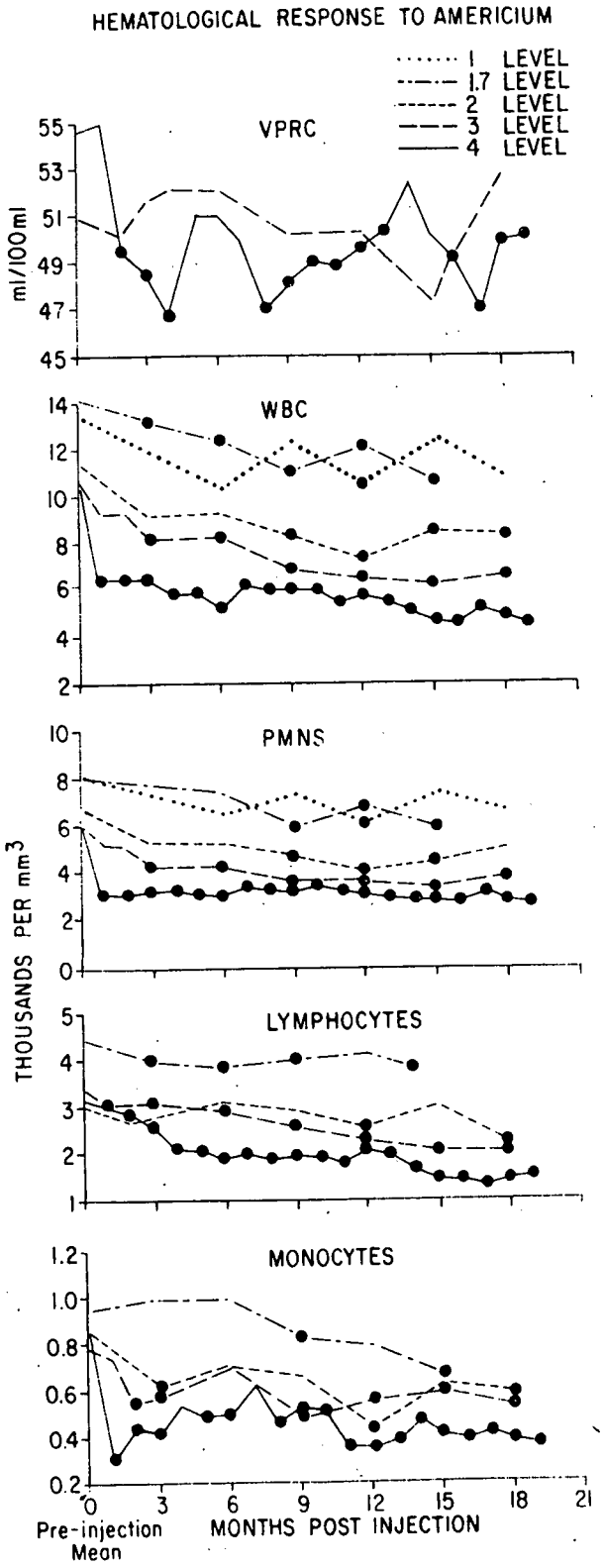

Figure 2. Hematological response of ^{241}Am injected dogs relative to pre-injection values. (Solid dot indicates $P < 0.05$).

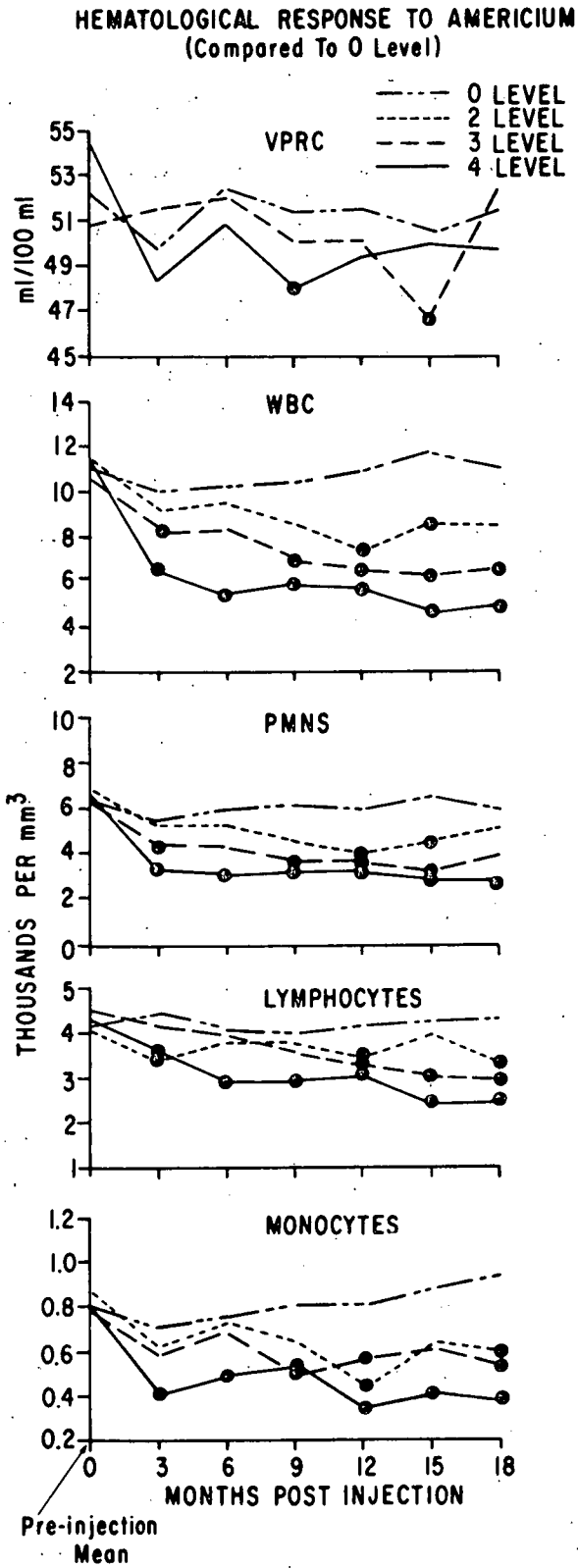

Figure 3. Hematological response of ^{241}Am injected dogs relative to 0-level controls. (Solid dot indicates $P < 0.05$).

was found to be significant most frequently in 4-level dogs. In Fig. 2 which shows monthly means, there seems to be a cyclic response with depression beginning at one month and then periods of recovery (e. g., at 4 and 5 months) and subsequent depressions. Fig. 3 shows the VPRC decrease is only significantly different from 0-level dogs at 9 months. The mean values of 3-level dogs show no significant change from pre-injection values (Fig. 2) although at 15 months it did differ from 0-level dogs (Fig. 3). The red cell changes are much less marked than those of the white cells.

The leukocyte changes are shown in both Figs. 2 and 3 for total WBC and the three most prevalent types of leukocytes. Eosinophils, which are present in fewer numbers, also were included in the analyses, but not graphed for lack of space. The depressions of eosinophils closely follow those of pmns. Blood basophils are present in very small numbers in the dog (less than 1/4%) so were not included in the analyses.

The results from both types of comparisons, i.e., pre-injection means and 0-level dogs, are generally quite similar. The main exception is the 1.7 level which had unusually high pre-injection white cell values and the observed, lower post-injection values are consequently statistically significant (Fig. 2). The white cell counts of these dogs are still within normal range and are not significantly different from 0-level dogs. Also, the pmns of 1-level dogs which did seem to decrease after injection (significant at 12 months, Fig. 2) did not at any time differ from 0-level controls.

In summary, all white cell values are depressed at all time intervals in the 4-level dogs with no tendency toward recovery. The 3-level shows much the same trend although the white cells fall more slowly and do not reach as low levels. The 2-level dogs (particularly when compared to 0-levels) do not show significantly low values until about 12 months post-injection. It is doubtful whether there is any real effect on white cell values of the 1.7 and 1-level dogs.

Since ^{241}Am and ^{239}Pu both deposit in the skeleton on bone surfaces [1] and since comparable amounts were injected at each dose level, it is of interest to compare their hematological responses. Leukocytes are depressed at the 2, 3, 4 and 5-dose levels for ^{239}Pu [2] as well as for ^{241}Am. The minimal mean values also are similar over the 18 months period studied. The red cells show an early as well as terminal depression only at the 5-level for both nuclides. There are not enough dogs injected with the 5-level dose of ^{241}Am to make any meaningful comparisons of the degree of anemia and leukopenia. Blood platelets show an early transient depression at the two highest levels for both nuclides.

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MODIFICATION OF TUMOR GROWTH WITH DRUG AND DIET MANIPULATION

D. H. Taysum and D. Brammer

Abstract: When heat labile constituents of a synthetic diet of soy bean and wheat flour plus non-iodized salt are subjected to 120° C for 24 hours and an ascorbic acid antagonist, d-glucoascorbic acid, is employed, tumor growth is retarded, onset is delayed and time to death is extended for B-16 melanoma in C57 Bl mice.

Introduction

In earlier experiments [1] with Bl6 melanoma implants in C-57Bl mice it appeared possible to significantly alter the growth rate as well as the relative number of tumor takes. The postulated mechanism was that interference with fibroblastic function and endothelial cell integrity by blocking synthesis of ascorbic acid and eliminating vitamin C from the diet produced the effect. In subsequent experiments reported herein, additional factors are implicated. These factors were revealed when the synthetic diet used in the first experiments was subjected to 120°C for 24 hours to further assure that there would be no ascorbic acid in it.

The cooked synthetic diet has had the heat labile materials it contained denatured. The effect of denaturing these materials is the alteration of growth of the implanted B-16 melanoma and the delaying of the visible onset of tumor growth. The cooking caused a loss of weight when fed to normal non-implanted mice as can be seen in Figure 1.

Materials and Methods

The antogonist of ascorbic acid was d-glucoascorbic acid (2, 3-enediol-d-glucohepton-1,4-lactone). This compound was a gift of the Wallerstein Company. It was used in the first series of experiments as an I.P. injection at a concentration of 0.1 molar or 2.0 milligrams in a 0.1 milliliter injection on a five day per week schedule.

In all following experiments the d-glucoascorbic acid was added to the drinking water in such concentration as to be equivalent to the amount received by daily injections.

The synthetic diet was made from 300 grams of soy bean flour, 600 grams of whole wheat flour and 16 grams non-iodized salt. This diet when pressed into a cake is well tolerated by the mice, and they present the appearance of healthy active mice. When it is cooked at 120° c for 24 hours, it is altered to the point that mice fed this diet will lose weight. The tumor was the B-16 melanoma which we have carried by serial animal passage for many years. The transplant method which is standard in this laboratory was as follows: a donor mouse with a tumor of approximately 2 grams was decapitated. The tumor site, the exterior of the right hind thigh, was swabbed with alcohol. The skin only was opened and laid back, and the tumor capsule was opened with a sterile scalpel; a 2.5 ml polypropylene syringe without needle was inserted and the tumor was drawn into the syringe. The volume of material in the syringe was adjusted to 1.0 ml by expressing air and tumor material. The syringe was then fitted with a 20 guage needle and the tumor

was forced into a 30 ml rubber-topped bottle containing 5 ml of saline. The syringe was then filled and emptied 10 times. This formed the suspension that was given in 0.2 ml aliquots. The implant was performed by anesthetising the recipient mouse and inserting the needle from the anterior of the mid right thigh through the muscles until the tip was just below the skin on the outer aspect. The plunger was depressed and the 0.2 ml of tumor suspension was deposited in the region of the outer mid-thigh.

The tumor implanted in this manner takes in almost 100% of routine implants and has never spontaneously regressed.

Only C57 Bl mice and a spontaneously arising albino mutant were used. The mice are derived from the C57 BL mice maintained by Professor T. F. Dougherty of this University. The animals were housed in plastic cages on butcher's sawdust bedding in an air conditioned room at 72 ± 2°F. Mice of 12 to 14 weeks of age were used in all experiments.

Results

Figure 1 is presented to illustrate the effect of the cooked diet on five normal mice. These mice were fed the regular laboratory diet, Purina Micro Mixed Chow, then after one week they were placed upon the cooked synthetic laboratory diet for eleven days and then returned to the regular colony diet.

Figure 2 is a graph illustrating the difference between two groups of tumor implanted mice when one group was given regular diet and tap water and the other group received cooked synthetic

diet plus tap water. The apparent weight gain of the mice on regular diet was largely due to the growth of the tumor as was the weight gain of the cooked-synthetic-diet mice when their tumors begin to grow toward the latter part of the experiment.

Figure 3 illustrates the growth pattern differences when mice were fed regular Purina Micro Mixed food as contrasted with the cooked synthetic diet with and without d-glucoascorbic acid in the drinking water. As can be seen, the cooked diet and the cooked diet with d-glucoascorbic acid modified the time course of tumor growth.

Figure 4 illustrates the apparent influence of factors other than ascorbic acid upon the growth pattern since the regular diet mice and the mice receiving a Nutrional Biochemical Co. vitamin C-free diet plus d-glucoascorbic acid were practically identical, and the cooked synthetic diet plus d-glucoascorbic acid mice showed a different onset time with increased longevity.

The cooked synthetic diet is probably deficient in many factors, most of which are vitamins, although the essential fatty acids could be oxidised by such treatment. What other nutritional factors could have been altered is not known.

Figure 5 illustrates the difference in growth pattern when the tumor was implanted in a spontaneously arising albino of C57 Bl parent mice. The difference once again between mice on regular Purina Micro Mixed food plus tap water and mice fed the cooked synthetic laboratory diet plus d-glucoascorbic acid in tap water is readily seen.

It is questionable as to how much effect is attributable to the

d-glucoascorbic acid when the cooked synthetic diet was fed. Figure 6 shows how similar the pattern is when the only difference was d-glucoascorbic acid given to one group and not to the other. It is noted however, that two mice in the d-glucoascorbic acid group did not develop tumors.

Figure 7 illustrates that cooking the diet and adding d-glucoascorbic acid to the drinking water did have an effect but that this was variable from experiment to experiment. Sometimes the largest seeming variation was produced by a single control mouse that succeeded in living as long or longer than the mice in the treated group.

Discussion

In an extensive paper by Drummond [2] the results of dietary manipulations as he and others employed them, gave little indication of success in preventing tumor growth by restricting the dietary intake of the host. It would seem that the reason for this lack of success lies in the fact that the blood stream in its continued circulation through the tumor is filtered, so to speak, of whatever metabolite the tumor requires, and that even when the host mobilizes body tissues in an effort to meet its own needs the tumor continues to intercept these materials, effectively removing them from the bodily economy.

The particular point that this paper would make is that if one considers the ground substance surrounding all cells as an extension of the vascular system and, further, that the efficiency of the capillary bed can be altered by withholding ascorbic acid and block-

ing its synthesis, then the efficiency of the tumor in removing from the circulation the materials required for its synthesis of more cells would be reduced. That this would result in an elimination of vigorously synthesizing cells is not expected. Instead, it might be expected that a significant change in the time course of the neoplastic proliferation would be observed. The question then becomes: if these cells can be denied the materials which they must have for synthesis of more cells, will synthesis stop, and will cell division be stopped? What becomes of a cell that does not stop dividing if it can not get the materials to build more cells? The work related in this study does not answer the above questions. These are to be answered by further studies when refinements have been made upon the process of reducing the efficiency of the blood-to-tumor cell transport of essential materials.

References

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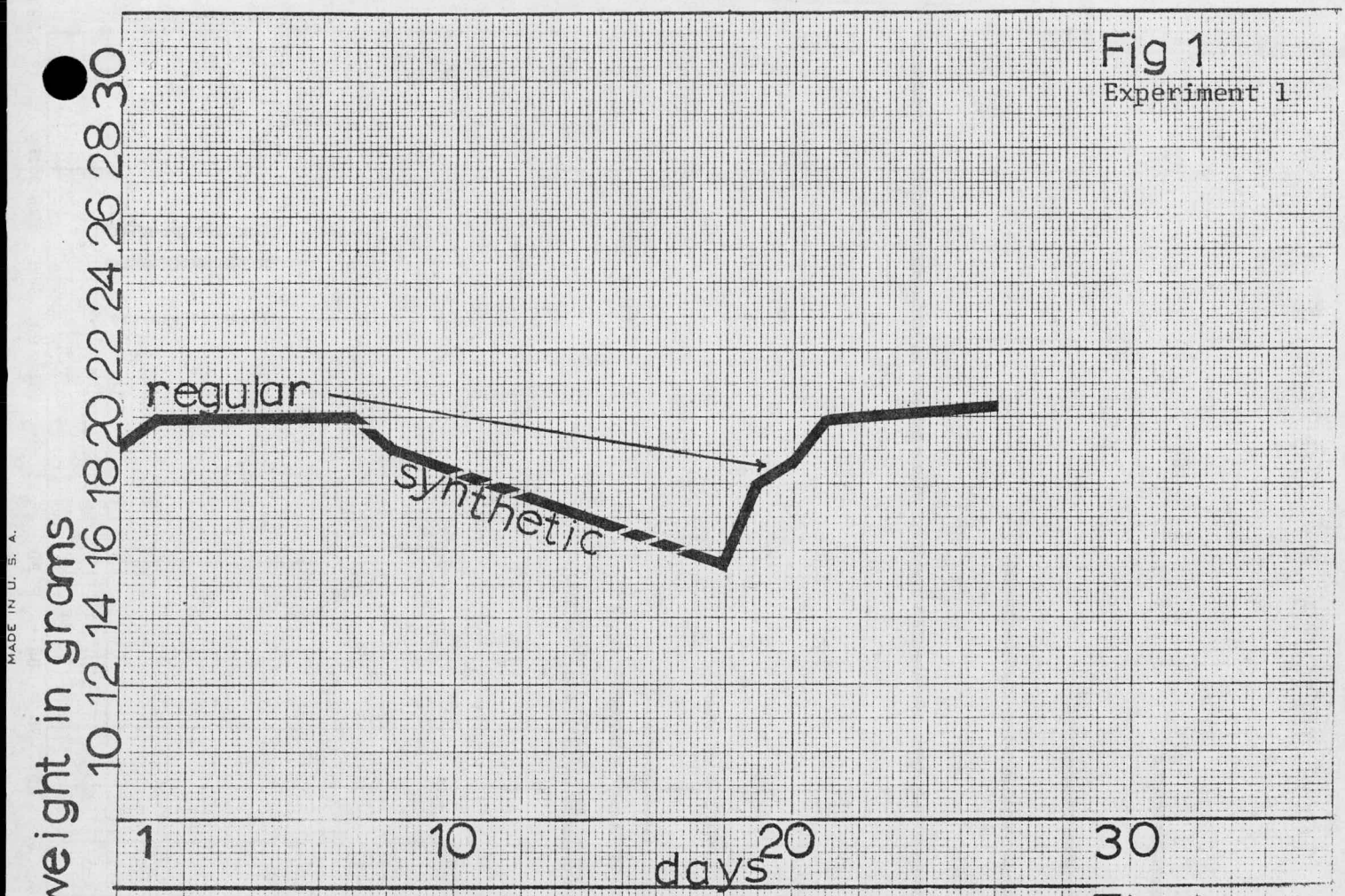
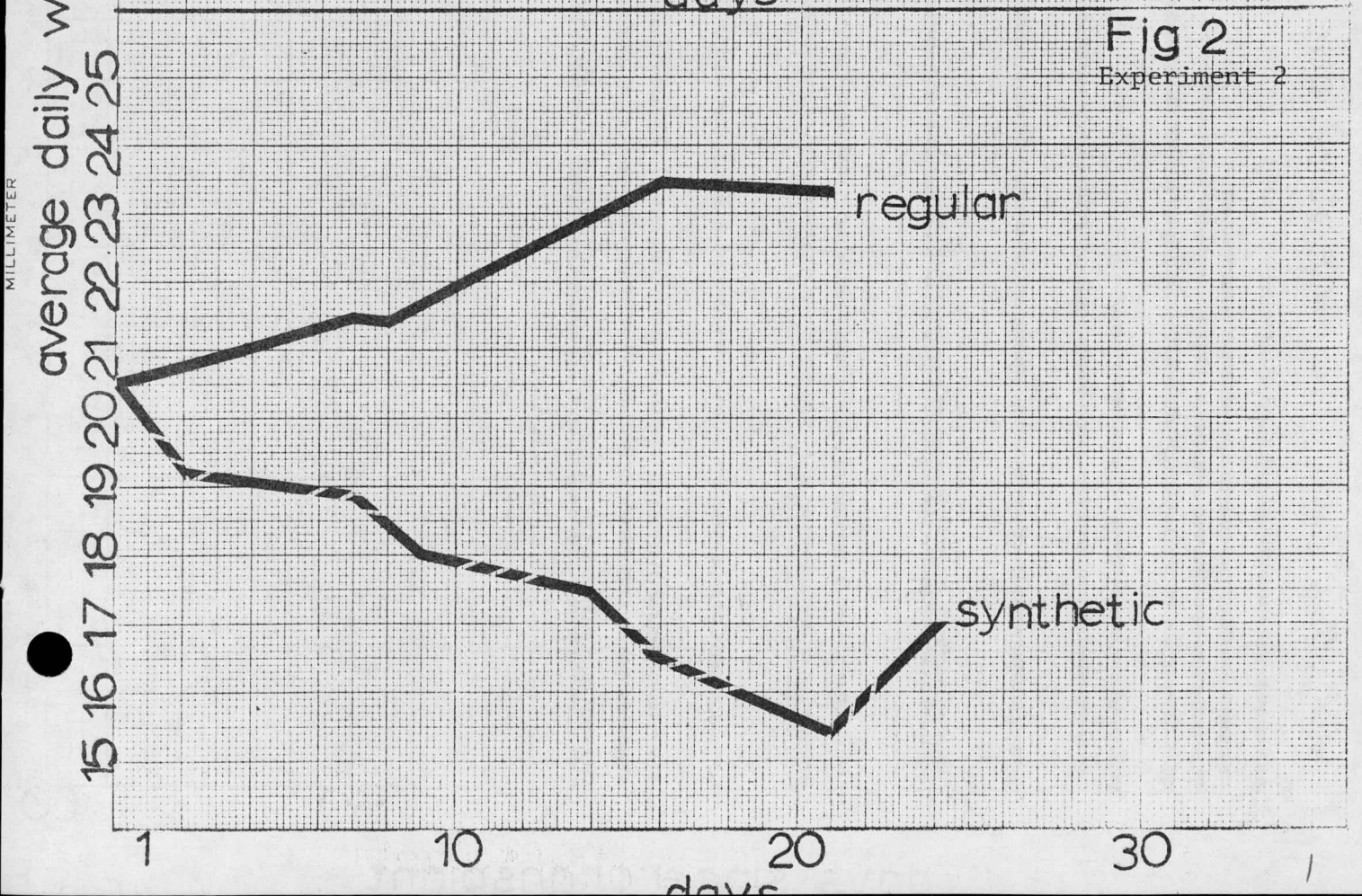
3. Note: Other than the article by Drummond in 1917, two articles of interest appeared in Cancer Research 29, (December 1969) by J. R. Bertino and P. F. Nixon (p. 2417) and by Charles A. Nichol (p. 2422). Both of these articles are upon the subject of the nutritional factors and manipulation of metabolism.





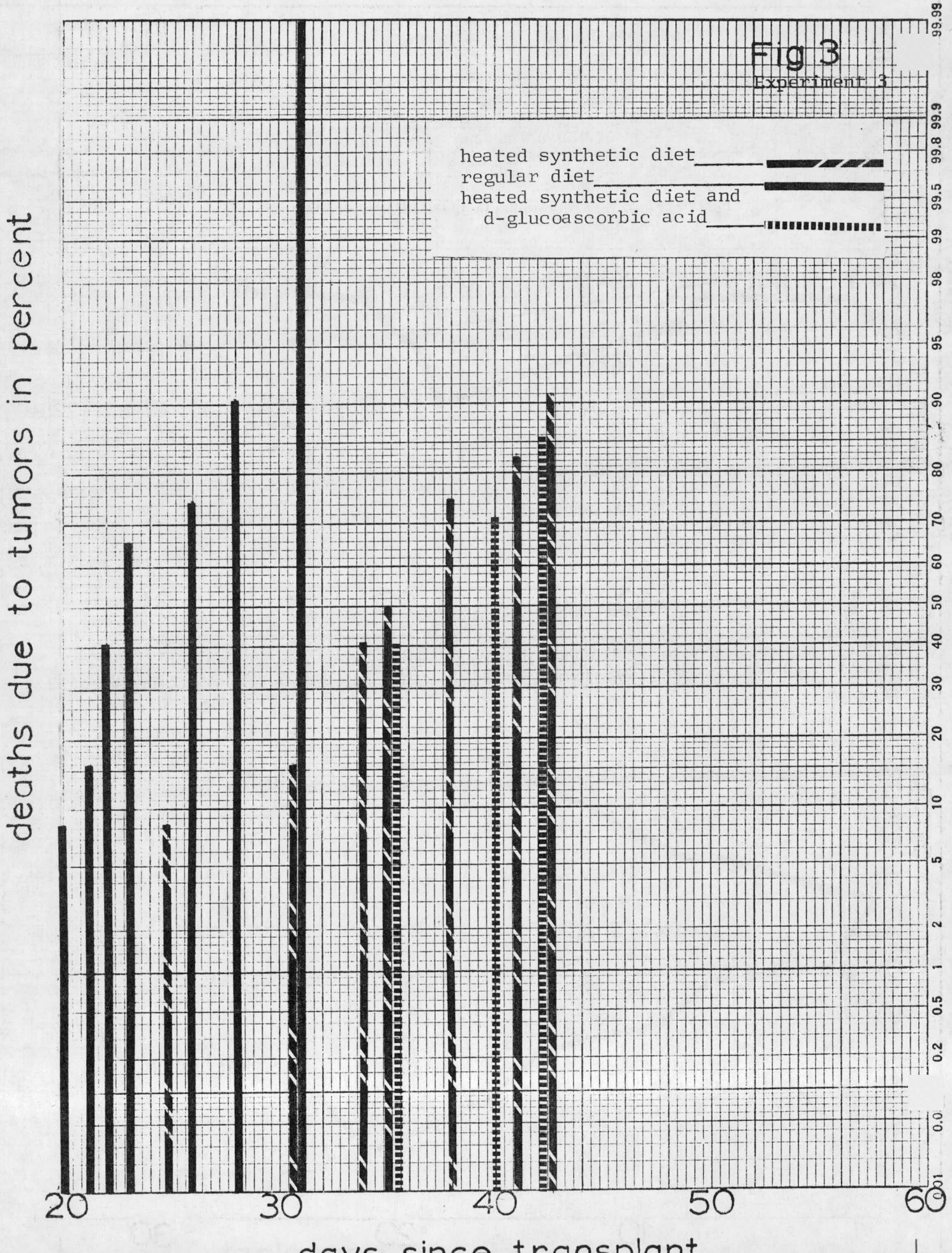
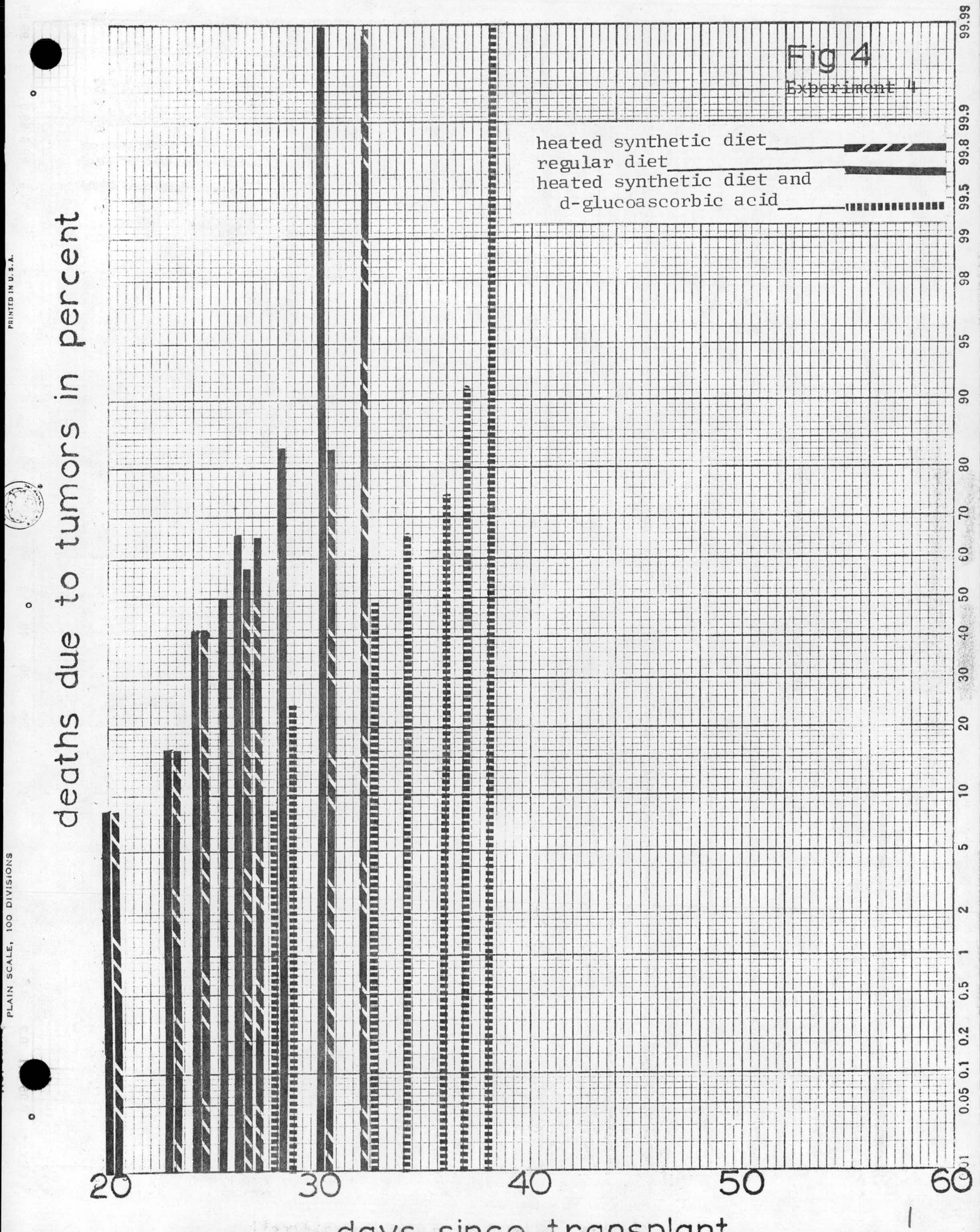


Fig 3
Experiment 3
heated synthetic diet
regular diet
heated synthetic diet and
d-glucoascorbic acid
deaths due to tumors in percent
99.99
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.0
20
30
40
50
60
days since transplant



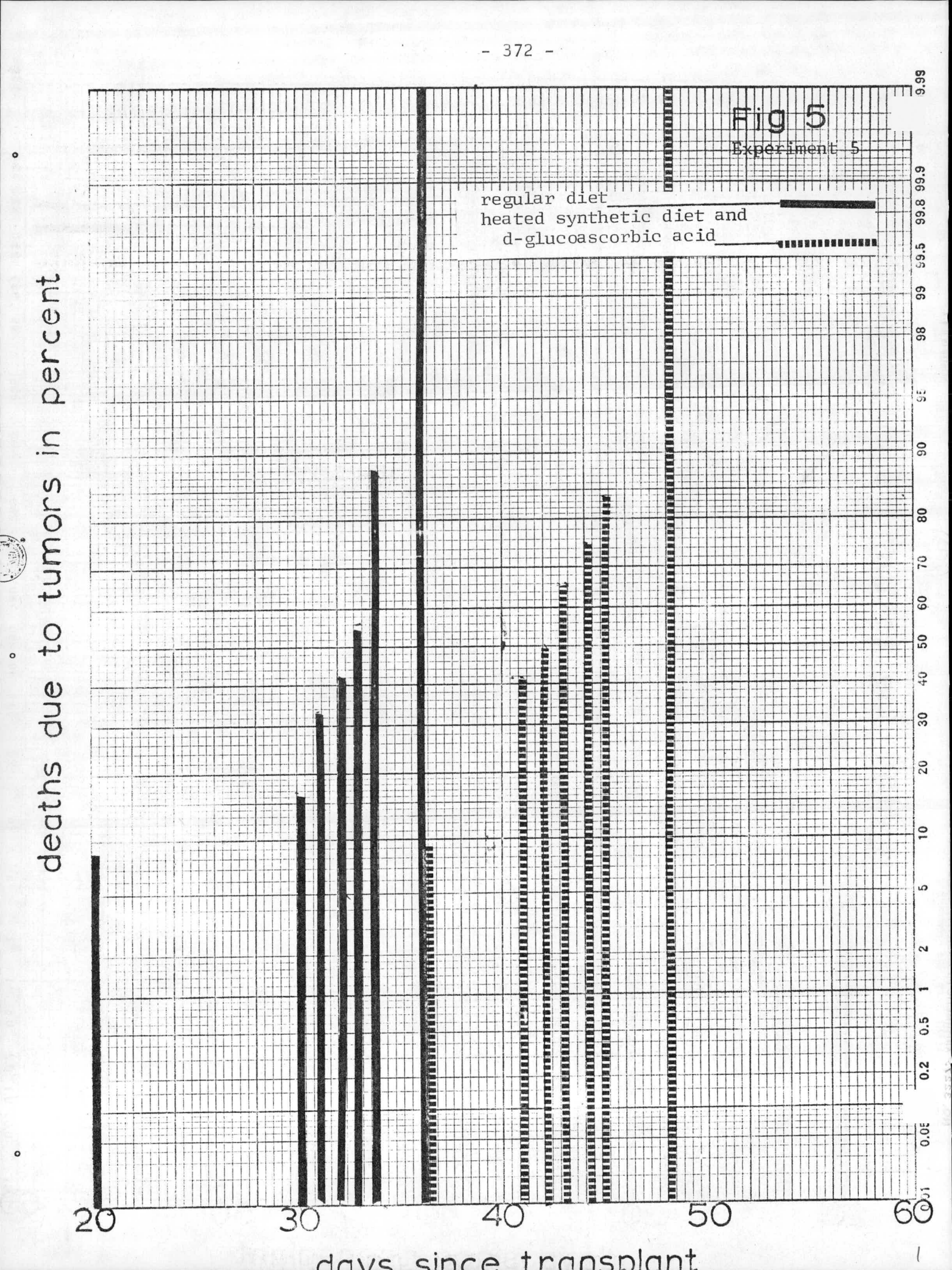


Fig 5
Experiment 5
regular diet
heated synthetic diet and
d-glucoascorbic acid
deaths due to tumors in percent
9.99
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.05
20
30
40
50
60
days since transplant

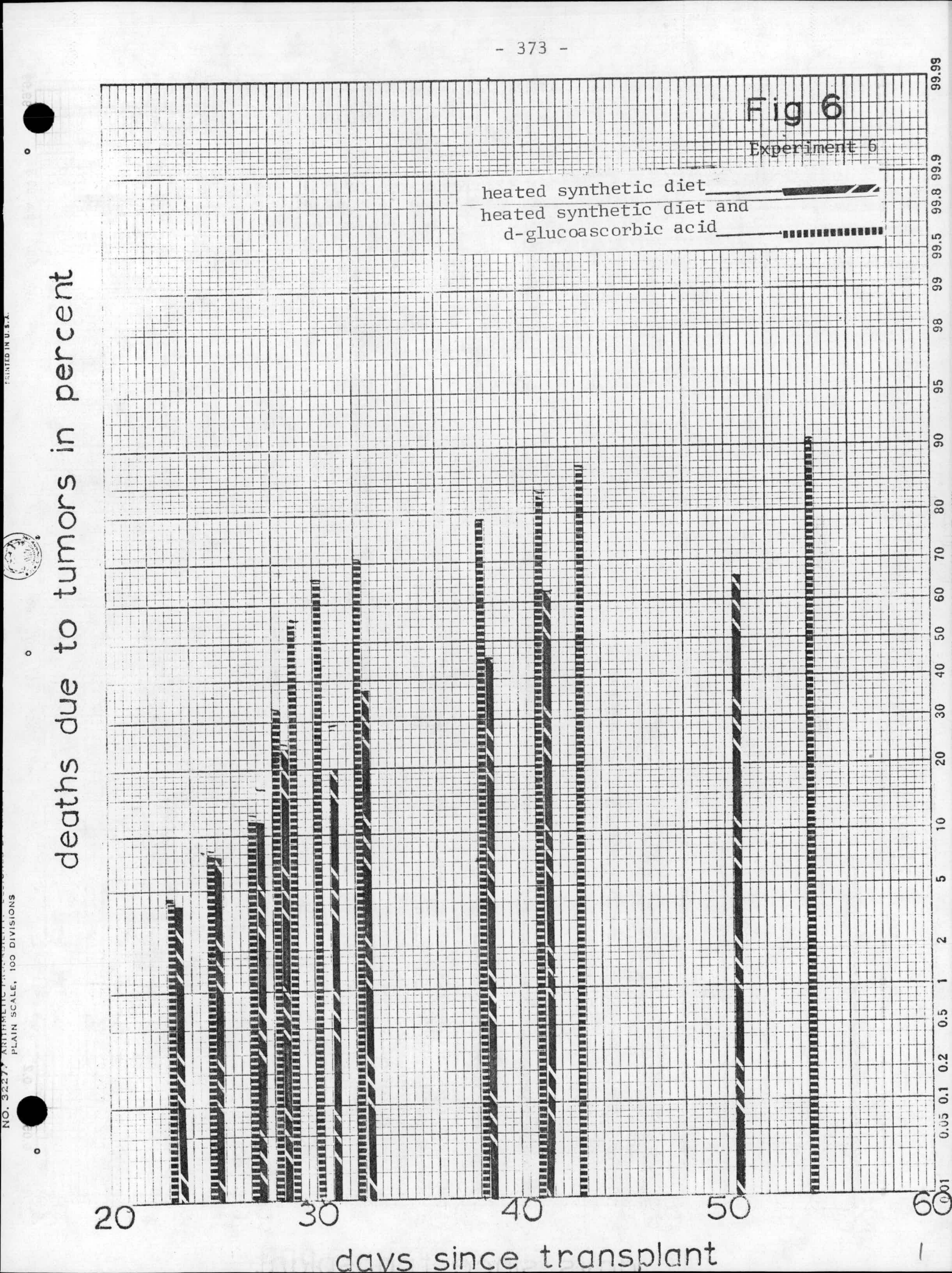


Fig 6
Experiment 6
heated synthetic diet
heated synthetic diet and
d-glucoascorbic acid
deaths due to tumors in percent
99.99
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
20
30
40
50
60
days since transplant

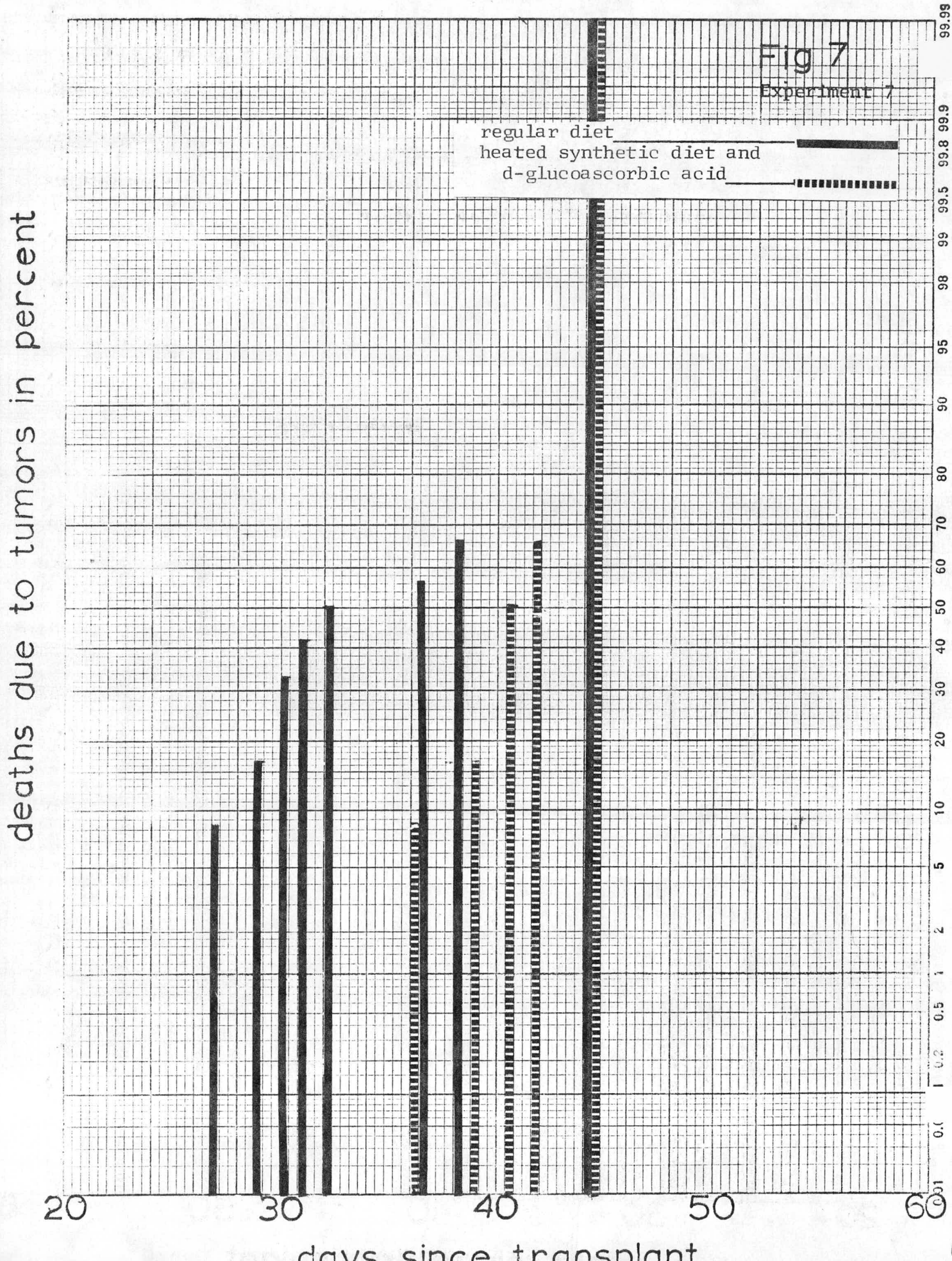


Fig 7
Experiment 7
regular diet
heated synthetic diet and
d-glucoascorbic acid
deaths due to tumors in percent
99.99
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
20
30
40
50
60
days since transplant

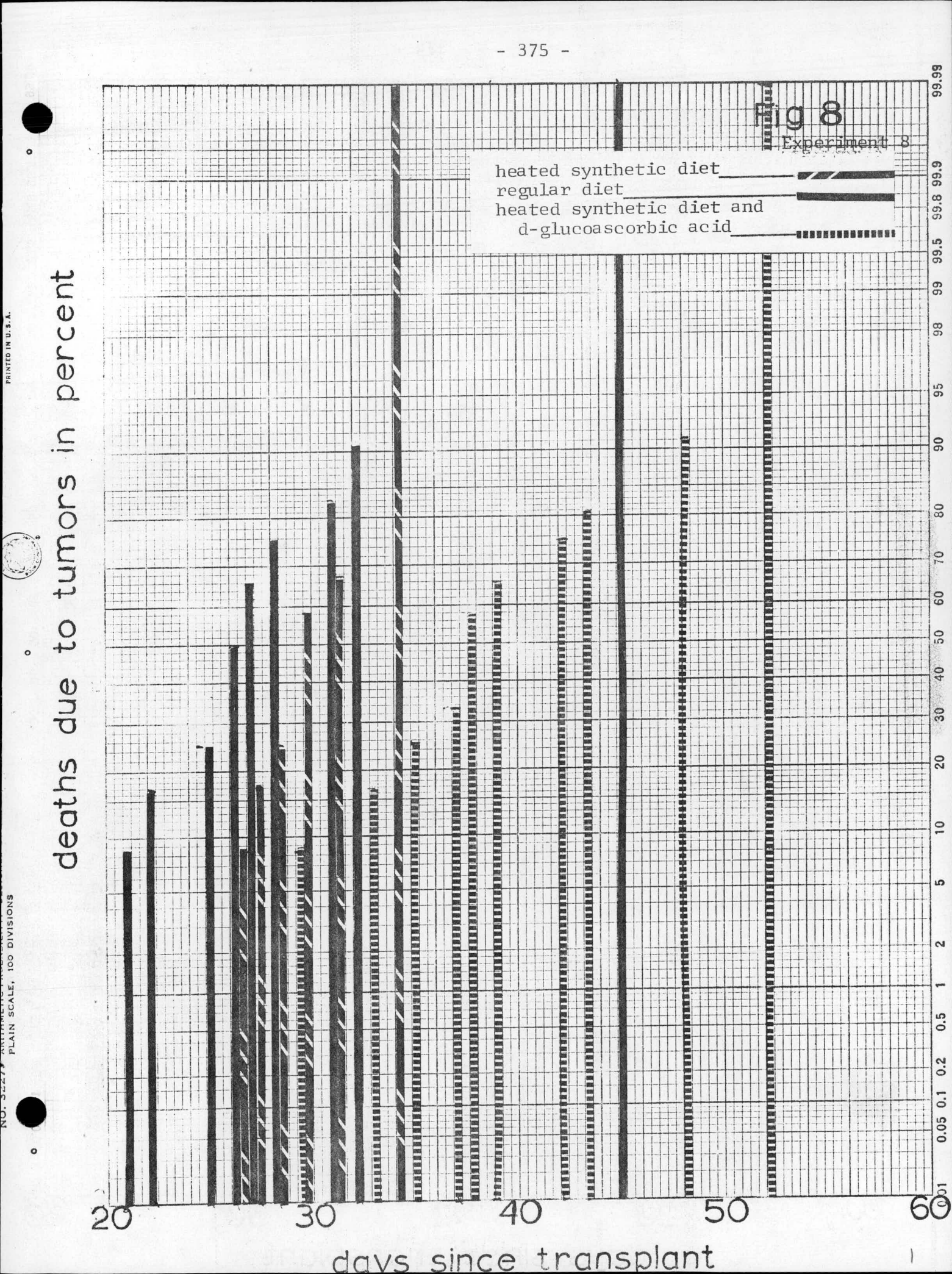


Fig 8
Experiment 8
heated synthetic diet
regular diet
heated synthetic diet and
d-glucoascorbic acid
deaths due to tumors in percent
99.99
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
20
30
40
50
60
days since transplant

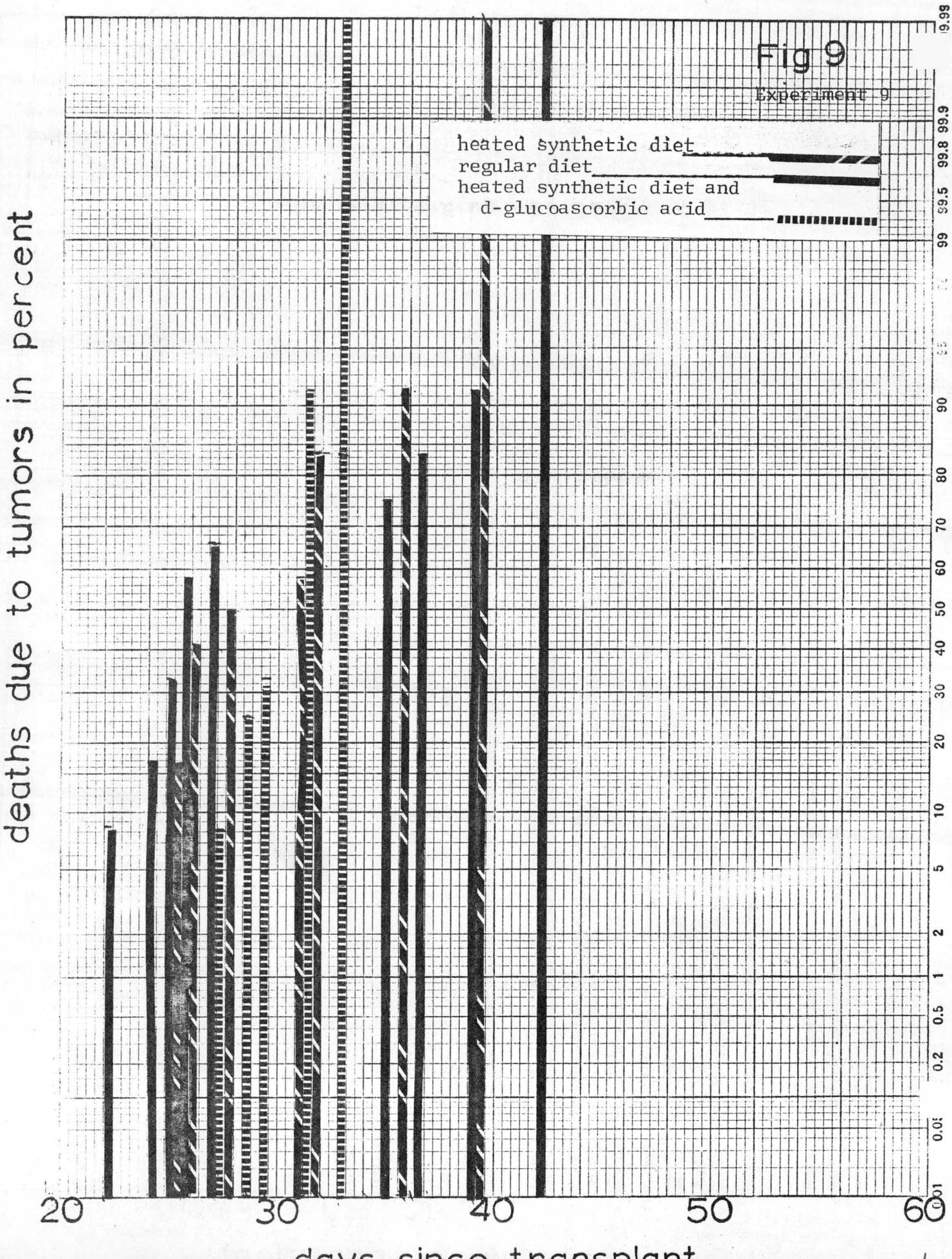


Fig 9
Experiment 9
heated synthetic diet
regular diet
heated synthetic diet and
d-glucoascorbic acid
deaths due to tumors in percent
99.99
99.9
99.8
99.5
99
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.01
20
30
40
50
60
days since transplant

RETENTION OF INJECTED ^{239}Pu BY CHINCHILLAS

Ray D. Lloyd, Charles W. Mays, Glenn N. Taylor

and David R. Atherton

Abstract - The total-body retention of ^{239}Pu has been determined in 6 adult male chinchillas. Measurements extended to about 1000 days after intravenous injection in citrate buffer. Retention is lower in chinchillas than in dogs. In these chinchillas ^{239}Pu was retained as if 63% of the injected atoms had a biological half-time of about 11 days and 37% had a half-time of 1475 days. In 2 animals dying 148 and 162 days after injection, about 85% of the retained plutonium was in the skeleton, 9% in the liver, and 6% in other soft tissues.

Methods

^{239}Pu was administered to 6 chinchillas (Table 1) as an intravenous injection in a sodium citrate citric-acid buffer solution (pH 3.5). Plutonium retention was determined by total-body counting for about 1000 days after injection, and the distribution of retained activity in the skeleton, liver and other soft tissue was determined in two animals.

Although ^{239}Pu emits gamma-rays (1-6) in only about 0.02% of its disintegrations*, body burdens of animals containing about 1 μCi or more can be determined by total-body counting (7). Retention measurements in this study were made in our low background chamber with 12 inch thick steel walls. The 100 keV photopeak was utilized in the determinations. Each chinchilla was counted inside a restraining cage made of 1/16 inch thick

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* Gamma-ray abundances (γ rays/disintegration) listed in Reference 1 are: 39 keV = 2×10^{-5}; 53 keV = 7×10^{-6}; 100 keV = 5.5×10^{-6}; 124 keV = 2.5×10^{-5}; and 384 keV = 1.5×10^{-5} (See Figure 1).

lucite which kept its body in a standard position with its midline 5 cm from the face of an 8 inch diameter x 4 inch thick NaI (Tl) crystal (Figure 2). A thin-walled glass ampoule containing a known activity of ^{239}Pu in 10 ml of solution was also counted at the same distance from the crystal. To approximate the photon self-absorption of a chinchilla's body, the ampoule was placed inside a 5.8 cm external diameter x 10.3 cm long cylindrical lucite absorber containing a 2 cm diameter axial tunnel. Calibration of this counting system was accomplished by counting 2 animals intact, then determining the total ^{239}Pu content of each following autopsy by counting the individual parts (defleshed skeleton, liver and residual soft tissue) under standard conditions of geometry and sample self-absorption. The partitioning of activity among these 3 compartments was also determined at the same time.

A semi-log plot was made of the percent biological retention of each animal as a function of time after injection (Figure 3). Lines connecting the points representing serial measurements of the same animal were drawn, and an average retention curve was fit through the data points roughly parallel to the slopes of the individual lines for all the animals.

Results and Discussion

The total-body content of ^{239}Pu was determined for each of the 6 animals until about the time of death. None of the deaths could be ascribed to the effects of ^{239}Pu. In these chinchillas, ^{239}Pu was retained during the first 1000 days as if 63% of the

injected atoms had a biological half-time of about 11 days and 37% had a half-time of about 1475 days (Table 2, Figure 3). The retention at 1000 days was about 22% whereas the 1000 day retention in the beagle is about 65% (8,9). A corresponding lower retention by chinchillas as compared to dogs injected with ^{226}Ra has also been reported (10).

The distribution of plutonium in 2 chinchillas is shown in Table 3. About 85% of the retained activity was in the skeleton, about 9% in the liver, and the remaining 6% in other soft tissues. It appears that in the chinchilla monomeric plutonium clears quickly from the liver. The highest concentration of ^{239}Pu activity, and therefore the highest average dose rate, is in the skeleton, which represents about 7.1% of total-body weight. A total of 37.7 g and 37.3 g of carefully defleshed and water soaked skeleton was found in 2 animals weighing 518.4 g and 539.0 g respectively (7.3% and 6.9%). The liver had only about 1/4 to 1/2 of the mean skeletal concentrations (12 chinchilla livers averaged 9.42 g each). Unfortunately, neither skeletal weights nor liver weights are available for the 2 animals listed in Table 3.

Acknowledgments

We are grateful to David H. Taysum and Richard L. Stair for the design and construction of the restraining cage and cage positioner.

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Table 1

Chinchillas Used in These ^{239}Pu Studies

Animal	Days of Age at Injection	Weight at Injection (kg)	Injected μCi ^{239}Pu/kg	Days after Injection at Death
C1P5	584	0.468	2.58	212
C3P5	598	0.510	2.59	1039
C1P6	472	0.460	7.73	61
C3P6	400	0.460	7.73	474
C1P7	524	0.404	23.2	116
C3P7	630	0.440	23.2	157

Table 2

Biological Retention of Injected ^{239}Pu in 6 Chinchillas Determined by Total-Body Counting

Days after Injection	Percent Retention					
	C1P5	C3P5	C1P6	C3P6	C1P7	C3P7
7	57.7	75.3	64.6	80.3	60.6	79.6
25	39.8	51.0	47.6	53.5	41.6	55.4
82	28.7	34.7		43.7	29.8	44.0
148	30.0	38.2		47.1	27.5	44.7
162						38.6
427		21.7		33.0		
987		22.0				

Table 3

Distribution of Retained ^{239}Pu in 2 Chinchillas

Animal	Days after Injection	Fractional Total-Body ^{239}Pu Retention	Percent of Retained ^{239}Pu		
			Skeleton	Liver	Other Soft Tissue
C1P7	148	0.275	80.7	12.2	7.1
C3P7	162	0.386	89.4	4.9	5.7
Average			85.0	8.6	6.4

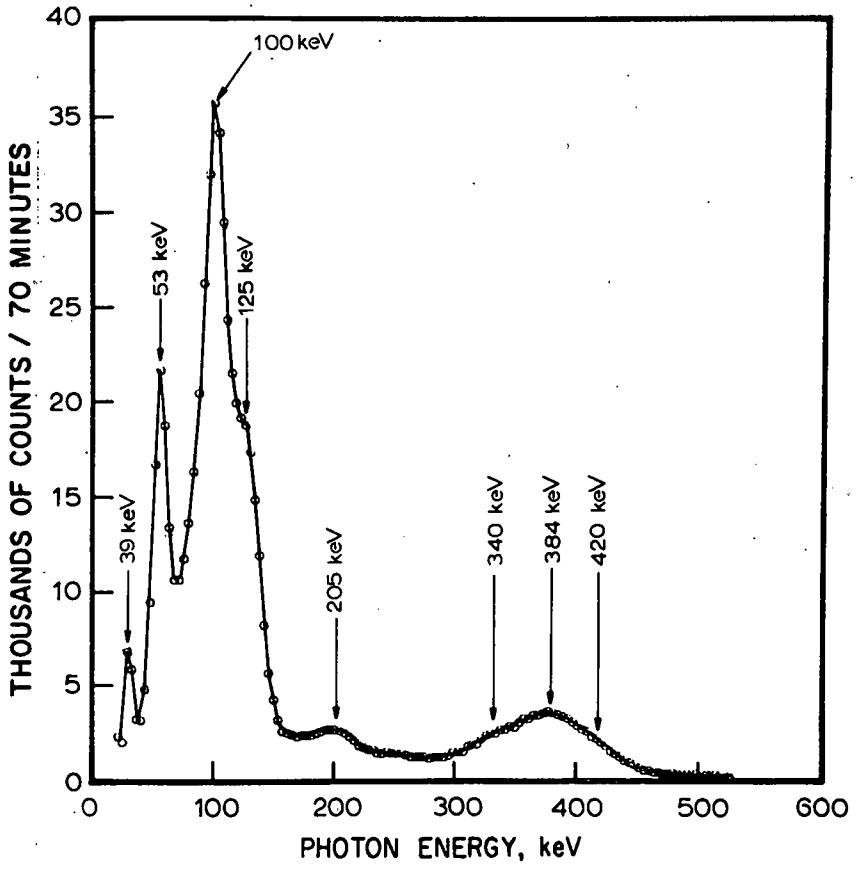

Figure 1. The photon spectrum of 54.4 μCi of ^{239}Pu in a 10 ml glass ampoule with an inside radius of 0.88 cm. Its center was 2.35 cm from the face of an 8 inch by 4 inch NaI (Tl) crystal in a 0.02 inch thick stainless steel container. The radiation was further attenuated by 1.5 cm of plastic.

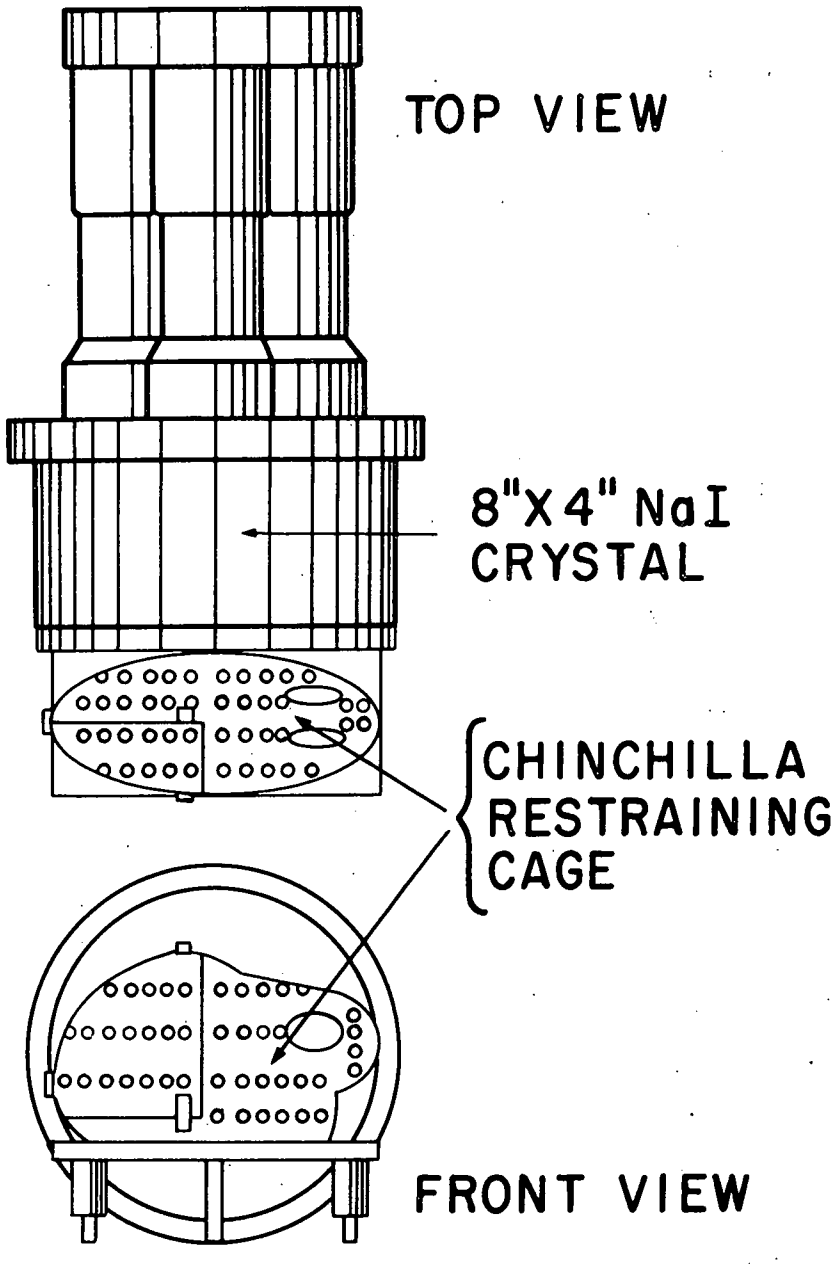

Figure 2. Representation of the total-body counting system for measuring ^{239}Pu in chinchillas.

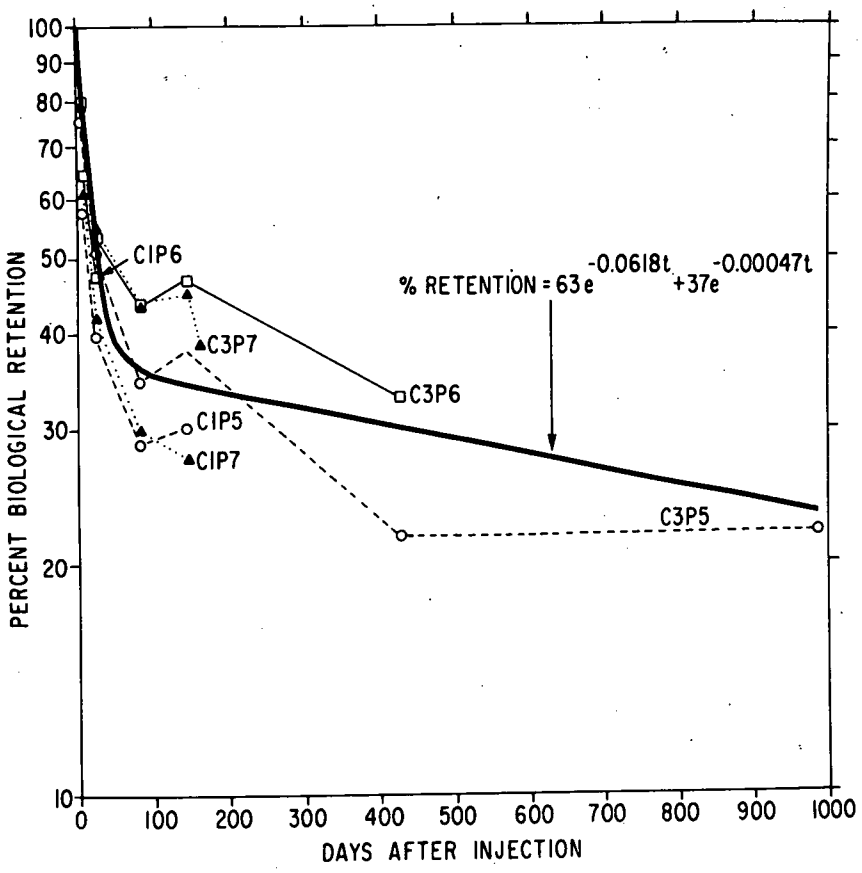

Figure 3. Biological retention of injected ^{239}Pu in chinchillas. The code number for each animal is shown, and the equation gives representative retention. We have elected to represent the average retention of ^{239}Pu in chinchillas by a sum of two exponentials. Other equations may fit the data at least as well.

BONE CANCER INDUCTION BY RADIONUCLIDES: INCIDENCE vs. DOSE

Charles W. Mays, Thomas F. Dougherty, Glenn N. Taylor, Betsy J. Stover, Webster S. S. Jee, William R. Christensen, Jean H. Dougherty, Walter Stevens, Jr., and Charles J. Nabors, Jr.

Abstract: Dose-response relationships are reviewed for several bone-seeking radionuclides in dogs, rodents and humans. This includes beagles injected with ^{239}Pu, ^{228}Th, ^{228}Ra (MsTh), ^{226}Ra, or ^{90}Sr at the University of Utah; rats injected with ^{90}Sr by Y. I. Moskalev et. al.; mice injected with ^{90}Sr or ^{226}Ra by Miriam Finkel et. al.; Dial painters and other humans containing ^{226}Ra and ^{228}Ra (MsTh) and studied by R. D. Evans et. al. and A. J. Finkel et. al.; and German patients injected with ^{224}Ra (Th-X) who are being followed by H. Spiess.

For the β-emitter ^{90}Sr, a sigmoid dose-response relationship is seen, characterized by a low-risk region in which few if any bone cancers have been induced. This suggests that considerable recovery from β-irradiation is possible, provided that the dose-rate is sufficiently low.

For the α-emitters, data on ^{226}Ra in humans support a practical threshold, below which few if any cancers should be induced; while data on ^{226}Ra in mice support a linear relationship in which cancer induction increases in direct proportion to dose, provided that the dose is not excessive. The other α-emitter results, each considered separately, can rule out neither the linear nor the threshold models although taken collectively, the threshold model seems better supported. It is possible that in general the most probable response at low dosage is a sigmoid relationship somewhere in between the linear and threshold models.

INTRODUCTION

The shape of the dose-response curves at low dosage is of practical importance in radiation protection, and of fundamental significance in understanding the mechanism of radiation-induced cancer.

RESULTS

Table 1 references the data to be presented in Figures 2-11 and their associated Tables. A linear dose-response was fit to each set of plotted data, such that the sum of sarcoma cases, predicted for all plotted points, matched exactly with their observed total. For the low-dose levels, the observed cases of bone sarcoma are compared with those predicted from the linear model.

Table 1. DATA SUMMARIZED IN THIS REPORT

Nuclides and route of administration	Figures	References
^{239}Pu citrate injected i. v. into adult beagles	2	(1,2,3,4)
^{228}Th citrate injected i. v. into adult beagles	3	(1,2,3,4)
^{228}Ra (MsTh) cit. inj. i. v. into adult beagles	4	(1,2,3,4)
^{226}Ra citrate injected i. v. into adult beagles	5	(1,2,3,4)
^{90}Sr citrate injected i. v. into adult beagles	6	(1,2,3,4)
^{90}Sr chloride injected i. p. into adult rats	7	(5)
^{90}Sr chloride injected i. v. into adult mice	8	(6,7)
^{226}Ra chloride injected i. v. into adult mice	9	(8)
^{226}Ra and ^{228}Ra (MsTh) in dial painters and other humans	10	(9,10)
^{224}Ra (Th X) chloride injected into children and adults	11	(11)

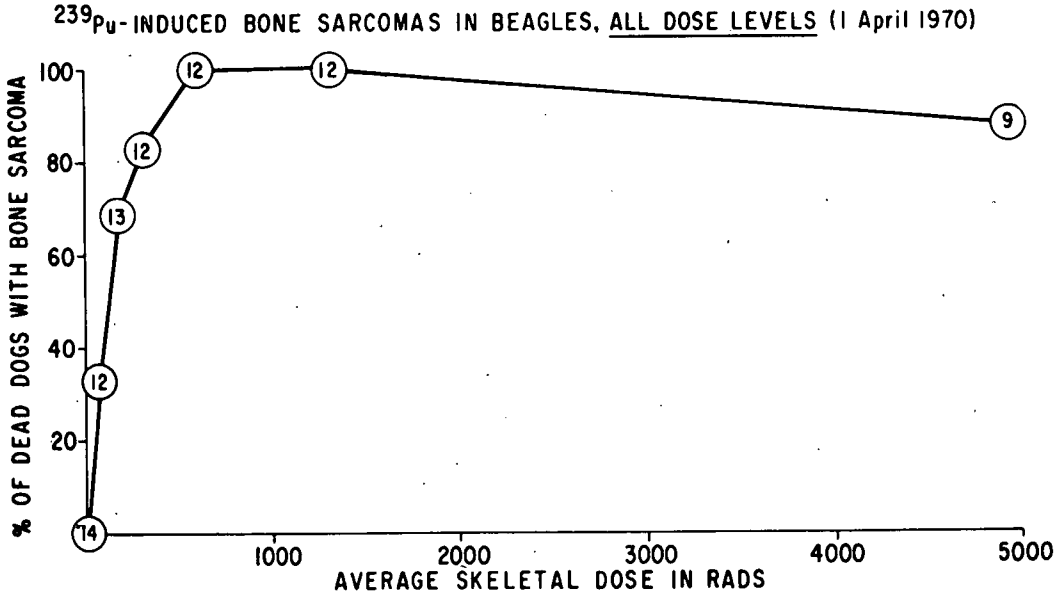

Figure 1. ^{239}Pu dose-response (all original levels plotted: see Table 2). The numbers inside the circles give the dead dogs at each incidence point. No bone sarcomas have occurred in our 74 life-span control dogs which have died. The natural incidence in beagles is extremely low, in the order of 1 bone sarcoma per 10,000 beagles living to old age. With increasing dose, the incidence of bone sarcoma increases to high "saturation" values, beyond which the incidence may decrease.

Table 2. ^{239}Pu-INJECTED BEAGLES (1 April 1970)

Inj. Level	μCi/kg Inj.	Inj. Dogs	Dead Dogs	Sar. Dogs	Incidence (Sar./dead)	Bone sarcoma dogs: Years from Inj. to death	Bone sarcoma dogs: Rads 1 yr Before death
5	2.88	9	9	7	78%	4.05	4930
4	0.909	12	12	12	100%	3.61	1310
3	0.296	12	12	12	100%	4.52	602
2	0.0951	12	12	10	83%	7.15	313
1.7	0.0477	14	13	9	69%	8.52	191
1	0.0157	14	12	4	33%	9.92	78
0	0	12	12	0	0%	----	0+
1*	0.0151	9	0	0	---	----	80+
0.7*	0.0101	9	0	0	---	----	53+
0.5*	0.00553	10	4	0	---	----	29+
0.2*	0.00189	10	0	0	---	----	10+
0.1*	0.00064	10	0	0	---	----	3+
0*	0	10	0	0	---	----	0+

* Recent levels injected after 1963: Older levels 1952-1958.
\+ For levels without bone sarcoma, rads at 10 years.

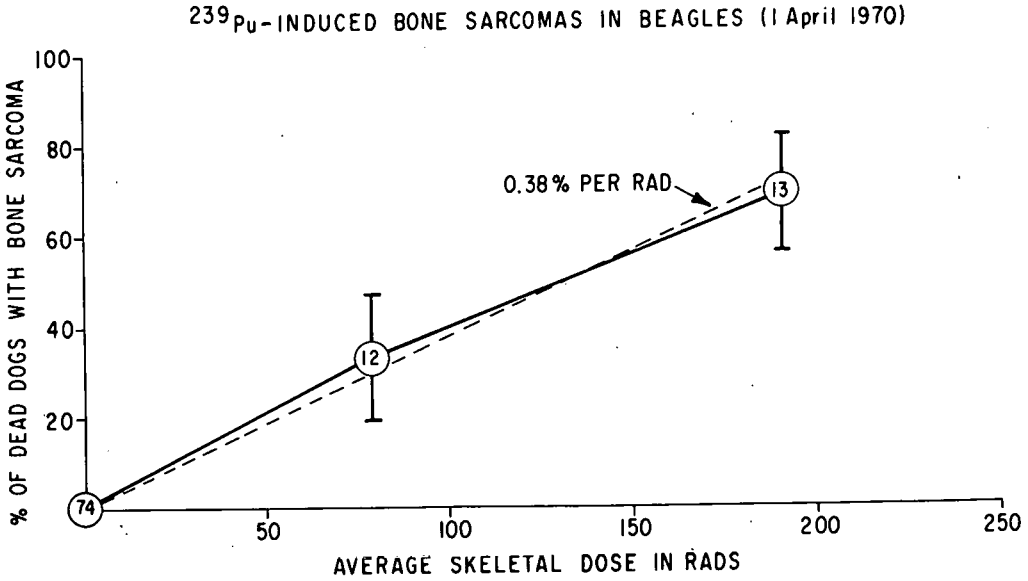

Figure 2. ^{239}Pu dose-response at the lower levels. High-dose "saturation" incidence points have been omitted from this and some of the subsequent graphs. Standard deviations, computed from the binomial relationship, are shown by vertical bars. Dogs injected since 1963 are not plotted, because they have not gone long enough to indicate the response between 0 and 80 rads.

Table 3. ^{228}Th-INJECTED BEAGLES (1 April 1970)

Inj. Level	μCi/kg Inj.	Inj. Dogs*	Dead Dogs	Sar. Dogs	Incidence (Sar./dead)	Bone sarcoma dogs: Years from Inj. to death	Bone sarcoma dogs: Rads 1 yr Before death
5	2.70	2	2	0	0%	----	----
4	0.858	4	4	2	50%	2.02	2870
3	0.290	12	12	12	100%	2.38	1150
2	0.0919	13	13	12	92%	3.26	516
1.5	0.0302	13	12	10	83%	6.52	249
1	0.0152	12	8	2	25%	8.75	130
0.5	0.00518	12	5	0	0%	----	47+
0.2	0.00171	13	1	0	0%	----	16+
0	0	13	6	0	0%	----	0+

* Dogs injected 1954-1963.
\+ For levels without bone sarcoma, rads at 10 years.

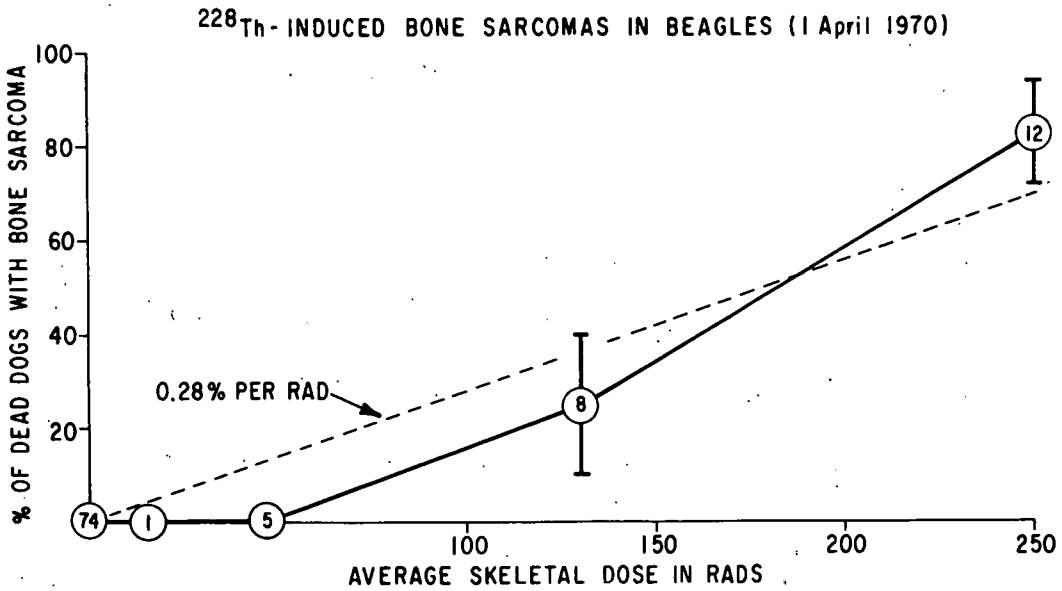

Figure 3. ^{228}Th dose-response at the lower levels. The indicated linear model predicts 0.7 sarcoma cases among the 6 dogs which have died without bone tumors at the 16 rad and 47 rad levels.

Table 4. ^{228}Ra (MsTh)-INJECTED BEAGLES (1 April 1970)
(Excludes dogs injected with over 1% ^{228}Th contamination)

Inj. Level	μCi/kg Inj.	Inj. Dogs*	Dead Dogs	Sar. Dogs	Incidence (Sar./dead)	Bone sarcoma dogs	
						Years from Inj. to death	Rads 1 yr Before death
5	8.49	4	4	1	25%	2.17	2830
4	2.62	5	5	4	80%	3.08	2948
3	0.973	9	9	9	100%	4.13	1650
2	0.309	9	8	7	88%	6.31	953
1.7	0.148	10	6	5	83%	7.99	485
1	0.0505	10	4	0	0%	----	226+
0.5	0.0177	12	0	0	----	----	79+
0	0	13	5	0	0%	----	0+

* Dogs injected 1957-1962.
\+ For levels without bone sarcoma, rads at 10 years.

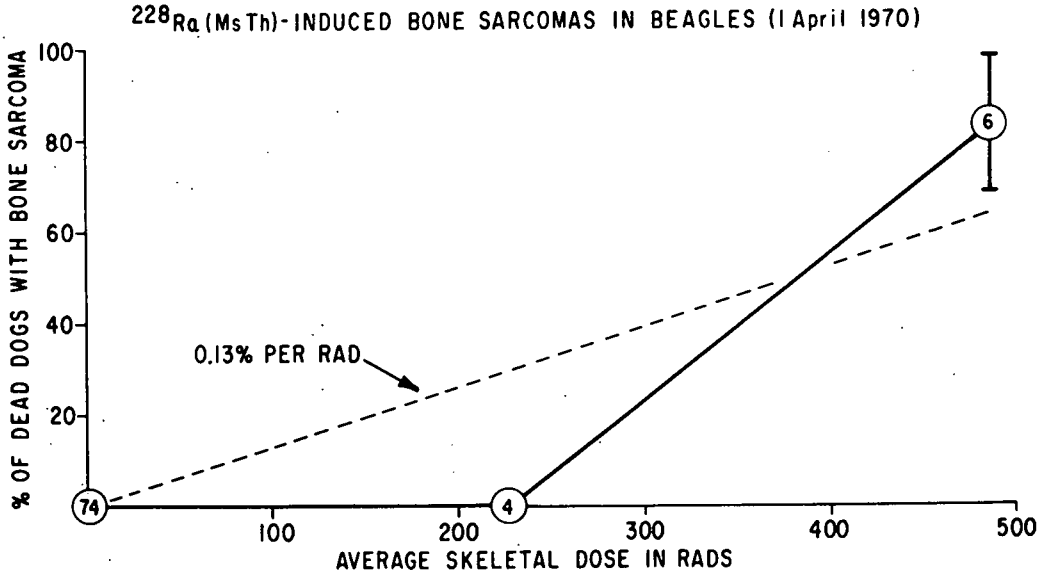

Figure 4. ^{228}Ra (MsTh) dose-response at the lower levels. The indicated linear model predicts 1.2 sarcoma cases among the 4 dogs which have died without bone tumors at the 226 rad level.

Table 5. ^{226}Ra-INJECTED BEAGLES (1 April 1970)

Inj. Level	μCi/kg Inj.	Inj. Dogs	Dead Dogs	Sar. Dogs	Incidence (Sar./dead)	Bone sarcoma dogs: Years from Inj. to death	Bone sarcoma dogs: Rads 1 yr Before death
5	10.4	10	10	9	90%	3.04	10900
4	3.21	13	13	12	92%	4.36	4530
3	1.07	12	12	11	92%	6.28	1940
2	0.339	13	13	5	38%	10.28	837
1.7	0.166	14	9	1	11%	11.25	458
1	0.0584	12	11	0	0%	-----	147+
0	0	12	12	0	0%	-----	0+
1*	0.0665	10	2	0	---	-----	167+
0.5	0.0220	10	0	0	---	-----	55+
0.2*	0.0074	10	0	0	---	-----	19+
0*	0	10	0	0	---	-----	0+

* Recent levels injected after 1963: Older levels 1953-1959.
\+ For levels without bone sarcoma, rads at 10 years.

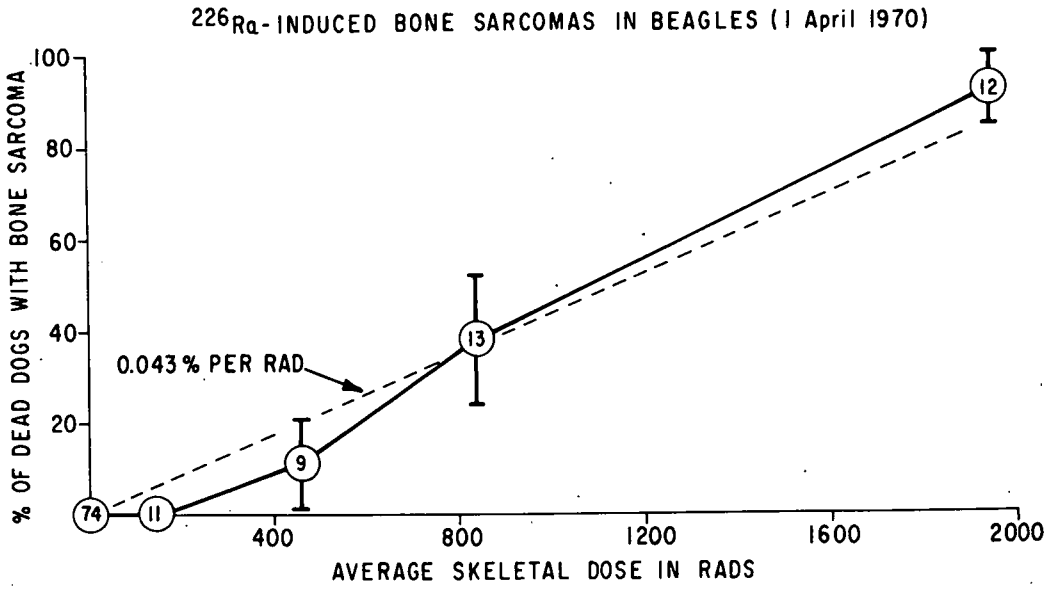

Figure 5. ^{226}Ra dose-response at the lower levels. The indicated linear model predicts 0.7 sarcoma cases among the 11 dogs which have died without bone tumors at the 147 rad level. Dogs injected since 1963 are not plotted.

Table 6. ^{90}Sr-INJECTED BEAGLES (1 April 1970)

Inj. Level	μCi/kg Inj.	Inj. Dogs	Dead Dogs	Sar. Dogs	Incidence (Sar./dead)	Bone sarcoma dogs: Years from Inj. to death	Bone sarcoma dogs: Rads 1 yr Before death
5	97.9	14	14	8	57%	4.02	9100
4.5*	63.6	12	2	2	100%	2.77	3920
4	32.7	12	7	0	0%	----	6000+
3	10.8	12	5	0	0%	----	1980+
2	3.46	12	8	0	0%	----	635+
1.7	1.72	13	6	0	0%	----	316+
1	0.571	12	4	0	0%	----	105+
0	0	13	6	0	0%	----	0+

* The 4.5-level injected 16 March 1966: Older levels 1955-1960.
\+ For levels without bone sarcoma, rads at 10 years.

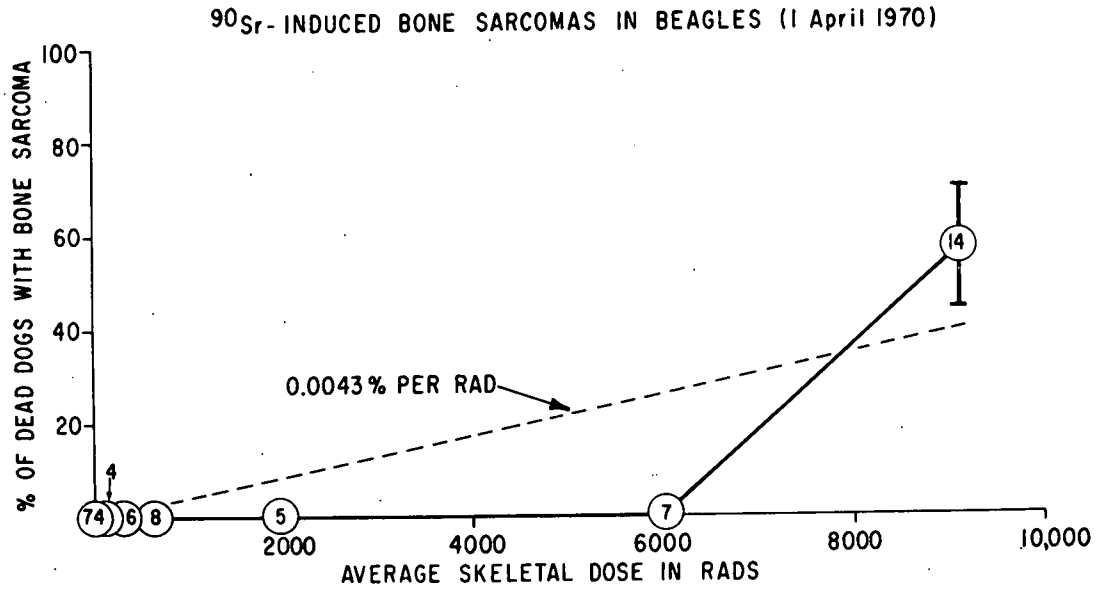

Figure 6. ^{90}Sr dose-response in beagles. Data have been omitted for the dogs injected with 63.6 μCi/kg in 1966, since insufficient time has passed to establish their incidence point with reliability. Omitting this data point, the indicated linear model (0.0043% per rad) predicts 2.5 sarcoma cases among the 30 dogs which have died without bone tumors at the 105 rad, 316 rad, 635 rad, 1980 rad, and 6000 rad levels. A similar linear relation (0.0051% per rad) based on all the dogs, including those injected with 63.6 μCi/kg, predicts 3.0 sarcoma cases for the non-tumor levels. These results, and those from the next 2 figures, strongly reject the linear model for bone sarcoma induction by β-emitting radiostrontium.

Table 7. ^{90}Sr-INJECTED RATS (MOSKALEV et. al. 1969)

µCi/kg Inj.	Dead Rats	Sar. Rats	Incidence (Sar./dead)	Days from inj. To sar. death	Bone dose at Death (rads)
500	43	22	51.2%	223	45 500
250	78	40	51.3%	356	29 400
75-100	158	6	3.8%	435	14 800
50	379	0	0	---	8 000
25	374	0	0	---	4 000
10	300	1	0.3%	500	1 600
5	300	1	0.3%	407	800
2.5	382	0	0	---	400
0.5	383	0	0	---	160
0.25	300	0	0	---	40
0.005	300	0	0	---	1
0	722	0	0	---	0

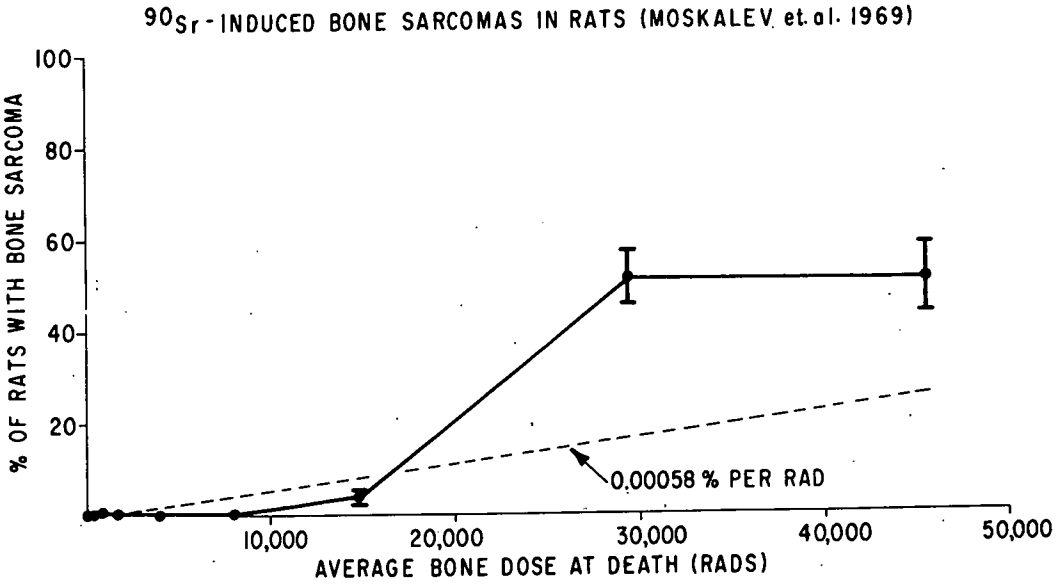

Figure 7. ^{90}Sr dose-response in Moskalev's strain of rats injected at 3 months of age. Note the non-linear nature of the actual dose-response curve. The indicated linear model predicts 32 sarcoma cases in the 2718 radioactive rats receiving 1-8000 rads, whereas only 2 bone sarcomas were actually observed among these levels. At low dosage, the true risk could be over 10 times smaller than predicted by the indicated linear equation. Although no bone sarcomas were observed among 722 control rats, it is possible that one or both of the 2 bone sarcomas observed in the 2718 lower-dose rats were spontaneous, rather than radiation-induced.

Table 8. ^{90}Sr-INJECTED MICE (M. FINKEL et. al. 1959 and 1960)

μCi/kg Inj.	Dead Mice	Sar. Mice	Incidence (Sar./dead)
9330	15	0	0
7000	30	0	0
4500	45	0	0
2200	30	19	63.3%
880	45	42	93.3%
440	45	32	71.1%
200	60	8	13.3%
88	75	3	4.0%
44	90	5	5.6%
8.9	105	0	0
4.5	120	3	2.5%
1.3	150	3	2.0%
0	150	4	2.7%

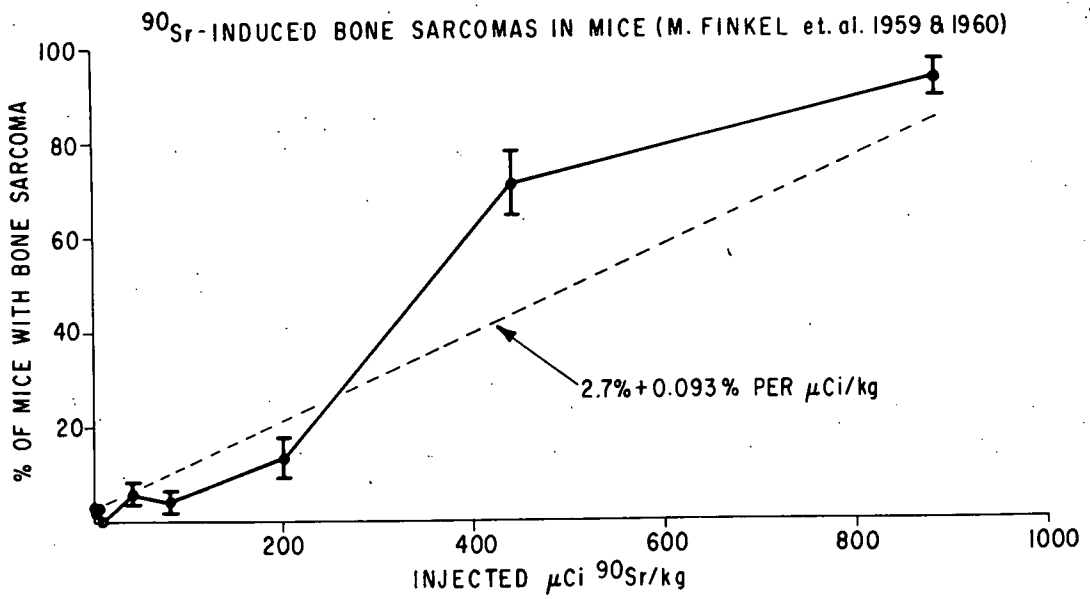

Figure 8. ^{90}Sr dose-response in CF 1 female mice injected at 70 days of age. The curve suggests a non-linear dose response relationship, but the high control incidence (2.7% in this experiment) makes it difficult at the very lowest dosage levels to distinguish radiation-induced bone sarcomas from those occurring naturally. The indicated linear equation predicts 11.3 radiation-induced plus 14.4 naturally-occurring sarcoma cases (25.7 total predicted cases) in the mice injected with 1.3-88 μCi/kg, whereas 14 cases of bone sarcoma were actually observed among these 540 mice.

Table 9. ^{226}Ra-INJECTED MICE (M. FINKEL et. al. 1969)

μCi/kg Inj.	Dead Mice	Sar. Mice	Incidence (Sar./dead)
120	45	14	31.1%
80	45	31	68.9
40	45	33	73.5
20	45	38	84.5
10	45	34	75.5
5	45	28	62.3
2.5	105	45	42.8
1.25	105	22	21.0
1.00	240	56	23.4
0.75	510	94	18.5
0.50	690	80	11.6
0.25	255	19	7.4
0.10	255	5	2.0
0.05	255	11	4.3
0	525	6	1.1

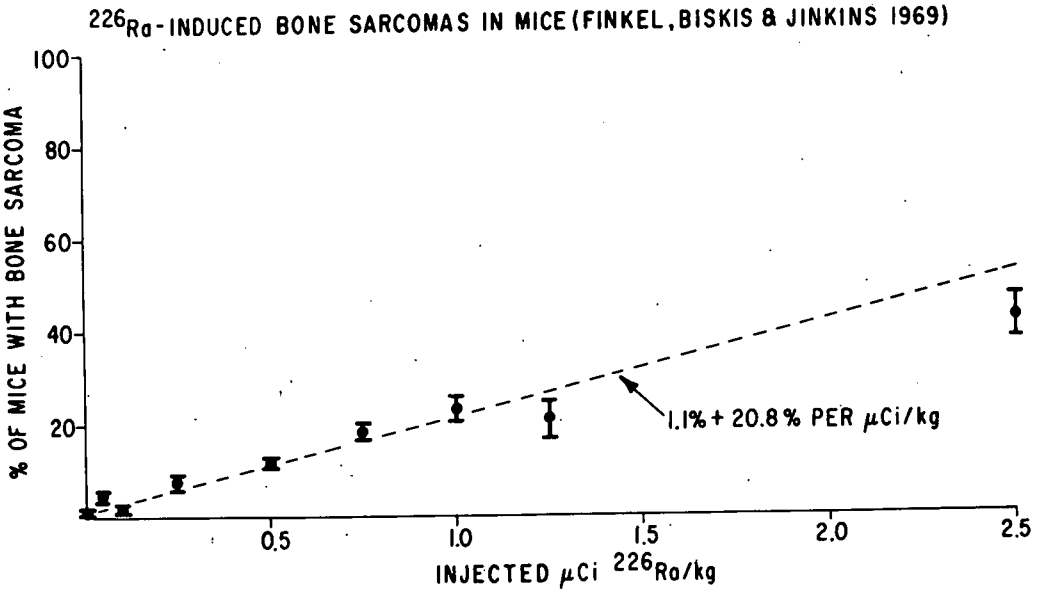

Figure 9. ^{226}Ra dose-response in CF 1 female mice injected at 70 days of age. Unlike the sigmoid curve for β-emitting radiostrontium, a linear curve represents the incidence from α-emitting radium very well, up to about 2.5 μCi/kg in these mice. The indicated linear equation predicts 93 radiation-induced plus 17 naturally-occuring sarcoma cases (110 total predicted cases) in the mice injected with 0.05-0.50 μCi/kg, whereas 115 cases of bone sarcoma were actually observed in these 1455 mice. It is unknown whether the shape of the dose-response would be different in another strain, such as CBA mice, which have a much smaller natural incidence of bone sarcoma.

Table 10. RADIUM CASES OF EVANS et. al.
(IDENTIFIED BY "SEARCH", NOT "SYMPTOM")

Av. skel. dose at death (rads) Range	Geometrical Mean	Persons	Sarcomas	Incidence (Sar./Person)
20,000 - 50,000	31,600	5	0	0%
10,000 - 20,000	14,100	8	1	12
5,000 - 10,000	7,070	12	2	17
2,500 - 5,000	3,540	22	3	14
1,200 - 2,500	1,730	12	4	33
1,000 - 1,200	1,100	5	0	0
600 - 1,000	775	6	0	0
300 - 600	424	17	0	0
100 - 300	173	41	0	0
50 - 100	71	28	0	0
1 - 50	7	170	0	0

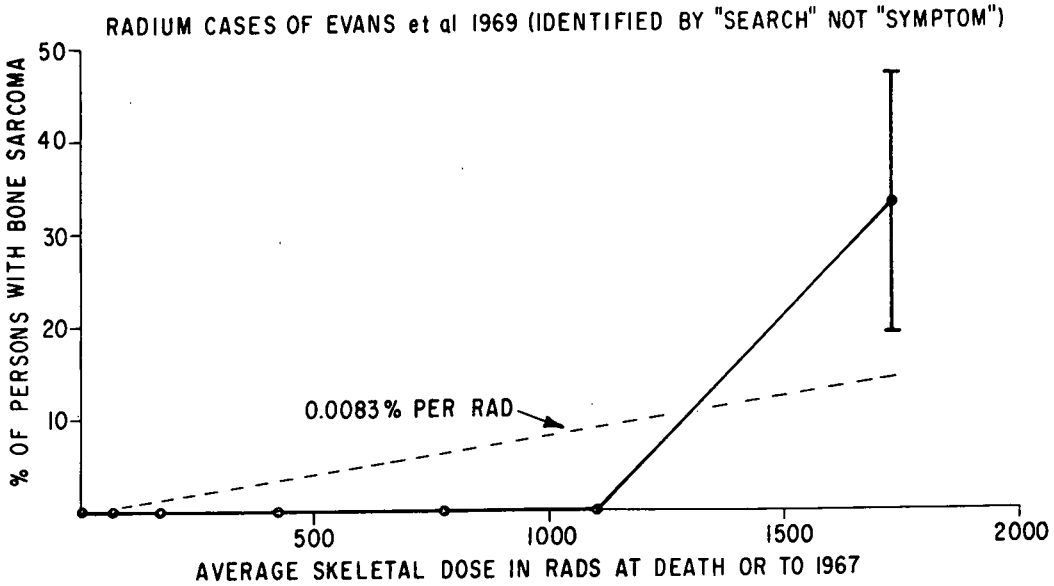

Figure 10. ^{226}Ra plus ^{228}Ra dose-response in humans (mostly dial painters). Only the R. D. Evans et. al. groups are plotted, with average skeletal doses extending up through 1730 rads. The geometrical mean of each dose range was used as a reasonable estimate for the mean average dose for each group. The indicated linear model based on plotted data predicts 2.3 sarcoma cases among the 267 total living and dead persons below 1200 rads in which no bone tumors have been observed at typical burden times of 40-50 years. No bone sarcomas have been observed below about 1200 rads in a similar study by A. Finkel et. al. involving a roughly equivalent number of exposed persons. Thus for the combined studies of Evans et. al. and Finkel et. al., the linear model predicts about 4.6 sarcomas among their non-tumor persons below 1200 rads.

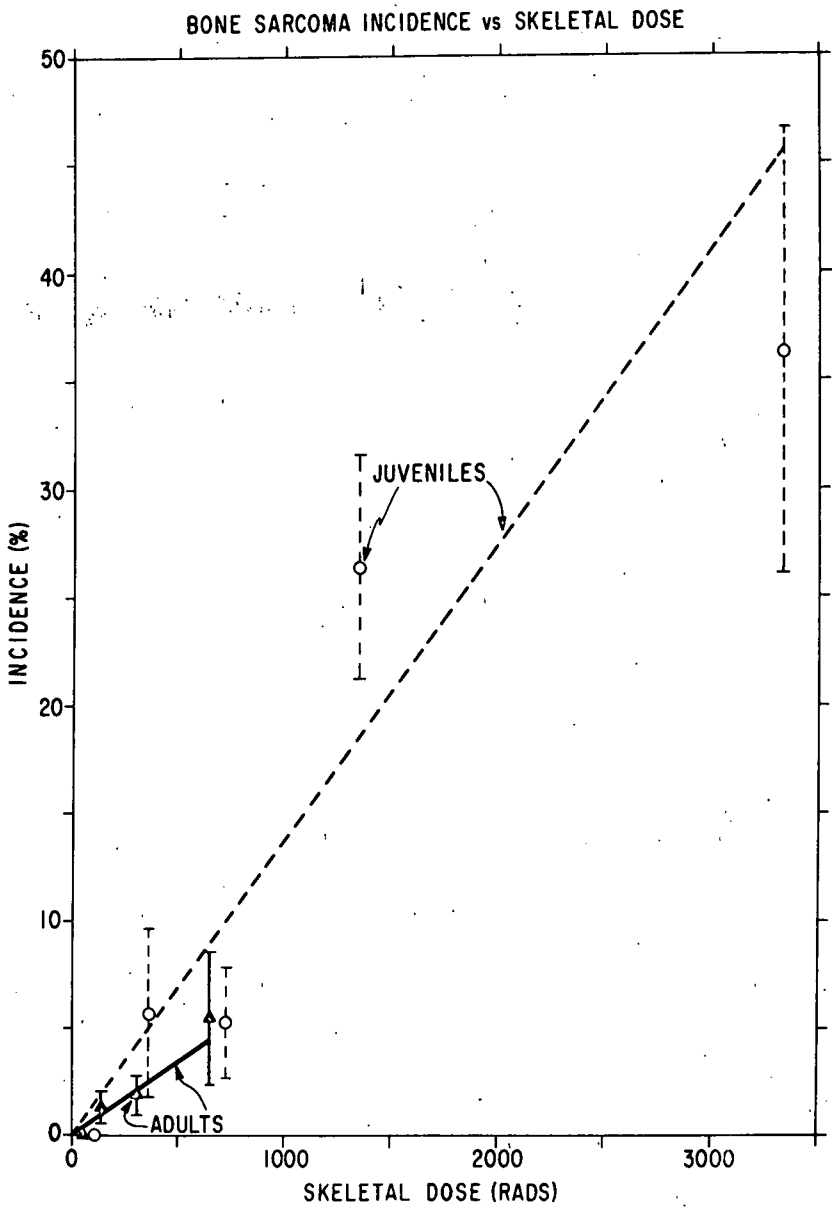

Figure 11. ^{224}Ra (Th X) dose-response in children and adults. These German patients were given repeated injections of ^{224}Ra after World War II for the treatment of tuberculosis, ankylosing spondylitis, and other diseases. For the linear responses shown, the risk of developing bone sarcoma during the first 14-21 years after the initial injection is 0.007% per rad for the adults and 0.014% per rad for the juveniles. These linear models predict 0.8 sarcomas among the 210 adults in their non-tumor 0-89 rad dose band and 0.2 sarcomas among the 12 juveniles in their non-tumor 0-199 rad dose band. The high toxicity of ^{224}Ra seems mainly due to its short 3.62 day half-life which causes a large fraction of the skeletal ^{224}Ra to decay while still on bone surfaces.

Table 11. ^{224}Ra (Th X)-INJECTED CHILDREN AND ADULTS (SPIESS AND MAYS 1970)

Age	Av. skel. dose in rads: Range	Mean Dose	Persons Inj.	Sarcoma Cases	Incidence (Sar./person)
Adults	500 - 999	650	55	3	5.5%
	200 - 499	306	214	4	1.9
	90 - 199	139	229	3	1.3
	0 - 89	53	210	0	0
Juveniles	2000 - 5750	3329	22	8	36.4%
	1000 - 1999	1345	72	19	26.4
	500 - 999	727	76	4	5.3
	200 - 499	363	35	2	5.7
	0 - 199	106	12	0	0

DISCUSSION

The observed cases of bone sarcoma at low dosage are compared with "linear" and "threshold" predictions in Table 12. The threshold predictions are the expected number of naturally-occurring cases. In the U. S. A. population, about 1 bone cancer is reported per 1000 deaths, (12) and in the dose bands without bone tumors, about 500 ^{226}Ra plus ^{228}Ra (MsTh) cases and about 200 ^{224}Ra cases are being studied. However, most of these cases are still living, so their expected number of "naturally-occurring" cases to date may be less than the 0.5 and 0.2 cases tabulated under the threshold predictions in Table 12.

Table 12. COMPARISON OF LINEAR AND THRESHOLD MODELS AT LOW DOSAGE

Species	Nuclide	Predicted sarcoma cases: Linear	Threshold	Observed Cases
Beagles	^{239}Pu	---	---	---
	^{228}Th	0.7	0	0
	^{228}Ra (MsTh)	1.2	0	0
	^{226}Ra	0.7	0	0
	^{90}Sr	2.5	0	0
Rats	^{90}Sr	32	0	2
Mice (CF 1)	^{90}Sr	26	14	14
	^{226}Ra	110	17	115
Humans	^{226}Ra & ^{228}Ra	~ 4.6	~ 0.5	0
	^{224}Ra (Th X)	1.0	~ 0.2	0

The ^{90}Sr results are the most decisive, so they will be discussed first. None of the ^{90}Sr data support the linear model, as can be seen for beagles, rats and mice. In our 30 dead beagles at the 105 rad, 316 rad, 635 rad, 1980 rad, and 6000 rad levels, 2.5 bone sarcomas were predicted from the linear model while none were actually observed. The probability of this occurring by chance is 7% (P = 0.07). A 93% chance of observing 1 or more cases should have existed if this linear model were true. The results in Moskalev's 2719 rats at 1-8000 rads are even more striking. The linear model predicted 32 cases of bone sarcoma while only 2 were observed. The probability of 2 or fewer induced sar. occurring by chance is incredibly small, $P < 10^{-11}$ (less than 1 chance in 100 thousand million). Thus, the linear model is strongly rejected for ^{90}Sr. However, this does not necessarily prove that the threshold model is absolutely correct, because other alternative models are possible, such as a sigmoid relationship in which the slope of the incidence curve steepens with increasing dose as the high incidence region is approached. Regardless of the exact shape of the dose-response curve for ^{90}Sr the following conclusion is inescapable: Low dose risk from induced bone sarcoma is considerably less than that based on a linear model force-fit through all of the incidence points. This suggests that the cells giving rise to induced bone cancer are capable of considerable recovery from low LET (linear energy transfer) radiation, such as from β-particles or X-rays, provided that the dose-rate is sufficiently low.

Results from α-radiation are less conclusive. For the total human ^{226}Ra + ^{228}Ra (MsTh) cases below 1200 rads, the linear model predicts about 4.6 radiation-induced sarcomas whereas none have been observed

below this dose. If the linear model is correct, this result could occur by chance with a probability of about 1% (P = 0.01). More low-dose patients who have been injected with ^{224}Ra (Th X) need to be followed before either the linear or the threshold models can be ruled out for this α-emitter. (About 2000 additional ^{224}Ra patients exist whose skeletal dosimetry has not yet been evaluated.) On the other hand, ^{226}Ra results in CF1 mice are in excellent agreement with the linear hypothesis, although it is unknown whether their high natural incidence (1-3%) of bone sarcoma could affect the shape of their dose-response. Certainly the mouse results demonstrate that the shape of the response to β-emitting ^{90}Sr and α-emitting ^{226}Ra is strikingly different. In beagles about 10 more years will be required to define the response in the newest low-dose levels of ^{239}Pu and ^{226}Ra. Results from the original levels taken separately do not at this time rule out linear possibilities: ^{228}Th, 0.7 predicted cases in 6 dead dogs (P = 0.48 for the observed zero cases); ^{228}Ra (MsTh), 1.2 predicted cases in 4 dead dogs (P = 0.24 for the observed zero cases); ^{226}Ra, 0.7 predicted cases in 11 dead dogs (P = 0.49 for the observed zero cases). However, when results are combined for the α-emitter levels at which no bone tumors have occurred, the 2.6 predicted cases in the 21 dead dogs are less compatible with the zero observed cases (P = 0.06). Additional valuable mortality data will become available within the next few years on beagles at the original levels which were injected 1952-1963, since the median post-injection life expectancy in our control beagles is about 11 years.

It is possible that for mammals with a low natural incidence of bone cancer, the most probable response from low doses of α-radiation

is a sigmoid relationship somewhere in between the linear and threshold models. If so, it is less curvilinear than the dose-response for β-radiation. Studies in humans and beagles are still in progress, and final conclusions must await the future. But with time and the necessary financial support, it should be possible to reduce the uncertainties as to the true risk from low doses of skeletal irradiation.

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