RADIOBIOLOGY LABORATORY

College of Medicine, University of Utah

Salt Lake City 12, Utah

SEMI-ANNUAL PROGRESS REPORT

September 30, 1957

Operated for the Atomic Energy Commission

by

The University of Utah

through the

Department of Anatomy of the College of Medicine

CONTRACT AT(11-1)-119

DISCLAIMER

AEC4-3583

Radiobiology Laboratory

College of Medicine

University of Utah

Salt Lake City 12, Utah

SEMI-ANNUAL PROGRESS REPORT

September 30, 1957

Operated for the Atomic Energy Commission

by

The University of Utah

through the Department of Anatomy of the College of Medicine

Contract AT(11-1)-119

Table of Contents

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 in groups of six or seven of the same sex (five or six dose levels plus one control). Litter mates are used whenever possible. Each animal receives the designated dose of one radioisotope in a single I.V. injection. The animals are injected at approximately 18 months of age after skeletal maturity has been demonstrated radiographically. Twelve such groups are planned for each radioisotope.

The five dose levels designated by integers are those specified at the early meetings of the consultants, and those designated by non-integers have been added by the local group. Since these dose levels were specified as "retained" doses,(1) the actual injected doses are 4 times the desired "retained" doses of Ra^{226}, Ra^{228} (Mesothorium), and Sr^{90}, and 1.11 times the desired "retained" doses of Pu^{239} and Th^{228} (Radiothorium). The desired "retained" doses are the same for all the isotopes except Sr^{90}, in which case they are greater by a factor of 10.

Dose level 1 is the basis of the scheme, and is 10 times the maximum permissible Ra^{226} in man. Level 1 = 0.0143 "retained" $\mu c/kg$

$$= 10 \times \frac{0.1\ \mu c\ Ra^{226}}{70\ \text{Kg man.}}$$

All other dose levels are simple multiples of level 1 as shown on the following page.

(1) The average fractional radium retention equals 0.25 at 330 days after injection; the average fractional retention of plutonium is 0.90 at six days after injection, but plutonium retention decreases more slowly during the first year than does radium retention.

Level 2 = 6 x level 1
Level 1.7* = 3 x level 1
Level 1.5/ = 2 x level 1
Level 0.5/ = 1/3 x level 1
Level 3 = 3 x Level 2 (= 18 x level 1)
Level 4 = 3 x Level 3 (= 54 x level 1)
Level 5 = 3 x Level 4 (=162 x 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 dogs (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.

Next comes a code letter for the radioactive isotope. (P = plutonium, R = radium, M = mesothorium, T = radiothorium, S = strontium). The final number is the dose level number as defined above and with 0 = control.

Example: M1P5 is a male animal in the first plutonium group at the top dose level.

Although M1P5, M1P4, M1P3, M1P2, M1P1, and M1P0 constitute a group and were injected at the same time, the tables are arranged according to dose level to facilitate comparison of all the P5 animals, all the P4 animals, etc.

* All the isotopes except Th^{228}.
/ Th^{228} only.

TABLE I

TOXICITY ANIMALS (AS OF 9-30-57)

A. Plutonium

Approx. Retained Dose (μc/kg)	Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Days since Injection
2.5	M1P5	14	8.86	2.67	12- 1-52	(dead at 1324 d)
	F2P5	38	8.75	3.30	3- 2-53	(dead at 1576 d)
	M3P5	17	8.10	3.00	6- 1-53	(dead at 499 d)
	M4P5	19	9.18	3.17	9-16-53	1475
	F5P5	23	8.77	2.77	10-14-53	1447
	F6P5	13	7.90	2.57	5-12-54	(dead at 1194 d)
	F7P5	16	8.33	2.99	10-25-54	1071
	M8P5	16	9.55	2.69	3-15-55	930
	F9P5	18	9.45	2.73	9- 9-55	752
0.81	M1P4	15	7.61	0.823	12- 1-52	(dead at 1724 d)
	F2P4	19	8.65	1.03	3- 2-53	(dead at 1556 d)
	M3P4	16	9.36	0.929	6- 1-53	(dead at 1198 d)
	M4P4	19	8.74	0.974	9-16-53	(dead at 1066 d)
	F5P4	21	7.05	0.872	10-14-53	(dead at 1245 d)
	F6P4	14	9.26	0.811	5-12-54	1237
	F7P4	16	8.45	0.963	10-25-54	1071
	M8P4	21	9.22	0.887	3-15-55	930
	F9P4	18	8.58	0.960	9- 9-55	752
	F10P4	17	6.48	0.868	11-22-55	678
	M11P4	20	9.56	0.927	4-24-56	524
	M12P4	20	11.4	0.838	5-29-56	489
0.27	M1P3	14	8.00	0.261	12- 1-52	(dead at 1476 d)
	F2P3	14	6.85	0.312	3- 2-53	1673
	M3P3	16	8.74	0.291	6- 1-53	1582
	M4P3	20	8.51	0.292	9-16-53	1475
	F5P3	21	8.22	0.288	10-14-53	1447
	F6P3	14	8.38	0.282	5-12-54	1237
	F7P3	16	9.00	0.314	10-25-54	1071
	M8P3	13	9.73	0.30	3-15-55	930
	F9P3	18	7.67	0.30	9- 9-55	752
	F10P3	20	8.94	0.298	11-22-55	678
	M11P3	20	10.5	0.309	4-24-56	524
	M12P3	20	10.2	0.308	5-29-56	489
0.086	M1P2	15	7.61	0.0853	12- 1-52	1764
	F2P2	14	7.73	0.112	3- 2-53	1673
	M3P2	16	10.5	0.0940	6- 1-53	1582
	M4P2	20	9.84	0.0862	9-16-53	1475
	F5P2	20	8.12	0.0846	10-14-53	1447
	F6P2	14	7.54	0.0902	5-12-54	1237
	F7P2	16	8.40	0.0996	10-25-54	1071
	M8P2	13	9.73	0.0957	3-15-55	930
	F9P2	18	9.72	0.101	9- 9-55	752
	F10P2	18	7.94	0.0968	11-22-55	678
	M11P2	20	10.3	0.0961	4-24-56	524
	M12P2	20	9.98	0.100	5-29-56	489

Approx. Retained Dose (μc/kg)	Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Days since Injection
0.043	M1P1.7	22	8.72	0.0475	6-26-56	461
	F2P1.7	17	8.62	0.0431	11-22-55	678
	M3P1.7	21	8.63	0.0493	6-26-56	461
	M4P1.7	22	8.37	0.0484	10-10-56	355
	F5P1.7	21	11.6	0.0459	6-26-56	461
	F6P1.7	21	10.3	0.0495	6-26-56	461
	F7P1.7	25	9.73	0.0481	10-10-56	355
	M8P1.7	22	13.6	0.0479	10-10-56	355
	F9P1.7	25	9.72	0.0485	10-10-56	355
	F10P1.7	24	10.6	0.0495	10-10-56	355
	M11P1.7	20	11.6	0.0486	4-24-56	524
	M12P1.7	22	9.41	0.0491	10-10-56	355
0.0143	M1P1	15	9.41	0.0150	12- 1-52	1764
	F2P1	14	6.85	0.0163	3- 2-53	1673
	M3P1	17	8.00	0.0165	6- 1-53	1582
	M4P1	20	9.97	0.0139	9-16-53	1475
	F5P1	20	8.80	0.0142	10-14-53	1447
	F6P1	14	7.38	0.0140	5-12-54	1237
	F7P1	17	6.36	0.0167	10-25-54	1071
	M8P1	15	10.6	0.0172	3-15-55	930
	F9P1	18	7.87	0.0168	9- 9-55	752
	F10P1	21	12.0	0.0152	11-22-55	678
	M11P1	20	8.90	0.0157	4-24-56	524
	M12P1	21	9.67	0.0167	5-29-56	489
Control	M1P0	15	9.70	0	12- 1-52	1764
	F2P0	14	6.36	0	3- 2-53	1673
	M3P0	17	10.8	0	6- 1-53	1582
	M4P0	14	10.7	0	9-16-53	1475
	F5P0	20	9.75	0	10-14-53	1447
	F6P0	14	5.59	0	5-12-54	1237
	F7P0	17	6.90	0	10-25-54	1071
	M8P0	19	10.9	0	3-15-55	930
	F9P0	19	9.35	0	9- 9-55	752
	F10P0	22	11.0	0	11-22-55	678
	M11P0	20	10.3	0	4-24-56	524
	M12P0	21	10.9	0	5-29-56	489

B. Radium

Approx. Retained dose (μc/kg)	Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Days since Injection
2.5	M1R5	16	9.87	10.5	4-20-53	(dead at 908 d)
	M2R5	15	8.85	10.8	11-16-53	(dead at 1380 d)
	F3R5	13	7.82	10.1	3-10-54	(dead at 481 d)
	M4R5	14	8.90	10.6	4- 7-54	(dead at 1091 d)
	M5R5	15	10.9	10.1	6-22-54	1196
	F6R5	16	9.66	10.2	7-27-54	(dead at 1015 d)
	M7R5	15	8.85	11.9	8-24-54	1133
	F8R5	16	7.76	9.68	12-21-54	(dead at 968 d)
	F9R5	14	9.16	9.48	4-11-55	903
	M10R5	17	10.7	10.2	7-27-55	796
0.81	M1R4	16	9.08	3.51	4-20-53	(dead at 1606 d)
	M2R4	15	9.53	3.55	11-16-53	1414
	F3R4	13	8.65	3.33	3-10-54	(dead at 490 d)
	F3R4A	20	7.20	3.10	11-30-56	304
	M4R4	14	8.83	3.47	4- 7-54	1272
	M5R4	15	13.2	2.42	6-22-54	1196
	F6R4	16	8.55	3.44	7-27-54	1161
	M7R4	15	9.55	3.88	8-24-54	1133
	F8R4	16	8.94	3.14	12-21-54	1014
	F9R4	18	8.53	3.02	4-11-55	903
	M10R4	17	10.8	3.28	7-27-55	796
	F11R4	16	10.4	2.84	12-20-55	650
	F12R4	16	9.61	2.81	1-17-56	622
0.27	M1R3	16	8.91	1.20	4-20-53	1624
	M2R3	15	9.02	1.21	11-16-53	1414
	F3R3	13	7.74	1.11	3-10-54	1300
	M4R3	14	11.7	1.16	4- 7-54	1272
	M5R3	15	13.0	0.846	6-22-54	1196
	F6R3	16	9.75	1.14	7-27-54	1161
	M7R3	17	12.3	1.29	8-24-54	1133
	F8R3	18	7.76	1.03	12-21-54	1014
	F9R3	18	8.02	0.987	4-11-55	903
	M10R3	17	10.1	1.06	7-27-55	796
	F11R3	16	12.9	0.938	12-20-55	650
	F12R3	16	11.4	0.883	1-17-56	622
0.086	M1R2	16	8.74	0.382	4-20-53	1624
	M2R2	19	8.22	0.387	11-16-53	1414
	F3R2	18	8.53	0.347	3-10-54	1300
	M4R2	14	10.5	0.361	4- 7-54	(dead at 328 d)
	M4R2A	14	10.6	0.306	4-11-55	903
	M5R2	15	11.5	0.267	6-22-54	1196
	F6R2	16	10.6	0.360	7-27-54	1161
	M7R2	17	11.1	0.413	8-24-54	1133
	F8R2	19	6.95	0.331	12-21-54	1014
	F9R2	19	9.38	0.317	4-11-55	903
	M10R2	17	9.95	0.345	7-27-55	796
	F11R2	16	9.30	0.310	12-20-55	650
	F12R2	16	10.3	0.281	1-17-56	622

Approx. Retained Dose (μc/kg)	Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Days since Injection
0.043	M1R1.7	17	9.98	0.137	1-17-56	622
	M2R1.7	20	7.85	0.163	11-30-56	304
	F3R1.7	16	13.1	0.165	12-20-55	650
	M4R1.7	17	6.20	0.163	12-20-55	650
	M5R1.7	17	10.1	0.151	12-20-55	650
	F6R1.7	16	7.90	0.152	12-20-55	650
	M7R1.7	20	7.17	0.163	11-30-56	304
	F8R1.7	16	9.50	0.154	12-20-55	650
	F9R1.7	20	7.55	0.168	11-30-56	304
	M10R1.7	19	9.57	0.167	11-30-56	304
	F11R1.7	20	8.17	0.165	11-30-56	304
	F12R1.7	19	8.95	0.167	11-30-56	304
0.0143	M1R1	16	8.48	0.618	4-20-53	1624
	M2R1	21	10.0	0.0876	11-16-53	1414
	F3R1	21	8.68	0.0576	3-10-54	1300
	M4R1	14	8.60	0.642	4- 7-54	1272
	M5R1	16	11.7	0.0436	6-22-54	1196
	F6R1	16	7.23	0.0484	7-27-54	1161
	M7R1	17	11.4	0.0651	8-24-54	1133
	F8R1	28	8.98	0.0559	12-21-54	1014
	F9R1	26	9.88	0.0521	4-11-55	903
	M10R1	17	11.5	0.0573	7-27-55	796
	F11R1	17	11.2	0.0522	12-20-55	650
	F12R1	17	9.71	0.0444	1-17-56	622
Control	M1R0	18	8.03	0	4-20-53	1624
	M2R0	16	14.3	0	11-16-53	1414
	F3R0	20	11.4	0	3-10-54	1300
	M4R0	15	11.0	0	4- 7-54	1272
	M5R0	15	6.57	0	6-22-54	1196
	F6R0	16	8.43	0	7-27-54	1161
	M7R0	17	11.0	0	8-24-54	1133
	F8R0	21	8.21	0	12-21-54	1014
	F9R0	23	11.7	0	4-11-55	903
	M10R0	17	10.9	0	7-27-55	796
	F11R0	18	10.2	0	12-20-55	650
	F12R0	16	8.68	0	1-17-56	622

C. Mesothorium

Approx. Retained Dose (μc/kg)	Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Days since Injection
1. Plus 15 per cent radiothorium (on an activity basis)						
2.5	F1M5	16	7.77	9.83	1- 4-54	(dead at 232 d)
0.81	F1M4	17	7.56	3.15	1- 4-54	(dead at 841 d)
0.27	F1M3	17	10.4	1.04	1- 4-54	(dead at 918 d)
0.086	F1M2	22	7.60	0.334	1- 4-54	1365
0.0143	F1M1	24	7.75	0.0561	1- 4-54	1365
Control	F1M0	24	7.33	0	1- 4-54	1365
2. Plus 3 per cent radiothorium						
2.5	F2M5	15	7.35	10.0	11-29-54	(dead at 780 d)
	M3M5	19	8.87	9.93	3-13-56	566
0.81	F2M4	15	6.95	3.41	11-29-54	(dead at 778 d)
	M3M4	19	9.65	3.22	3-13-56	(dead at 418 d)
0.27	F2M3	15	6.70	1.12	11-29-54	1036
	M3M3	19	10.4	0.921	3-13-56	566
0.086	F2M2	17	8.25	0.355	11-29-54	(dead at 965 d)
	M3M2	19	11.0	0.342	3-13-56	566
0.043	F2M1.7	18	9.90	0.173	3-13-56	566
	M3M1.7	19	11.0	0.172	3-13-56	566
0.0143	F2M1	15	8.25	0.0593	11-29-54	1036
	M3M1	19	13.8	0.0574	3-13-56	566
Control	F2M0	18	6.94	0	11-29-54	1036
	M3M0	19	13.0	0	3-13-56	566
3. Plus 0.6 per cent radiothorium						
2.5	M4M5	16	7.29	9.82	1-15-57	258
	F5M5	22	11.1	10.2	3- 5-57	209
	M6M5	19	7.53	10.1	4-23-57	160
	F7M5	16	7.35	9.77	6- 4-57	118
0.81	M4M4	20	7.84	3.07	1-15-57	258
	F5M4	17	9.63	3.21	3- 5-57	209
	M6M4	17	9.49	3.10	4-23-57	160
	F7M4	18	8.40	2.92	6- 4-57	118
	M3M4A	16	7.34	2.89	6- 4-57	118
0.27	M4M3	20	10.2	1.13	1-15-57	258
	F5M3	17	8.51	1.13	3- 5-57	209
	M6M3	17	9.09	1.11	4-23-57	160
	F7M3	18	9.94	0.993	6- 4-57	118

Approx. Retained Dose (μc/kg)	Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Days since Injection
0.086	M4M2	20	9.88	0.351	1-15-57	258
	F5M2	17	8.30	0.359	3- 5-57	209
	M6M2	17	12.4	0.357	4-23-57	160
	F7M2	18	10.1	0.327	6- 4-57	118
0.043	M4M1.7	20	8.94	0.178	1-15-57	258
	F5M1.7	22	12.8	0.175	3- 5-57	209
	M6M1.7	17	10.0	0.168	4-23-57	160
	F7M1.7	18	10.2	0.160	6- 4-57	118
0.0143	M4M1	20	9.90	0.0600	1-15-57	258
	F5M1	22	8.80	0.0602	3- 5-57	209
	M6M1	17	10.6	0.0545	4-23-57	160
	F7M1	18	9.89	0.0550	6- 4-57	118
Control	M4M0	20	10.3	0	1-15-57	258
	F5M0	22	11.2	0	3- 5-57	209
	M6M0	16	7.56	0	4-23-57	160
	F7M0	13	8.71	0	6- 4-57	118

D. Radiothorium

Approx. Retained Dose (μc/kg)	Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Days since Injection
2.5	M1T5	16	9.48	2.76	2- 8-54	(dead at 212 d)
	M2T5	16	8.22	2.63	9-28-54	(dead at 97 d)
0.81	M1T4	16	8.32	0.882	2- 8-54	(dead at 645 d)
	M2T4	15	8.32	0.916	9-28-54	(dead at 833 d)
	F3T4	15	7.25	0.800	6- 6-55	(dead at 763 d)
	M4T4	20	8.81	0.835	10-18-55	713
0.27	M1T3	10	9.15	0.301	2- 8-54	(dead at 988 d)
	M2T3	15	11.9	0.301	9-28-54	(dead at 859 d)
	F3T3	16	12.0	0.272	6- 6-55	(dead at 547 d)
	M4T3	20	9.69	0.285	10-18-55	713
0.086	M1T2	16	10.2	0.0976	2- 8-54	(dead at 1282 d)
	M2T2	16	9.16	0.0875	9-28-54	1098
	F3T2	16	7.87	0.0908	6- 6-55	847
	M4T2	19	13.0	0.0900	10-18-55	(dead at 78 d)
	M4T2A	21	10.6	0.0899	9- 7-56	388
0.0285	M1T1.5	23	7.95	0.0289	9- 7-56	388
	M2T1.5	15	10.0	0.0293	9-28-54	1098
	F3T1.5	20	10.3	0.0303	6- 6-55	847
	M4T1.7	19	8.59	0.0299	10-18-55	713
0.0143	M1T1	16	9.36	0.0146	2- 8-54	1330
	M2T1	23	9.27	0.0146	9- 7-56	388
	F3T1	24	8.84	0.0145	9- 7-56	388
	M4T1	23	8.27	0.0146	9- 7-56	388
0.00476	M1T0.5	23	14.3	0.00496	9- 7-56	388
	M2T0.5	15	10.5	0.00490	9-28-54	1098
	F3T0.5	22	8.59	0.00485	6- 6-55	847
	M4T0.5	17	8.58	0.00503	10-18-55	713
Control	M1T0	16	8.24	0	2- 8-54	1330
	M2T0	16	7.28	0	9-28-54	1098
	F3T0	26	11.6	0	6- 6-55	847
	M4T0	19	8.10	0	10-18-55	713

E. Strontium

Approx. Retained Dose (μc/kg)	Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Date since Injection
25.0	F1S5	14	9.38	93.6	1-18-55	(dead at 960 d)
	M2S5	18	12.2	111.	2-14-56	(dead at 255 d)
8.1	F1S4	15	8.74	30.7	1-18-55	986
	M2S4	19	11.2	32.6	2-14-56	594
	M3S4	20	9.83	34.1	9-11-57	19
2.7	F1S3	15	7.36	10.8	1-18-55	986
	M2S3	19	9.62	11.8	2-14-56	594
	M3S3	16	11.4	10.4	9-11-57	19
0.86	F1S2	17	5.59	3.33	1-18-55	986
	M2S2	19	8.97	3.51	2-14-56	594
	M3S2	16	7.82	3.49	9-11-57	19
0.43	F1S1.7	17	7.41	1.81	2-14-56	594
	M2S1.7	19	11.6	1.94	2-14-56	594
	M3S1.7	16	9.19	1.74	9-11-57	19
0.143	F1S1	50	6.84	0.505	1-18-55	(dead at 308 d)
	F1S1A	17	9.38	0.576	2-14-56	594
	M2S1	19	8.81	0.605	2-14-56	594
	M3S1	16	10.9	0.593	9-11-57	19
Control	F1S0	17	8.48	0	1-18-55	986
	M2S0	20	11.1	0	2-14-56	594
	M3S0	16	9.03	0	9-11-57	19

TABLE II

TEST ANIMALS (AS OF 9-30-57)

A. Plutonium

Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Dead at (days)	Comments
T0P5	21	11.4	3.05	6-24-52	1	Detailed distribution, radioautographic study.
T1P5	52	12.7	3.04	10-13-52	29	Retention.
T2P5	30	11.9	6.85	9-15-52	44	Retention.
T3P5	31	9.65	3.22	10-13-52	610	Distribution, excretion.
T4P5	33	8.78	3.02	10-13-52	365	Distribution, excretion.
T5P5	16	10.4	2.69	12-14-54	400	Development of bone pathology.
T6P5	17	6.16	2.73	12-14-54	406	Development of bone pathology.
T7P5	16	7.40	2.68	12-14-54	777	Development of bone pathology.
T8P5	17	8.32	2.67	12-14-54	863	Development of bone pathology.
T9P5	19	10.3	2.80	11-22-55	15	Radioautographic studies.
T10P5	18	11.9	2.74	11-23-55	15	Radioautographic studies.
T11P5	17	12.1	2.76	11-22-55	28	Radioautographic studies.
T12P5	16	9.23	2.74	11-23-55	28	Radioautographic studies.
T13P5	19	8.27	3.16	4-24-56	3	Radioautographic studies.
T14P5	19	9.38	2.43	4-24-56	7	Radioautographic studies.
T15P5	24	8.32	2.79	10-15-56	1	Radioautographic studies.
T16P5	22	10.7	2.85	10-10-56	92	Radioautographic studies.
T17P5	24	11.1	3.01	2-12-57	210	Radioautographic studies.
T18P5	24	8.16	2.83	2-12-57	217	Radioautographic studies.

B. Radium

T1R5	33	11.1	10.3	12- 1-52	1074	Retention & excretion.
T2R5	30	8.40	4.39	1-12-53	1368	Retention & excretion.
T3R5	48	8.29	4.76	1-12-53	428	Retention & excretion.
T4R5	15	10.0	10.6	7- 6-53	1	Distribution.
T5R5	4	6.14	11.7	10- 6-53	1	Distribution (Ca^{45}).
T6R5	4	6.14	11.4	10- 6-53	1	Distribution.
T7R5	4	6.14	11.8	10- 6-53	1	Distribution.
T8R5	10	5.52	1.92	5-10-53	58	In vivo bone radon retention
T9R5	75	10.4	1.94	5-10-53	58	and distribution study.
T10R5	1	1.02	1.98	5-10-53	49	" "
T11R5	1	1.58	1.91	5-10-53	49	" "
T12R5	13	12.3	9.72	5- 9-56	225	" "
T13R5	13	7.59	9.76	5- 9-56	188	" "
T14R4	17	8.13	3.17	7-11-56	72	Phantom.
T15R4	22	9.03	3.11	7-11-56	446*	Phantom.
T16R5	20	12.4	9.68	7-11-57	12	In vivo bone radon retention and distribution study.

* Days since injection.

C. Mesothorium

Exp. No.	Age at Injection (mos.)	Weight at Injection (kg)	Injected Dose (μc/kg)	Date Injected	Dead at (days)	Comments
T1M4.5	17	9.13	5.55	9- 8-54	314	Translocation of Th^{228}
T2M4.5	15	8.93	5.60	9- 8-54	755	Translocation of Th^{228}
T3M5	19	9.15	10.1	3-13-56	566*	Translocation of Th^{228}

D. Radiothorium

T1T5	20	9.30	4.88	12- 1-53	23	Excretion, retention,
T2T5	16	8.48	2.56	2- 8-54	77	distribution, and
T3T4	14	10.4	0.870	2- 8-54	820	translocation
T4T5	15	8.92	2.59	9-28-54	113	"
T5T5	15	10.1	2.32	9-28-54	65	"
T6T4	19	7.01	0.884	10-18-55	651	"
T7T3	19	9.23	0.298	10-18-55	713*	"

E. Strontium

T1S0	5	7.71	Sacrificed	3- 5-54		Hematology control
T2S0	5	6.85	148.	3- 5-54	11	Hematology, radioautography
T3S5	5	6.19	148.	3- 5-54	18	Hematology, radioautography
T4S5	5	7.05	148.	3- 5-54	41	Hematology, radioautography
T5S5	5	5.25	148.	3- 5-54	116	Hematology, radioautography
T6S5	5	7.01	87.	3-16-54	(1 hr.)	Hematology, radioautography
T7S5	5	6.74	87.	3-16-54	2	Hematology, radioautography
T8S0	8	7.0	Sacrificed	11- 4-54		Hematology control.
T8SH	2	2.10	**	9-27-55	66	Distribution following
T9SH	2	2.16	**	9-27-55	66	multiple doses.
T10SH	2	1.59	**	9-27-55	132	"
T11SH	2	2.58	**	9-27-55	132	"
T12S3	20	10.6	10.8	9-11-57	5	Phantom

F. Aging Controls

MAC35 Sacrificed 2-17-56, 17 months old.
MAC37 Sacrificed 7-12-56, 11 years old.
FAC1 Sacrificed 5-14-57, 45 months old.

* Days since injection
** Doses of 1 μc each from 9-27-55 and weekly thereafter for 10 weeks.

CLINICAL SECTION REPORT

C. E. Rehfeld, G. N. Taylor, W. Fisher, N. B. Nebeker

I. Tabular summary regarding toxicity and test animals that were allowed to live until degenerative changes seriously impaired normal function. This will include that group of dogs which developed clinically apparent tumors and another group in which tumor formation was not apparent and in which death was due to anemia and/or other causes.

II. Effect of radioactive isotopes upon the rate of narrowing of pulp cavities of teeth. This study was made through the use of radiographs. This work was done by G. N. Taylor in consultation with William R. Christensen and C. E. Rehfeld.

III. Case histories of the dogs which have been sacrificed or have died from various causes in the period March 15, 1957 through September 17, 1957.

IV. Current census of beagle colony.

I. Summary of Observations on Toxicity Animals and on Test Animals Treated as Toxicity Animals.

Data listed here includes:

1. Days of age when given the radioactive isotope burden.
2. Days following isotope injection when first clinical and/or radiographic evidence obtained for presence of a tumor - latent period of tumor formation.
3. Days following isotope injection when death occurred by euthenasia or spontaneous causes.
4. Anatomical location of tumor which dictated euthenasia; reason for euthenasia other than the presence of a tumor; or cause of death other than by euthenasia.

Radium studies - tumor formation

	1.	2.	3.	4. (location of tumor)
T1R5	995	974	1074	proximal right tibia
T2R5	919	1331	1368	body of 6th cervicalvertebra
M1R5	473	869	908	arch of 6th lumbar vertebra
M2R5	470	1293	1380	distal left tibia
M4R5	408	1031	1091	body of 7th lumbar vertebra
F6R5	486	983	1015	distal right humerus
F8R5	474	917	968	distal right ulna
M1R4	471	1513	1606	left carpus, left maxilla, proximal left tibia

Radium studies - no tumor formation

	1.	2.	3.	4. (reason for death or euthenasia)
F3R5	380		481	encephalitis
F3R4	384		490	encephalitis
M4R2	414		325	perforated infarcted ileum

Plutonium studies - tumor formation

	1.	2.	3.	4. (location of tumor)
M1P5	412	1288	1324	proximal 1/3 left femur
F6P5	407	1129	1194	shaft of right ilium
M1P4	442	1701	1724	proximal right humerus, proximal left femur, fracture of left mandible.
F2P4	567	1537	1556	sacrum
M3P4	475	1171	1198	near head of left tenth rib
M4P4	567	1007	1065	neck of right femur
F5P4	650	1141	1245	body of 3rd lumbar vertebra
M1P3	417	1460	1476	body of 4th lumbar vertebra, body of 5th cervical vertebra.

Plutonium studies - no tumor formation

	1.	3.	4. (reason for death or euthenasia)
F2P5	1151	1576	fracture of left mandible, extreme osteolytic degeneration of entire skeleton
M3P5	515	499	chronic hepatitis and erythropenia

Mesothorium studies - tumor formation

	1.	2.	3.	4. (location of tumor)
F2M4	460	763	777	dorsal process of 13th thoracic vertebra
F1M3	458	828	918	body of 3rd lumbar vertebra

Mesothorium studies - no tumor formation

	1.	2.	3.	4. (reason for death or euthenasia)
F1M5	494		232	aplastic anemia and uremia
F2M5	460		780	bilateral fractures of tibiae
F1M4	510		841	multiple fractures
M3M4	579		418	strangulated inguinal hernia
F2M2	517		965	acute hepatitis

Radiothorium studies - tumor formation

	1.	2.	3.	4. (location of tumor)
M1T4	480	(unknown)	645	mid-shaft left radius, fractures of femurs bilaterally
M1T3	314	942	988	proximal left femur near head of left 11th rib
M2T3	458	859	859	body of 5th cervical vertebra (killed in dog fight
F3T3	471	511	547	body of 13th thoracic vertebra
M1T2	491	1229	1282	shaft of right ilium

Radiothorium studies - no tumor formation

	1.	2.	3.	4. (reason for death or euthenasia)
M1T5	480		212	febrile uremia, aplastic anemia
M2T5	483		97	aplastic anemia, radiation hyperthermia, bacteremia
M2T4	458		832	multiple fractures
F3T4	461		763	aplastic anemia
T3T4	429		820	multiple fractures and acute anemia
T6T4	591		651	multiple fractures
M4T2	553		78	trauma and shock (dog fight)

Strontium studies - tumor formation

	1.	2.	3.	4. (location of tumor)
F1S5	434	870	960	distal right femur

Strontium studies - no tumor formation

	1.	2.	3.	4. (reason for death or euthenasia)
M2S5	540		255	strangulated inguinal hernia
F1S1	1524		308	considered unsuitable for inclusion in toxicity group because following injection it was necessary to perform a hysterectomy and an orbital enucleation and age at injection was undesirable.

Animals which have been replaced, according to research numbers are: F3R4, M4R2, M3M4, M4T2 and F1S1.

II. Effect of Radioactive Isotopes upon the Rate of Narrowing of Pulp Cavities of Teeth.

Introduction: Since March 1956, an occlusal film of the left mandible has been included in the semi-annual radiographic studies of each experimental dog and subsequently this bone and related dental parts have been studied in detail for radiographic changes resultant to the isotope burden. One of the features being investigated is the rate of narrowing of the pulp cavities of the teeth and the influence, if any, of radioactive elements. This study evaluates this feature in only one tooth root.

Three groups of beagles are used: Control dogs, five level plutonium dogs, and five level radium dogs. The pulp cavity measurements are summarized in graphical form and the significance of changes in the cross-sectional diameter, as the age and treatment vary, is evaluated statistically.

Methods: All of the measurements were made by viewing the films with a binocular dissecting scope with a calibrated eye piece. Only one measurement of the pulp cavity was made and the widest cross-sectional diameter in the anterior root of the first molar of the left mandible was arbitrarily selected for this measurement. This tooth was chosen for the preliminary investigation because of the relatively long period it is retained in the animals mouth, its freedom from radiographic distortion, and its relatively large size.

Cross-sectional measurements were taken from 46 radiographs of control dogs and 42 radiographs of 5-level plutonium dogs of comparable ages. In most instances several films are from the same dog but spaced by significant time intervals. Eleven subclasses were formed within each class by arbitrarily grouping the dogs, whose ages ranged from 19 to 84 months, into six-month age groups. The groups were then compared by computing the "F" value at the 95 percent confidence interval, using the method of analysis of variance with disproportionate subclass numbers as outlined by Snedecor.[1]

[1] Snedecor, George W. Statistical Methods, 4th Edition, The Iowa State College Press, Ames, Iowa.

The same procedure was used in comparing the 5-level radium dogs with the control group except 53 radiographs of the treated group and 45 radiographs of the control group were used. Also, the ages ranged from 19 to 78 months, being divided into ten six-month age groups. Mrs. Betty Chamberlain made the statistical computations.

For non-treated dogs we have radiographs of a wider age span, six to 116 months. This is because of the minimum age requirements that are set for injection with the isotope and also the reduced longevity that occurs among the treated groups. Figure 1 shows the age-diameter relationship of the widest age interval for which we have radiographs. Eighty-two occlusal films were used in plotting this curve- all from non-treated beagles.

Results: The "F" values[1] for the effect of age and treatment on the cross-sectional diameter of the 1st molar of the left mandible are summarized in the following table.

Source of Variation	Degrees of Freedom	"F" value computed from the data	"F" value necessary to be significant (95% confidence interval)
5-level plutonium	1 & 66	16.9	252
Age	10 & 66	9.5	2.6
5-level radium	1 & 78	.05	253
Age	9 & 78	6.65	2.77

The above tabulation suggests that the effect of a 5-level quantity of either plutonium or radium administered intravenously by a single injection is not significant as a factor in causing narrowing of the pulp cavity of the specified root. However, the values for the effect of age on the same root indicate this to be a significant factor. The term "age", presented in the above table under the heading "source of variation", is not used in the sense of a single entity or a process but as an inclusive term indicating the duration of

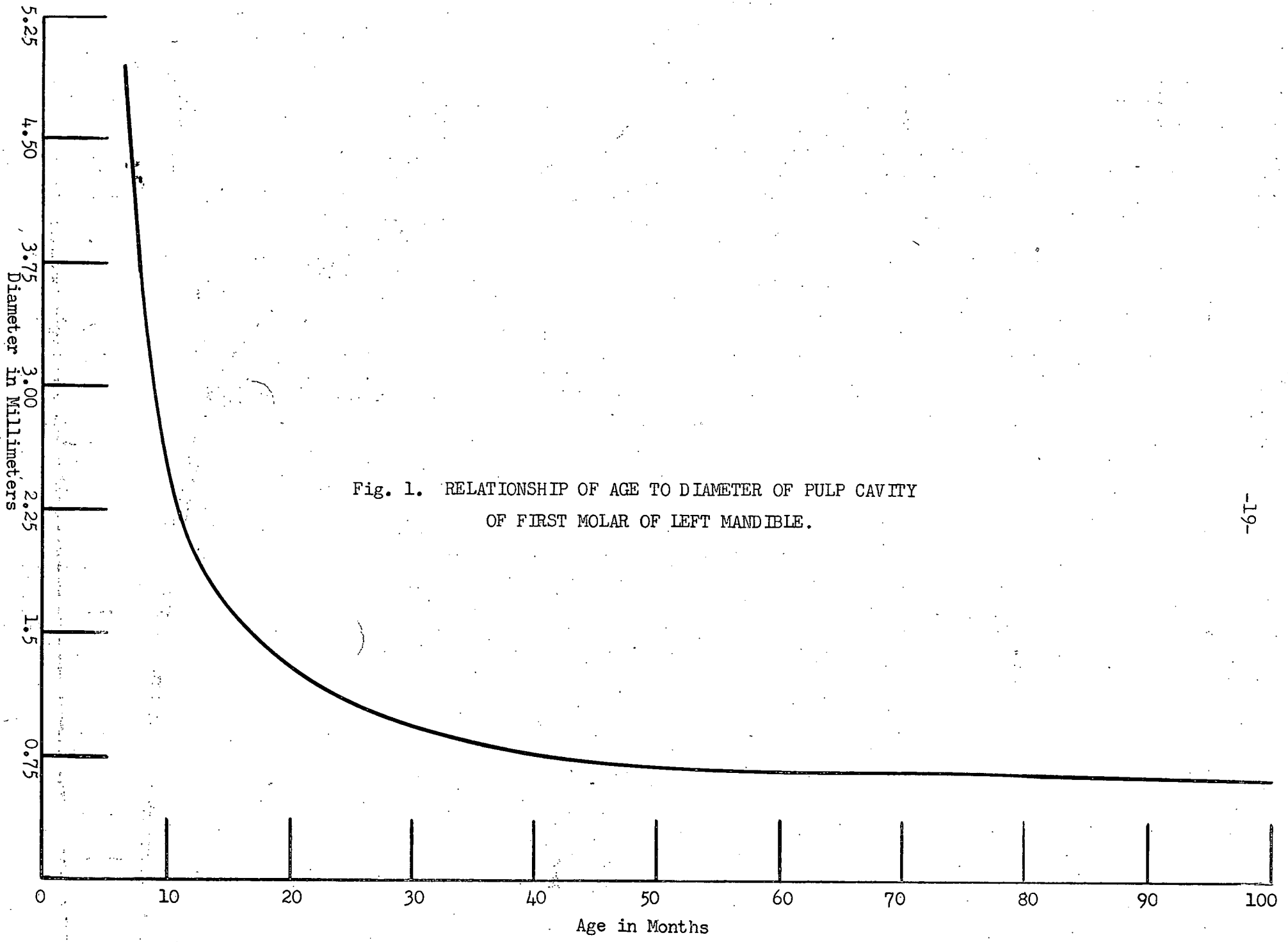


Fig. 1. RELATIONSHIP OF AGE TO DIAMETER OF PULP CAVITY OF FIRST MOLAR OF LEFT MANDIBLE.

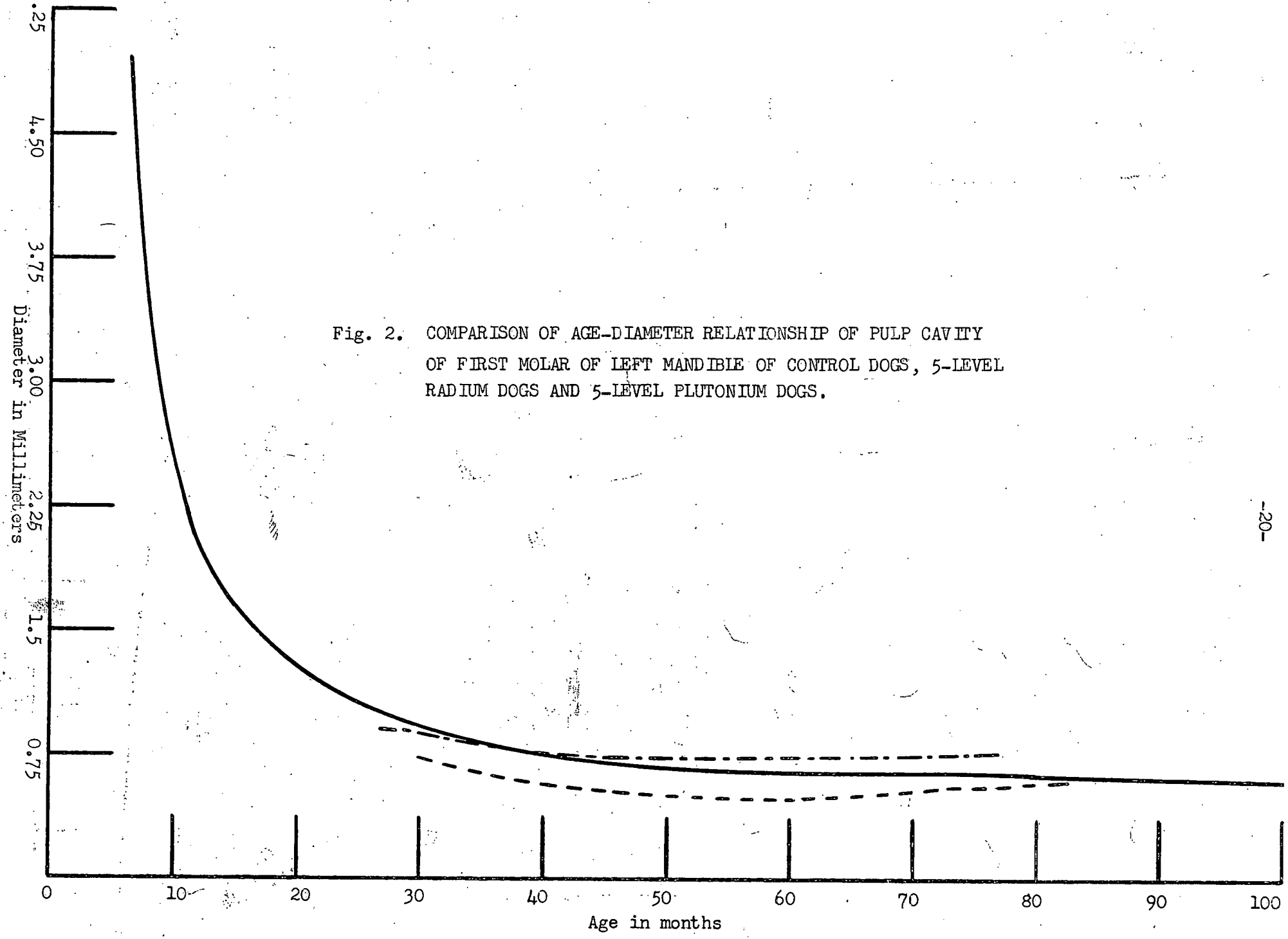


Fig. 2. COMPARISON OF AGE-DIAMETER RELATIONSHIP OF PULP CAVITY OF FIRST MOLAR OF LEFT MANDIBLE OF CONTROL DOGS, 5-LEVEL RADIUM DOGS AND 5-LEVEL PLUTONIUM DOGS.

one to many factors.

Figure 2 substantiates the above statistical results. It is even more suggestive of the importance ofthe age factor because it shows the rapid rate of narrowing that occurs between the ages of 6 and 15 months- such period not included in the statistical analysis for reasons already given.

Conclusions: Five level plutonium and radium burdens have no significant effect on the narrowing process of the pulp cavity of the first molar of the mandible, as observed radiographically. This process appears to be primarily a function of age.

III. Case Histories

Included here are brief reviews of pertinent clinical information of all dogs which died or were euthenized since March 12, 1957. The cases are presented in chronological order in reference to time of euthenasia.

M4R5, Tatoo No. 110: This male beagle was one of three siblings born on February 23, 1953. It was injected with 10.6 μc/kg of radium^{226} on April 7, 1954 when it was 408 days of age. Euthenasia was performed 1091 days after injection because a tumor of the seventh lumbar vertebra caused urinary and fecal incontinence resulting in acute toxemia.

Review of weight changes: Dog weighed 8.9 kg at injection and gained 4.99 kg in the next 706 days, but weighed 8.96 kg 385 days later when euthenized.

Anesthesia: Employed at least 18 times throughout the period of time when the dog carried a burden of radium.

Study with other isotopes: Red blood cell life span was studied by means of chromium^{51} tagging 709 days after injection of the radium burden.

Fractures revealed by radiology and clinical examination: 693 days post-injection, fractures of the second and third thoracic vertebra involving only the dorsal processes. 727 days, additional fracture of one of the first ribs. 777 days, 1 additional rib fracture. 927 days, fracture of the left humerus. That is, there was a defect in the left humerus which appeared as an old well healed fracture, but there were no clinical symptoms of the fracture at this time. 942 days, 1 additional rib fracture. 958 days, fracture of left humerus. 974 days, fracture of right tibia and right fibula. Approximately 48 to 60 days later, the dog developed a urinary and fecal incontinence. This indicated the presence of a tumor arising in the vertebral column. 1085 days, tumor arising from seventh lumbar vertebra confirmed by radiographic examination.

Post-mortem observations: Osteogenic sarcoma of the seventh lumbar vertebra, metastastic processes were seen as follows. There were two closely adjacent to

the diaphragm and the mediastinum; two nodules in the right ventricle of the heart, one at the atrio-ventricular junction, at the anterior lateral aspect measuring 8mm in diameter and the second nodule was more posterior measuring 2 to 3 mm in diameter. There were also numerous nodules from 1 to 3 and 4 mm in diameter seen in all lobes of the lung, these were predominantly at the periphery of the lung.

T8P5, Tatoo No. 168: This male beagle was one of five siblings born on July 5, 1953. It was given a plutonium burden of 2.67 μc/kg at 527 days of age. This animal was euthenized 863 days later as one of a series of 5-level plutonium test dogs.

Weight summary: Injection weight 8.32 kg, autopsy weight 10.62 kg. There was no illness which required that the animal be hospitalized.

Study with other isotopes: This animal was used to study the life span of red blood cells through chromium^{51} tagging and also was given a tracer dose of strontium^{90} 24 hours before sacrifice.

Radiology observations: 477 there was definite pathology due to the radioactive material as reflected in architectural changes in the bone. 787 days, two unhealed rib fractures. 863 days, post mortem observations, five rib fractures, one of which was recent and two of which were well healed. The other two rib fractures showed a regular callus formation and false joint formation . The shaft of the left humerus had changes which suggested a fracture had occurred at some time in this bone but was then well healed. However there was no physical disability due to a fracture in this left humerus at any time.

Observations of soft tissues at autopsy:

The heart: There was a peculiar endocarditis involving the first portion of the pulmonary artery immediately above the valves. The valve ring and valves were normal but the endocarditis had manifested itself as small vari-colored nodules, suggesting calcification and varied from less than 1 mm to 1mm in diameter. These nodules did not extend down to the main branches of the pulmonary artery. There were focal yellow calcified nodules and a thickening in the endocardium of the left atrium as well, particularly around the ostia of the pulmonary vein.

There were glistening nodules 1/2 to 1 mm in diameter located along the line of closure of the mitral valves. There were focal areas of graded calcified nodules less than 1 mm in diameter located in the sinuses of Valsalva above the aortic valves.

The liver: The liver was characterized by mottled contrasting shades of brown in a fine pattern suggesting a disorganized architecture and fine scaring. There were no well defined adenomata.

Kidney: The kidney was seen to have as follows; yellow areas of calcification in the mid portion of the papilla which measured less than 1 mm in diameter and were poorly circumscribed.

M3M4 Tatoo No. 291: This male beagle was one of 4 siblings born August 12, 1954. The mesothorium burden was given (3.22 μc/kg) at 579 days of age. This dog survived 418 days after injection at which time it died from toxemia resulting from the effects of a strangulated inguinal hernia.

Weight changes: The injection weight was 9.65 kg and just before the final illness the weight approximated 10.28 kg and the autopsy weight was 8.18 kg.

Study with other isotopes: 11 days pre-injection this dog was used for a chromium^{51} red blood cell survival time study. 30, 104 and 230 days post-injection blood volume studies and red cell survival time studies were performed using chromium^{51} and iodine^{131}.

Health: This dog suffered 2 periods of illness, one occurring at 248 days and the other at 417 days which was the final illness. 248 days, this dog had been under rather close observation for several days prior to this time and was then brought in for hospitalization at 248 days as it had an extremely debilitated appearance and was suffering partial aneorexia. A thorough clinical pathological study yielded no positive results or indication as to the source or cause of its condition. However a change of diet caused some improvement so the dog was released in a better state of health within two weeks. 417 days, it was reported that the dog had a complete lack of appetite and a brief examination soon revealed

that the animal had a strangulated inguinal hernia. The dog was obviously a very poor surgical risk but surgical intervention is dictated in such cases and was completed within a matter of hours. The dog died the next day at 418 days of acute toxemia and shock and was submitted immediately for post-mortem examination.

Post-mortem observations:

Skeleton: Post-mortem radiographic study revealed moderate to advanced changes in the skeleton due to effects of the radioactive isotope. There was a moderate amount of pathological remodelling of the bone including some osteolytic rarefraction which was present throughout the cortex of the long bones. In regard to fractures there was a single unhealed fracture of the spinous process of one of the thoracic vertebra and the transverse process of a lumbar vertebra. There was one unhealed rib fracture and two fractures of the fatigue type in the body of the scapula.

Soft tissues: Alterations were limited to changes characteristically resulting from stasis in the gastro-intestinal tract. The premortem toxemia may have obliterated minor changes in other of the soft tissues.

F6R5 Tatoo No. 134: This female beagle was one of six siblings born on March 28, 1953. This animal was given 10.2 μc/kg of radium^{226} on July 27, 1954 when it was 486 days of age. 1015 days after injection, euthenasia was dictated by the presence of a large tumor of the distal right humerus and a fracture of the left mandible which interfered with normal ingestion.

Review of weight changes: This dog weighed 9.66 kg at the time it was injected and gained a total of 6.3 kg to its maximum weight and then it lost a total of 7.78 kg from that maximum weight in the last 475 days of its life.

Anesthesia: The dog was anesthetized between 27 and 30 times in the entire period of its life.

Radiology and clinical observations: 542 days post-injection there was a large amount of pathologic remodelling in the bone structure as well as a fracture of the right sixth rib. 574 days, this dog suffered a fractured left mandible in

a dog fight as well as multiple bite wounds. The fracture of the mandible was successfully reduced by use of a single heavy wire suture. At this time there was advanced depigmentation of the iris and the choroid had markedly altered in appearance. 615 days, there were 5 fractures of the dorsal processes of thoracic vertebra all unhealed, but some had some callus formation. 658 days, both fibulae had fractured near the proximal terminations and in addition there were two ribs which had marked enlargements along their length which indicated the possibility of an extra bony growth or of old well healed fractures. Also at this time it appeared that one of the dorsal processes of the thoracic vertebrae had completed healing. 706 days, there appeared to be well healed fractures of the 6th, 8th and 9th rib of the right side. There was abundant callus formation of the dorsal processes of the thoracic vertebrae and appeared to be similar action of the left fibula. All of these had resorted to false joint formation. Advanced osteolysis had occurred at the fracture site in the left mandible and the wire suture was serving no purpose then, so 3 days later this suture was removed. 723 days, two additional well healed fractures of the ribs appeared but there was no indication of fracture at these positions. There was also a fracture of one of the coccygeal vertebrae with no signs of healing. In the left mandible there was a zone of total loss present with a gap of approximately 1 cm which was radiolucent. 768 days, there developed a definite lameness of the right pectoral limb which proceeded without remissions to develop a characteristic radial paralysis with advanced atrophy of the musculature, and a noticeable difference in the temperature of this limb from the opposite normal limb. 792 days, one of the fibulae had healed completely. 983 days, there was a gross irregular zone of rarefraction with some calcification in surrounding soft tissues present at the distal end of the left humerus. This appearance suggested an osteogenic sarcoma at this position.

Post-mortem observations:

Skeleton: The dorsal processes of the first five thoracic vertebrae were fractured with no indication of healing and little callus formation and in addition

the one fibula was still fractured and the coccygeal vertebra showed no signs of healing. Otherwise, healing had occurred with renewed trabecular pattern in the ribs and other fractures, including that of the dorsal process of one of the thoracic vertebrae. The tumor of the right distal humerus had reached dimensions approximately 9 x 9 cm in extent.

Soft tissues: The liver contained numerous small pale poorly defined foci or nodules which averaged about 1 mm to less in diameter. There was a light yellow discoloration of the inner cortex of the kidney and a discoloration of most of the lymph nodes of the body suggesting that they contained hemosiderin deposits.

FAC1 Tatoo No. 172: This female beagle was born on July 31, 1953 and it was given a strontium^{90} burden 24 hours before sacrifice. This animal was euthenized at 1383 days of age to serve as a source of normal tissues. There was nothing remarkable in the history or seen grossly on post-mortem examination.

F2P4 Tatoo No. 7: This female beagle (no siblings) was born August 12, 1951. It was given a plutonium burden of 1.034 μc/kg in March 2, 1953 when it was 567 days of age. This dog survived 1556 days from the time of injection to sacrifice and then was euthenized because of complications caused by an osteogenic sarcoma of the sacrum.

Congenital condition: This dog had suffered no illness prior to the final phase except for a congenital torticollis which caused it to rotate its head slightly to the left.

Review of weight changes: This dog weighed 8.65 kg at the time it was injected and attained a maximum weight of 11.53 kg 531 days pre-mortem and then declined gradually to an autopsy weight of 8.53 kg.

Study with other isotopes: This dog was used three times for a study of the life span of red blood cells by means of chromium^{51}. It was used once for a blood volume study using chromium^{51} and iodine^{131}.

Radiology and clinical observations: 1101 days, first unequivocal evidence of radioactive isotope toxicity affecting the bone causing pathologic remodelling.

All subsequent radiographs indicated that this condition advanced steadily throughout the study period of the animal. At no time were there any clear cut fractures apparent. 1500 days, mild lameness of the left forelimb which at this time did not require hospitalization. 1537 days, definite indications of mild discomfort in left forelimb and there was some weakness of the rear limbs (pelvic). 1542 days, marked incoordination of the pelvic limbs and continued lameness of the left forelimb. Soon thereafter the animal's appetite failed and sacrifice was recommended. However, euthenasia could not be arranged immediately and the animal was placed under constant sedation. During the next 10 days real discomfort seemed apparent only when the tail was moved for any purpose. 1382 days, in the pre-mortem examination there was little to see which has not already been described except that there were some minor alterations in the tapetum which indicated destruction of some of the irridiocytes.

Post-mortem observations:

Liver: The liver was studded with poorly circumscribed nodules which varied in size up to 2 cm in diameter.

Kidney: The kidney had numerous shallow scars over the surface which measured approximately 1 mm in greatest diameter, and there was a zone of yellowish discoloration in the inner cortical region. The aorta showed variable thickness in the wall in the ascending arch but this was not reflected on the surface of the aorta since both surfaces were smooth and unaltered.

F2P5 Tatoo No. 000: This female beagle (number of siblings unknown) was born January 6, 1950. It was given a burden of 3.298 μc/kg of plutonium on March 2, 1953 when the dog was 1151 days of age. This dog was euthenized 1576 days following injection because of extreme degeneration of the skeleton and a fractured mandible which prevented normal ingestion of food.

Weight changes: The injection weight was 8.75 kg, the dog gained up to a maximum weight of 13.98 kg and weighed 8.98 kg at sacrifice. The period of decline as shown by loss of weight from maximum to sacrifice was 571 days. Anesthesia

was employed approximately 25 times.

Study with other isotopes: At 687 days post-injection a Sr^{85} tracer dose was given to the dog. $Chromium^{51}$ was used twice to study the life span of the red blood cells.

Radiology and clinical observation: 711 days post-injection there appeared the first unequivocal evidence of bone pathology due to radioactive material. 842 days, the spinous processes of three thoracic vertebrae were fractured. 1060 days, there was another spinous process fracture and one rib fracture. 1177 days, there were alterations in the body of the left ilium which made it appear that there was a well healed fracture present at that site. 1290 days, the single rib fracture appeared to have healed satisfactorily. 1330 days, there were at least 5 fractures of the ribs and the majority of these appeared to have healed satisfactorily, however, the spinous processes still had not shown signs of healing. The dog was hospitalized at this time for a period lasting 23 days. The animal at this time was rapidly deteriorating in general condition and health as reflected in weight loss, general attitude, appetite and the fact that it was losing hair very rapidly. All clinical pathological studies done at this time were negative but a change in diet appeared to cause much improvement. 1359 days, the animal was again hospitalized for a brief examination, period of treatment, and change in diet, with good results. 1376 days, the dog was again placed under close observation and constant care. The animal maintained a satisfactory appetite for the remainder of the time it was studied, approximately 202 additional days.

Pre-mortem examination: The iris appeared to be definitely mottled but it could not be said with certainty that this was a depigmentation process. However, the tapetum appeared granular, particularly at the periphery as though some of the irridiocytes had been destroyed in this area. There was also a structure at the distal extremety of the left radius which indicated this might possibly be an osteogenic sarcoma.

Post-mortem observations: The liver was grossly abnormal with complete obliteration of the usual lobe formation and had a variable color with small pale nodules presented in contrast to the intervening dark parenchyma. The largest nodules were up to .5 cm in diameter and most of them being several mm in size. These were separated by the intervening scar tissue and parenchyma.

Kidney: There was a yellow discoloration of the inner zone of the cortex of the kidney. The medulla contained a few yellow white streaks throughout extending down towards the papillae. Mammary tissue contained several very well circumscribed nodules which seemed to be fibrous in consistency. Parathyroids were unusual in that they were quite small and had petechial hemorrhages evenly distributed over their surfaces. Dura mater, the cranial dura mater was closely adherent to the calavarium so that it was freed with considerable difficulty.

F3T4 Tatoo No. 253: This female beagle was one of 5 siblings born March 2, 1954. This dog was given a radiothorium burden of .8 μc/kg on June 6, 1955 when the dog was 461 days of age. This dog was euthenized 763 days after injection because of an anemia so extreme that the volume of pack red cells was only 1/3 normal.

This dog weighed 7.25 kg at the time it received the radiothorium burden and advanced to a maximum weight 10.79 kg and from that point declined to 7.19 kg at the time of euthenasia. The period of decline as shown by a steady loss of weight from the maximum weight was 227 days.

Anesthesia: This dog was anesthetized approximately 11 times, nine of these times for the purpose of obtaining radiographs.

Study with other isotopes: Chromium^{51} was used to study the life span of red blood cells on August 10, 1955, October 20, 1955, April 25, 1956 and June 1, 1956.

Radiology and clinical observations: 262 days, the first evidence of changes in the bone were noted as limited to sub-periosteal new bone formation, and this was confined to the mid-sahft of the tibiae bilaterally. 478 days, there was a spontaneous fracture of the left ulna but this bone promptly healed so the dog could be discharged 63 days later. The dog reached its maximum weight at the end

of this particular hospitalization period. 641 days, the volume of packed red cells was reported to be extremely low so treatment was initiated but without success. 660 days, a rather rapid loss of weight, hair coat very dull, considerable alopecia, almost constant production of exudate from the nasal cavity and rather frequent fluctuation of appetite through poor, indifferent, and normal. The rather constant production of nasal exudate has not been observed in any other animal in this present study to date and it must be noted that there were no other clinical symptoms in connection with it and it never responded successfully to antibiotic therapy. 731 days, one rib fracture; fracture of the right ulna; marked alteration of structure in the shaft of the left radius.

Pre-mortem observation: There was advanced depigmentation of the iris, a marked state of dehydration and an obvious icterus.

Post-mortem observaticn: There was practically no functional bone marrow in the entire skeleton but what there was was located in the shafts of the humeri and the femur. The lymph glands were quite atrophic and the spleen was very light in color and also atrophic.

F2M2 Tatoo No. 166: This female beagle was one of 8 siblings born June 30, 1953. It was given an injection of .355 μc/kg of mesothorium on November 29, 1954 when it was 517 days of age. This dog died of acute hepatitis 965 days after receiving its mesothorium burden.

This dog weighed 8.25 kg when it was injected and reached a maximum weight of 12.87 kg 547 days after injection, and then weighed 10.68 kg when the autopsy was performed at 965 days.

Anesthesia: This dog was anesthetized 6 times for radiographic study and about 3 times for whole body radiation measurement.

Radiology: There were no marked changes in the bone as shown by radiographic study so the fatal illness was of interest because of its brevity and its unusual nature as seen in our experience here.

Symptoms, therapy and course of illness: On July 20, 1957 about 10:15 a.m. this dog was observed in the runs to be so weak that it was unable to stand. It was obvious that at least 100 ml of a serosanguinous fluid had drained from the rectum when the dog was found and more was lost similarly during the succeeding several hours. The dog became comatose shortly after being found in the weakened condition but was revived with 150 ml of whole blood given intravenously and another 200 ml of supportive fluids given similarly. There was no evidence of an intestinal tract blockage or of an acute gastritis or enteritis obtained by palpation and radiographic examination. There was no vomiting or nausea, there was an icterus and a body temperature of 103 degrees F. The dog responded very rapidly to the treatment given and was walking about quite alertly within two hours. Precaution was taken of giving both orally and rectally a mixture which coats the intestine with an antibiotic and a protective colloid.

Four hours after being found the dog was still somewhat depressed but very responsive so was given additional supportive fluids intravenously as well as rather large amounts of penicillin and dihydro-streptomycin. In spite of this rather good appearance and cause for optimism the dog died approximately 20 hours after first being seen in the weakened condition in the runs.

Post-mortem observations:

Liver: The liver had a rather characteristic so-called morroco-leather appearance and this later proved to be primarily a centra-lobular necrosis. The biliary duct system appeared to be patent. All fatty tissues throughout the body cavities were definitely icteric. Gastro-intestinal tract; the ileum was dialated about 5 cm anterior to the ileocecal valve when first observed but this soon contracted so that it was no greater in diameter than the rest of the ileum. The stomach was distended and about 1/2 filled with a grayish cloudy fluid. The spleen was very dark and filled with a large amount of blood. The lungs were somewhat edematous and the bronchial nodes were very dark and congested. The kidneys had a cooked appearance and the cortex was somewhat darker than normal.

Brain; although no pressure measurements were taken the fluids in the ventricles of the brain appeared to under an increased pressure. Bone marrow; the general marrow state of the dog was good and no unequivocal changes could be detected in the architecture of the bone. Lymphoid tissue; the lymph tissue was very black in color wherever seen and different in this respect from any other dog seen here to date. Particularly striking was the appearance of the lymphoid tissue of the gastro-intestinal tract, the peyers patches, the lymph nodes in the cecum and the rectum and colon were all a dull black in color, outlining extremely well the presence of and the extent of lymphoid tissue distribution in the the lower intestinal tract.

T16R5 Tatoo No. 022: This male beagle was purchased for use as a test animal. It was given a 5-level radium burden on July 11, 1957 when it was 604 days of age, weighed 12.39 kg, and was sacrificed when it had carried the burden for 12 days. It weighed 12.22 kg at sacrifice and a post-mortem examination of the animal revealed it to be grossly normal in all respects.

T6T4 Tatoo No. 257: This male beagle was one of 7 siblings born March 6, 1954. It was given a burden of radiothorium (.884 μc/kg) on October 18, 1955 when it was 591 days of age. This dog as a test animal was sacrificed 651 days post-injection following a fairly long period of illness which dictated sacrifice to prevent loss of tissues. He was extremely anemic and had multiple fractures responsible for the prolonged final illness.

Summary of weight changes: This dog weighed 7.01 kg at injection, gained to a maximum weight of 7.90 kg and then declined to a weight at autopsy of 7.22 kg. The weight had dropped considerably at one time during the illness and then re-gained to the high point only 11 days before sacrifice.

Anesthesia: This dog was anesthetized 10 times for radiographic study and 10 times for whole-body radiation counting.

Radiology and clinical observations: 335 days post-injection, there was a pathologic remodelling of the bone and 2 unhealed fractures of the ribs. In

addition there were 2 irregular zones of increased density localized at the upper end of the ulna bilaterally and there appeared to be a definite enroachment on the medullary canal at this point. 470 days, there was a marked lameness or weakness of the rear limbs, there were a total of at least 8 rib fractures, all unhealed, and in addition there was what appeared to be a healed fracture of the body of the left ilium and perhaps a fracture through the roof of the acetabulum. 487 days, there was definite lameness and tenderness in the left pelvic limb. 490 days, a radiograph demonstrated what appeared to be old fractures of the body of the ilium bilaterally. In the left ilium this appeared to have healed with abundant callus formation but a central irregular zone of osteolytic rarefraction was present. There was also some irregularity and deformity in the region of the pubic synphysis. 514 days, there were a total of at least 11 unhealed rib fractures. There also appeared to have been old fracture injuries of the right foreleg, the body of the ilium bilaterally and the body of the scapula. 529 days, there was a definite limp of the left pectoral limb and also a stilted movement of the rear limbs. Within 10 days it again became very active and alert and there was little abnormality in ambulation. 549 days, there was an additional unhealed fracture of the left ulna. 543 days, the left pectoral limb was not used at all in ambulation. 562 days, the dog was again walking with fair normality and a hard callus was obvious about the fractures which had been noted in both the radius and the ulna of the left forelimb. This callus became larger and immobilized the leg throughout the next 3 weeks. (It must be noted that the dog resisted all attempts to immobilize this left forelimb by means of supportive casts). 597 days, a summary of fractures included at least 13 rib fractures essentially all unhealed and the unhealed fractures of the right ulna, left radius and ulna and the body of the right ilium. 613 days, it was noted that the dog had rapid involuntary movements of the mandible or a chatter which had been previously noted in other dogs in this stage, particularly those bearing radiothorium and in a debilitated condition. This mandible chatter was stimulated particularly when the dog

lowered the head from a raised position and the condition continued throughout the remaining 38 days the dog lived.

Pre-mortem examination: The iris was moderately depigmented and the tapetum lucidum had a coarse granular nature. There was obvious atrophy of the temporal and masseter muscles. There was an advanced abnormal graying over the entire coat of hair and the hair was abnormally long. There was a lateral deviation of the left radius and ulna at mid-point giving the leg a bowed appearance.

Post-mortem observations: The muscle tissues were generally atrophic. The wall of the duodenum and the ileum appeared to be thickened and there were numerous petechial hemorrhages in the mucosa in this area. The aorta and vessels in general had normal surfaces but there was intermittent thickening in the wall of the ascending arch of the aorta.

M1T2 Tatoo No. 80: This was a male beagle (no siblings) born October 5, 1952. It was injected with .097 μc/kg on February 8, 1954 when it was 491 days of age. Euthenasia was performed 1282 days post-injection because a tumor of the right ilium had advanced sufficiently far into the pelvic vanal to prevent normal elimination.

Summary of weight changes: This dog weighed 10.2 kg at injection time, it attained a maximum weight of 11.96 kg and declined to a sacrifice weight of 8.46 kg. There was a nearly constant but slow loss of weight during the last 309 days.

Anesthesia: This dog was anesthetized 9 times for whole body radiation activity study and 8 times for radiology.

Radiology and clinical observations: 1163 days, there was pathologic remodelling of the bone and gross zones of osteolytic rarefraction were apparent. 1169 days, there was an infection in the right forelimb resulting in marked edema but the condition was corrected in 9 days. 1229 days, the dog had been limping intermittently during the previous two weeks (the right pelvic limb) and was hospitalized at this time for closer observation. An examination revealed the possibility of a tumor forming on the shaft of the right ilium which resulted in

extreme weakness of the pelvic limbs or perhaps a slight paralysis of the right pelvic limb. There was also some atrophy of the musculature in this affected limb. A review of radiographs taken 66 days before this had shown that there were gross zones of osteolytic rarefraction apparent in the right side of the pelvis and especially in the region of the right femoral neck. 1232 days, increasing osteolysis of the ilium and the anterior lip of the right ascetabulum. The head and neck of the femur and the ischial region of the pelvis also appeared involved. There was very little use of the right pelvic limb at this time. 1267 days, the right half of the pelvis with the exception of the wing of the ilium was largely radiolucent so that there appeared to be very little to support the femur in its attachment to the pelvis. There was considerable enroachment on the canal by soft tissue which promised to soon occlude this canal.

Post-mortem observations: There was a generalized icterus. The lung contained small firm nodules 1 to 2 mm in diameter scattered sparsely throughout all lobes and these were presumed to be metastatic tumors. There was focal thickening of the wall of the descending portion of the arch of the aorta but no evidence of intimal wrinkling in the aorta. The tumor of the right ilium had practically occluded the pelvic canal. Apart from the pelvis the skeletal tissue showed only minor degenerative changes with no fractures apparent. The bone marrow in mid shaft of the long bones was light pink in color in contrast to the usual finding of a rather deep red marrow in this position in other dogs. The marrow elsewhere as in the vertebral bodies and sternum was normal in appearance.

F8R5 Tatoo No. 195: This female beagle was one of 4 siblings born September 3, 1953. It was injected with 9.68 μc/kg on December 21, 1954 when the dog was 474 days of age. Euthenasia was performed 968 days following injection because a tumor of the right distal ulna had impaired circulation of the carpal and metacarpal region.

Summary of weight changes: The injection weight was 7.76 kg and the dog gained to a maximum weight of 11.16 kg 934 days post-injection. The sacrifice

weight was 9.72 kg. There was a steady loss of weight from the maximum attained during the last 34 days of the study period.

Anesthesia: This dog was submitted to whole body radiation measurements twice and was radiographed 12 times.

Study with other isotopes: Total blood volume study was performed on August 1, 1956 by means of chromium^{51} and iodine^{131}.

Radiology and clinical observations: 400 days, the skeleton appeared essentially normal. 478 days, there were at least six rib fractures and two fractures of the spinous processes of thoracic vertebrae. In addition there were gross changes in the architecture of the long bones, particularly of the radius and ulna bilaterally. 498 days, there were two additional rib fractures. 517 days, there were now 11 rib fractures and an additional fracture of a spinous process, the other spinous processes had healed and oneor more of the ribs had healed satisfactorily at this time. 646 days, all fractures appeared to have healed satisfactorily except for two of the ribs and one spinous process where there was false joint formation. 664 days, still two unhealed rib fractures and one new rib fracture. 678 days, the most recent rib fracture reported appeared to be healing but there were three additional new rib fractures, none of which were showing signs of repair. 741 days, no new rib fractures seen and the healing had progressed very well in all the others even to the extent that one of the ribs which had the appearance of a false joint for several months had now healed satisfactorily. 896 days, there appeared a defect (an interuption in the continuity of a portion of the cortex) in the ulna of the right foreleg at the juncture of the middle and the distal 1/3 of the bone. 917 days, the dog limped heavily on the right forelimb and there was some swelling over the distal lateral surface of the right ulna. This swelling was somewhat sensitive to palpation or pressure at this time and did not change in this respect throughout the next 51 days. 932 days, it was very obvious that the right ulna was showing considerable disorganization in bone structure as well as osteogenesis. 935 days, the tumor of the ulna

caused some lateral deviation of the foot. Throughout the remainder of the 51 terminal days, the tumor enlarged at a steady rate and caused increasing complications in the circulation of the foot. Otherwise the dog remained normal and quite active. The tumor eventually reached a size which was 5 1/2 cm by 6 cm and roughly oval in shape.

Pre-mortem examination: There was advanced depigmentation of the iris as well as a rather marked destruction of the tapetum cellulosum and the development of a moderate condition resembling albuminuric choroiditis in appearance. The edema of the right fore-foot was extreme.

Post-mortem observations: Skeleton; all fractures had healed satisfactorily with one exception, there was a false joint formation in one rib fracture. Lymph nodes; generally well replaced by fat and none appeared to contain tumors. Kidney; there was a yellow substance stippled on the inner portion of the cortex of the kidney.

F6P5 Tatoo No. 124: This female beagle was one of 6 siblings born March 31, 1953. It was given a burden of 2.57 μc/kg of plutonium on May 12, 1954 when the dog was 407 days of age. It survived 1194 days from the time of injection when a massive tumor originating from the shaft of the right ilium occluded the pelvic canal causing constipation, acute toxemia and death. The autopsy was performed the day following death.

Summary of weight changes: This dog weighed 7.90 kg at time of injection and achieved a maximum weight of 11.36 kg at 413 days post-injection, but it was given a reducing diet and reached an optimum weight of 10.23 kg at 890 days. The dog was then put on a maintainence diet but in spite of this it lost weight to 7.64 kg at the time of death.

Anesthesia was employed 12 times for radiology.

Study with other isotopes: Chromium^{51} was employed once to determine red cell life span and a combination of chromium^{51} and iodine^{131} was used 3 times for blood volume studies.

Radiology and clinical observations: 583 days, there were fractures of a first rib and of the dorsal process of one thoracic vertebra. 639 days, this dog developed an intestinal herniation into the left inguinal canal. This hernia was surgically corrected 13 days later and recovery was uneventful. 692 days, there were fractures of ribs 1 and 4 on the left side and ribs 7 and 8 on the right side. 877 days, there was good healing and union in one rib fracture but there were 2 additional fractures so that now there were fractures at the hot line in ribs 8, 9 and 10 on the right side. 897 days, there was obvious union in some of the rib fractures even at the hot line, and all of them had good callus formation. 1023 days, there began a marked lameness of the left pelvic limb which lasted for 80 days. The cause of this was not diagnosed. 1028 days, fracture at the hot line of the left ninth rib, all other fractures appeared to have resorted to false-joint formation or to have healed satisfactorily. 1034 days, weakness in the pelvic limbs which may have been referable to a defect in the shaft of the left ilium which had the appearance of an old fracture. 1129 days, there was a severe lameness of the right pelvic limb, otherwise the dog appeared quite normal. 1157 days, there were symptoms of a definite arthritis in the right stifle or knee joint. 1185 days, there was acute constipation caused by a partial occlusion of the pelvic canal. Radiographs indicated that there was a tumor arising from the ischium and the pubis of the right side. The constipation caused a toxemia which could not be successfully corrected.

Post-mortem observations: Comments upon the soft tissue have little value due to the condition of the dog pre-mortem and the delay in post-mortem examination. (Considerable comment has been included regarding the progress of fracture occurrence in this dog so a final status of rib fractures was included.) Ribs 8, 9 and 10 on the right side were fractured at the hot line, the 11th rib had healed from an early fracture at its hot line and the 12th rib was fractured just above the hot line. There had been a fracture of the 7th rib near its vertebral attachment but union was so perfect that the fracture site was scarcely demonstrable

by radiograph. On the left side there was a fracture midway in the first rib, at the hot line in the 3rd rib also in the 7th, 8th and 10th ribs. While healing had occurred in a previous fracture in the 9th rib. The fracture of the dorsal process of the 3rd thoracic vertebra had proceeded to false joint formation.

M1P4 Tatoo No. 3: This male beagle was born September 1951 and was one of the test animals purchased for toxicity studies. It was injected with .823 μc/kg of plutonium on December 1, 1952 when 442 days of age. A fracture of the left mandible, a tumor of the proximal right humerus and a tumor of the proximal left femur dictated euthenasia after a trial period of 1724 days.

Summary of weight changes: The weight at injection was 7.61 kg, and 9.9 kg at time of euthenasia. It reached a maximum weight of 11.53 kg at 1095 days, but still weighed 10.6 kg at 590 days later, only 39 days before sacrifice.

Anesthesia: This dog was anesthetized 15 times for radiographic study, once for surgery and one time for dental work.

Study with other isotopes: This dog was used once for erythrocyte life span study with chromium^{51}. Note- At one time prior to September 1955 this dog suffered from severe alopecia, but hair growth was restored after about a year when it had been on a beef fat supplemented diet for several weeks or months.

Radiology and clinical observations: 1316 days, first definite indication of injury to skeletal tissue through changes seen in the architecture of the bone. 1618 days, the dog was observed to tilt his head to the left when eating and the cause proved to be a fracture, at the level of the first premolar, of the ramus of the left mandible and considerable osteolysis in this area. There did not seem to be pain associated with this condition so the diet was changed resulting in the fact that the dog gained about 1 kg in the next five to six weeks. No swelling was ever apparent at the fracture site. 1665 days, there had been a gradual strophy of the temporalis and the masseter muscles. 1701 days, the animal started to limp heavily on the left pelvic limb and radiographs indicated an area of osteolysis in the left femur. At this time there were also suggestions of a bone

tumor forming at the proximal end of the right humerus. 1709 days, the left pelvic limb was held in extreme flexion at all times and tumors were now palpable on both the left femur and right humerus. Also at this time there were more definite signs of disorganization in the architecture of the bone of the left proximal femur.

Pre-mortem examination: It should be noted that the appetite was never seriously impaired in this animal. There was a peculiar condition in the fundus of the eye of this animal which made it appear that there were rust colored irridescent cells overlying the tapetum cellulosum. No fractures were ever apparent in this animal, other than in the zone of osteolysis in the left mandible.

Post-mortem observations: There were poorly circumscribed light yellow nodules varying up to 2 cm in diameter located throughout all lobes of the liver.

M2R5 Tatoo No. 54: This male beagle was one of 4 siblings born August 3, 1952. It was injected with 10.8 μc/kg on November 16, 1953 when it was 470 days of age. This dog survived 1380 days after injection when it was euthenized because it had developed a massive tumor of the distal left tibia.

Summary of weight changes: The injection weight was 8.85 kg and maximum weight was 11.93 kg at 683 days post-injection. This animal fluctuated often in its weight and still weighed 10.65 kg at 1355 days while the sacrifice weight was 9.89 kg. The period of decline from 10.65 was only 25 days, but this dog weighed as little as 7.2 kg at 1182 days or 198 days pre-mortem.

Anesthesia: This animal was anesthetized 24 times for the purpose of obtaining radiographs, at least 4 times fro surgery and 10 times for whole body irradiation measurement and once for dental work.

Chronic ear infection: This dog suffered from a chronic ear infection throughout most of its test period. There were three periods of time when more vigorous treatment was necessary than at other times. The first episode lasted from September 14, 1955 to February 10, 1956. The second episode from May 24, 1956 to July 7, 1956 and the last episode from January 29, 1957 through July 13,

1957. During the second episode the irritation was so severe as to stimulate violent shaking of the head causing a hematoma to form in each ear, and the one ear required surgical correction. The presence of this ear infection had some significance since there was some correlation between the ear infection and a poor hemogram appearing in the animals history.

Radiology and clinical observations: 20 days post-injection there was a foot injury which healed without complication. 674 days, there occurred an oblique fracture of the left humerus and it was observed at the same time that there were fractures of the 4th, 5th and 6th ribs on the left side and the 5th rib on the right side. 784 days, an additional fracture occurring in the 8th left rib and of the dorsal process of the 5th thoracic vertebra. 911 days, all fractures had healed with good bridging except the right 5th rib. 955 days, there were no new fractures but a peculiar formation of the bone on the left 13th rib and the right 9th rib made it appear that these ribs had been fractured at one time. 976 days, there was another similar structure indicating old fracture below the previous fracture on the right 5th rib and the previously noted fracture of the right 5th now had a callus formation although good bridging had not occurred. 1114 days, there occurred a fracture of the right femur and there now appeared to be a large osteolytic zone in the proximal left tibia. 1297 days, there was some attempt at bony bridging in the fracture of the right femur. 1353 days, there was obviously an osteogenic sarcoma of the proximal left tibia and the adjacent distal femur.

Pre-mortem examination: The eyes had undergone advanced destructive changes which included a marked depigmentation of the iris and the disappearance of irridiocytes in the tapetum cellulosum. There had developed a condition which is identical in appearance to the condition known as albuminuric choroiditis.

Post-mortem observations: Comments will be included in another report at a later date.

F1S5 Tatoo No. 219: This female beagle was injected with 93.6 μc/kg when it was 434 days of age. Euthenasia was performed 960 days post-injection because of the development of an osteogenic sarcoma of the distal right femur.

Summary of weight changes: The injection weight of F1S5 was 9.38 kg, it reached a maximum weight of 12.73 kg at 631 days post-injection and then declined to 8.12 kg at termination. This period of decline as shown by constant loss of weight was 329 days.

Anesthesia: Employed four times for whole body radiation measurements and ten times for radiology.

Radiology and clinical observations: 24 days post-injection this animal experienced an episode of radiation hyperthermia which was treated successfully so that the dog was apparently normal again within 17 days. Treatment consisted of aureomycin given at the rate of 200 to 400 mlg daily and 10 to 20 ml of nicothiamin given orally daily. The course of the toxicity period was entirely uneventful until 870 days post-injection when a definite lameness of the right pelvic limb with a slight edema or swelling over the stifle or knee joint indicating some disorder in the distal femur. Radiographs at this time indicated a large osteolytic area and a roughened cortical margin at the distal femur suggestive of a bone tumor. Succeeding radiographs throughout the next 90 days showed a gradually developing characteristic osteogenic sarcoma. At no time was there an impairment of the appetite or other normal functions. The only therapy employed in the course of this 90 day period was the use of analgesics and tranquilizing drugs because this dog was unusual in that there appeared to be obvious pain associated with the formation of the tumor. It should be noted that the pain associated with the tumor was stimulated primarily upon palpation of the tumor at infrequent intervals. There did not seem to be pain associated with ambulation or other normal functions, however, therapy was employed to eliminate the possibility of there being pain associated with this tumor at all times.

Pre-mortem examination: The lymph nodes in general were somewhat smaller than normal. The tapetum cellulosum in the fundus of the eye was altered sufficiently to indicate that there had been some damage to this organ in that perhaps some of the irridiocytes had been distroyed.

Post-mortem observations: Radiographs of the bones would indicate that osseous tissue was entirely normal with the exception of the tumor growth.

Lungs; normal texture but there were numerous small nodules evident over the surface, these were less than 1 mm in diameter.

Lymphoid tissue: The right popliteal and the internal iliac chains of lymph nodes were hemorrhagic and three to four times normal in size. The enlarged lymph nodes were those associated with the tumor bearing limb.

T17P5 Tatoo No. 358: This male beagle was one of three siblings born February 4, 1955. It was injected with a 5-level burden of plutonium on February 12, 1957 when it was 739 days of age. This animal was euthenized 210 days after injection so as to complete a series of plutonium test animals.

Summary of weight changes: The injection weight was 11.14 kg and weight at sacrifice was 10.20 kg.

Clinical observations: At 137 days of age (and pre-injection) this animal experienced a pneumonia and enteritis which was treated successfully in approximately 11 days. The clinical history during the post-injection period was with out events of importance but a pre-mortem examination indicated that this animal possessed an abnormally large spleen.

Post-mortem observation: The only thing worthy of note here was that the spleen was enormously enlarged so that it weighed just half as much as did the liver or 125 gms. This was approximately 3 to 4 times the size normally seen in other dogs.

M1R4 Tatoo No. 16: This male beagle was one of 3 siblings born January 5, 1952. It was injected with 3.51 μc/kg when it was 471 days of age. Euthenasia was employed at 1606 days because tumors had developed on the left maxilla, the left

carpus and the left proximal tibia.

Summary of weight changes: Injection weight was 9.08 kg and the dog gained to a maximum weight of 10.68 kg at 1279 days and then declined to a sacrifice weight of 6.59 kg at 1606 days. There was a slow and steady decline in weight during the last 327 days.

Anesthesia: Employed three times for whole body radiation measurement, twelve times for radiology and twice for surgery and dental work. S

Study with other isotopes: Chromium^{51} was used at 877 days to measure the life span of red blood cells.

Radiology and clinical observations: 1513 days or 93 days before euthenasia this dog was observed to have a swelling over the left carpus which caused little or no inconvenience. 1535 days the first real abnormality in ambulation was noted and seem referable to the pelvic area, particularly the action of either or both pelvic limbs. This inconvenience was of very short duration and ambulation appeared nearly normal thereafter for some days. 1557 days there developed a fairly large swelling over the left maxillary bone anterior lateral to the left eye. This swelling had all the characteristics of an abscess in connection with one of the upper cheek teeth but there proved to be no connection there. This swelling persisted with little change then for some weeks. 1589 days, there was a pronounced lameness of the left pelvic limb and a quickening increase in the size of the swelling over the left maxillary bone. Thereafter the condition of the animal deteriorated rapidly so euthenasia was recommended immediately and accomplished 16 days later.

Pre-mortem examination: There appeared to be some depigmentation of the iris and there was also some alteration in the tapetum cellulosum. There had been a condition develop in the fundus of the eye which resembled very well that termed albuminuric choroiditis in the human.

Post-mortem observations: There was little noteworthy to record as seen regarding the gross appearance of the tissues.

T12S3 Tatoo No. 418: This male beagle was one of 4 siblings bonr January 27, 1956. It was given a three level burden of strontium September 11, 1957 when it was 593 days of age. This animal was not suitable for use in a regular toxicity group because of the history of illness prior to an age suitable for assignment to a regular group. Euthenasia was performed 5 days post-injection and no autopsy was performed since the animal became than a phantom for use by the physics group.

Clinical observations: 424 days, traumatic injury to the right foreleg causing moderate edema but recovery was entire within 13 days. 444 days, severe bronchitis and possibly pneumonia complicated by a marked edema of the lower portions of all limbs with symptoms of a generalized arthritis or neuralgia. Vigorous treatment with erythromycin required 17 days to cause recovery. 470 days severe bronchitis and generalized neuralgia, 25 days required for recovery. A markedly elevated and stubborn temperature was characteristic of both of these episodes, that is the 2nd and 3rd episodes. 518 days, severe bronchitis and generalized neuralgia, very similar to the second respiratory episode, dramatic improvement and recovery was accomplished by the use of meticorten or prednisone. 592 days, there again appeared a temperature elevation and a generalized neuralgia so meticorten treatment was initiated immediately. The animal was given a strontium burden the next day. The animal was euthenized 5 days later.

T18P5 Tatoo No. 360: Male beagle one of three siblings born February 4, 1955. It was given a 5-level plutonium burden on February 12, 1957 when it was 739 days of age. It was sacrificed 217 days post-injection to complete a series of test dogs.

Clinical observations: 24 days post injection, there developed a characteristic radiation hyperthermia with a temperature of 105.5 or 4 degrees above normal and other symptoms characteristic of this condition. This dog was treated successfully with rather massive doses of aureomycin so that the temperature was reduced to normal range within 4 days. 180 days, approximately, there was incoordination of the pelvic limbs an episode of short duration with apparent

complete recovery without therapy.

The dog appeared entirely normal in a pre-mortem examination.

IV. Current Census of Beagle Colony

Radium	75
Test Radium	1
Plutonium	71
Mesothorium	42
Test Mesothorium	1
Radiothorium	21
Test Radiothorium	1
Strontium	26
Aging Control	41
Radiology	2
Unassigned	38*
	319

* Does not include all puppies.

THIS PAGE

WAS INTENTIONALLY

LEFT BLANK

HEMATOLOGY REPORT

The report from the hematology group includes the following data:

1. Pre-sacrifice hematology values on toxicity dogs sacrificed since the March 1957 Annual Report.
2. Graphs summarizing the hematological response to radiothorium and mesothorium for twenty-four months following injection.
3. Bone marrow differential counts on long bones of toxicity dogs autopsied to date.

1. Pre-sacrifice hematology determinations have been made on thirteen toxicity dogs since the last annual report. We were not able to obtain terminal values on three dogs which died unexpectedly on weekends when personnel was not available (dogs F6P5, F2M2, M3M4.)

PRE-SACRIFICE HEMATOLOGICAL VALUES OF TOXICITY DOGS

Dog No.	Days	VPRC	Hgb.	RBC	Retic	Sed. Rate	WBC	Seg. Pmn	Non-Seg. Pmn	Lympho-cyte	Mono-cyte	Direct eos. Count	Plate-let
Normal mean		49.4	16.3	7.35	0.6	2.5	13.9	7.95	0.31	4.00	0.86	384	419
Pu													
M1P4	1724	49.5	15.9	6.67	0.1	28.0	8.3	5.98	0.21	1.47	0.58	50	267
F2P4	1556	41.5	13.0	6.16	0.5	50.5	14.5	9.97	1.74	1.89	0.87	19	428
F2P5	1576	33.0	10.7	4.63	0.3	55.0	12.2	8.02	1.19	2.35	0.64	25	221
F5P4	1245	45.0	14.4	6.15	0.6	16.0	12.5	9.75	0.13	1.59	0.81	15	372
Ra													
M1R4	1606	42.0	13.8	5.91	0.2	28.5	9.7	7.30	0.12	1.48	0.73	28	336
M2R5	1380	38.5	11.7	5.19	0.5	51.0	21.5	16.39	1.40	1.08	2.63	15	138
M4R5	1091	29.5	9.3	3.97	1.7	61.0	17.4	12.53	2.26	2.04	0.57	35	413
F6R5	1015	35.5	11.1	5.00	1.0	34.0	11.6	6.00	1.33	3.28	0.93	25	208
F8R5	968	38.5	12.5	5.59	0.5	52.5	9.3	5.63	0.49	1.86	1.33	9	484
RdTh													
M1T2	1282	44.5	14.0	6.55	0.7	44.0	9.2	7.20	0.12	0.97	0.90	15	266
M2T3	859	41.5	13.5	5.60	0.4	6.5	4.9	3.03	0.17	1.29	0.29	62	-
F3T4	763	18.0	6.4	2.37	0.8	77.0	3.0	0.59	1.00	1.32	0.10	9	47
Sr													
F1S5	960	44.0	13.8	6.40	0.4	40.0	7.7	5.83	0.29	1.12	0.37	28	159

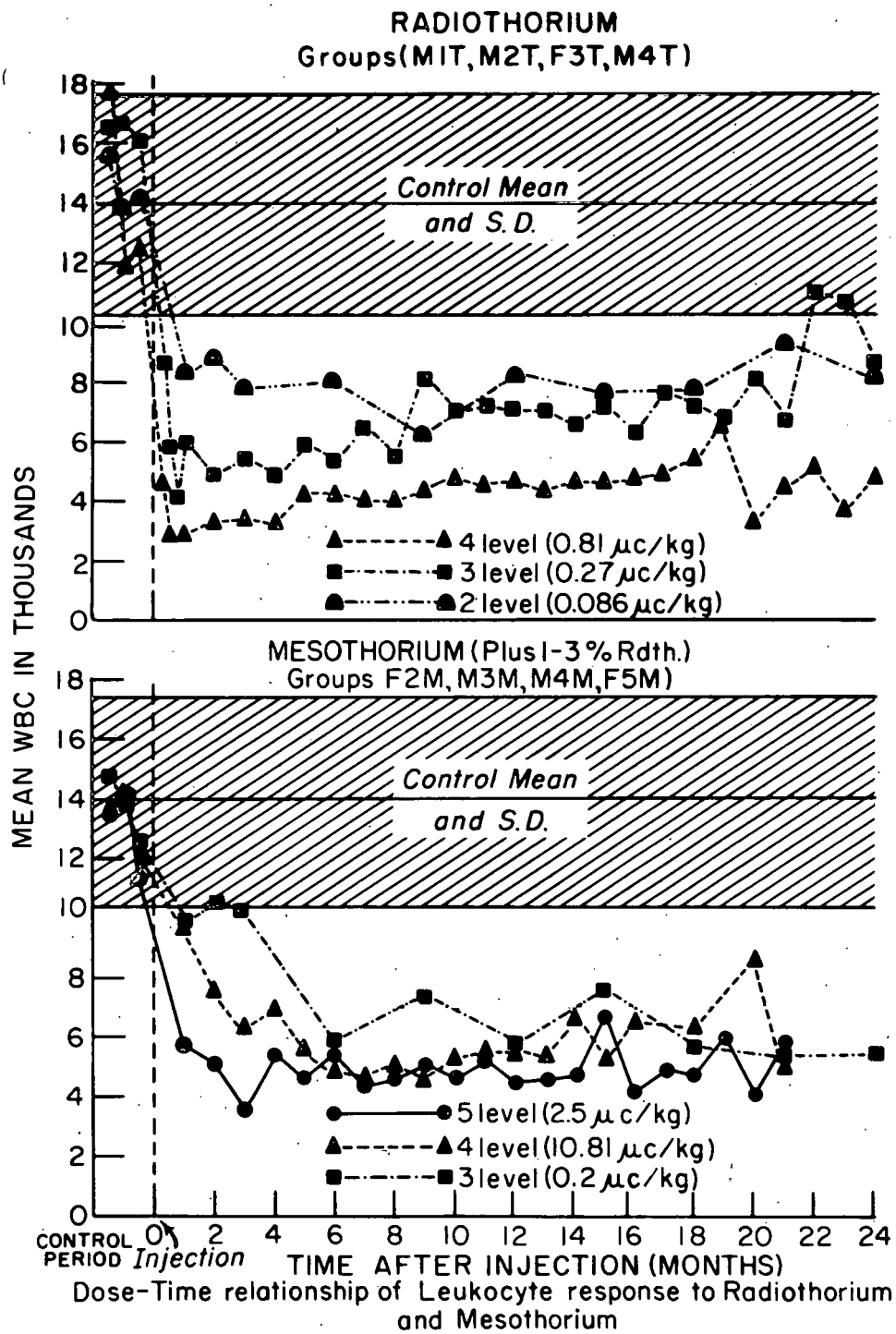
Comments: As mentioned in previous reports the most consistent findings prior to sacrifice are anemia, increased sedimentation rate, lymphopenia and eosinopenia.

As the general condition of a dog deteriorates due to fractures, tumors, osteolytic processes in bone, inanition, etc., the white blood cell count (WBC) tends to rise from leukopenic levels to normal or above normal numbers due to an increase in mature as well as immature pmns. The higher level radiothorium dogs (M2T3, F3T4) did not show this rise in pmns terminally but had a deteriorating blood picture from injection to sacrifice. Only three of the above dogs (M2R5, F3T4, F1S5) had low platelet numbers at the time of sacrifice.

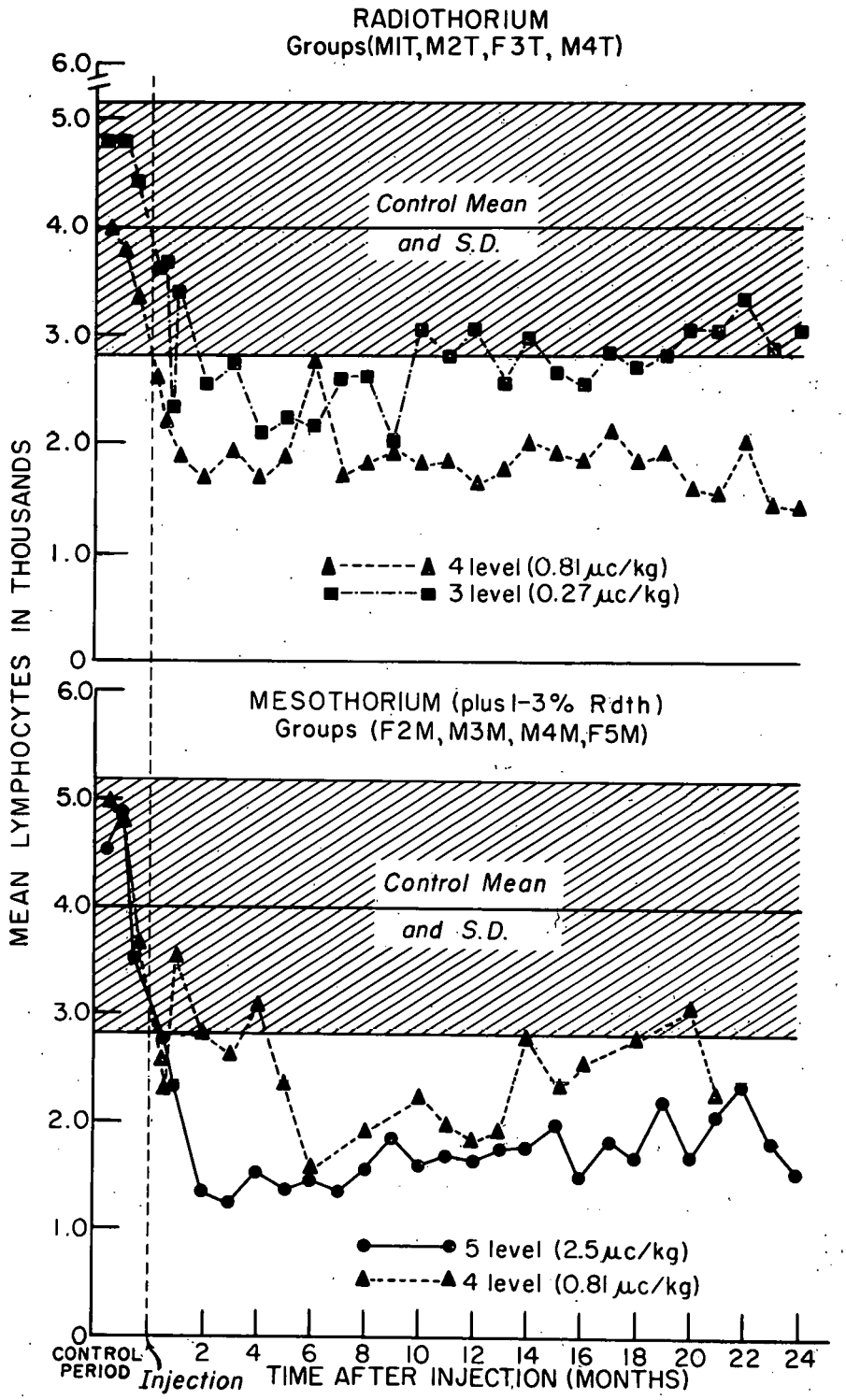
2. Preliminary comparison of the hematological response to mesothorium and radiothorium for a period of twenty-four months.

In annual reports the hematological findings on dogs injected with mesothorium or radiothorium have been given at yearly intervals. The graphs on the following page give mean values of WBCs and lymphocytes at one month intervals for the first four groups of dogs on each radioisotope.

Comments: The initial decrease in the leukocyte count of the 5-level mesothorium dogs is comparable in degree to that of the 4-level radiothorium dogs although the time of maximum depresion is later for the mesothorium injected dogs (3 months as compared to 1 month for RdTh). The leukocyte changes shown above are due mainly to changes in pmns. Eosinophils show a similar curve. Dogs injected with the 3- or 4-levels of mesothorium also develop a leukopenia which becomes maximum at three to six months. The leukocyte count remains at subnormal values for the two year period reported here. The leukocyte depression in 2- and 3-level radiothorium dogs appears earlier and the count remains low until toward the end of the second year.



Dose-Time relationship of Leukocyte response to Radiothorium and Mesothorium



Dose Time relationship of Lymphocyte response to Radiothorium and Mesothorium

Comments: A decrease in lymphocyte numbers occurs after injection of the highest levels of radiothorium and mesothorium (M3, T4). This lymphopenia is maximal for both radioisotopes around two months following injection and persists through the two year period shown above. There appears to be a slight decrease in lymphocytes in 3-level radiothorium dogs during the first six months with normal values thereafter. There is a definite drop in lymphocyte numbers in 4-level mesothorium dogs at six months with a rise to normal values at about fourteen months.

3. Bone marrow studies on femurs of toxicity dogs autopsied to date.

Bone marrow smears are taken at autopsy from various marrow sites ie., rib, sternum, costo-chondral junction, proximal-, mid- and distal-femur and humerus. At least 500 cells are counted (1000 cells are preferable) and a ratio is obtained to express the relative proportion of developing myeloid (white) to erythroid (red) cells, the myeloid/erythroid ratio. It is possible to have a normal myeloid/erythroid ratio but to have an abnormal distribution of the different types of immature cells. Since the pattern of radioisotope deposition varies in different types of bone, it is possible also that the qualitative picture of hematopoiesis may differ from bone to bone and even within the same bone. This has been evident in test-run plutonium dogs reported in the March 1957 Annual Report in which long bones show a different response than flat bones.

In the table on the following page detailed marrow differential counts are given for the femurs of toxicity dogs. The dogs are grouped according to radioisotope and in order of increasing time on experiment before sacrifice.

Some general trends appear from analyzing the bone marrow values. The M/E ratio in many of the injected dogs is above normal with the higher values in the dogs which lived the longest. Exceptions to this are dog M1T5 which had extreme bone marrow exhaustion and a very high ratio and dogs having severe regenerative anemias at time of death with stimulation of erythropoiesis and consequently low M/E ratios. Developing eosinophils are normal in percentage despite a decrease

BONE MARROW VALUES* OBTAINED AT AUTOPSY FROM TOXICITY DOGS

	No. Dog	No. Days Expt.	Developing Pmns: Myelo-blast	Promyelo cyte	Myelo-cyte	Meta-myelo-cyte	Seg.	Developing Eosinophils: Pro-myelo-cyte	Myelo-cyte	Meta-myelo-cyte	Seg.	Total Myeloid Cells	Developing Red Blood Cells: Pro-normo-blast	Baso-normo-blast	Late Normo-blast	Total Erythroid Cells	Total Misc. Cells	M/E Ratio
	Control 3 Dogs		2.3	3.6	13.0	14.7	10.2	0.2	0.9	0.6	0.5	46.1	0.2	0.3	49.9	50.3	3.5	0.94
	Range		0.8-3.6	3.0-4.4	8.4-19.0	12.0-18.0	4.8-18.2	0.0-0.4	0.6-1.6	0.4-0.8	0.0-1.0	39.0-53.2	0.0-0.6	0.0-0.8	43.9-57.4	44.0-58.0	2.4-5.0	0.67-1.20
Pu	M4P4	1065	3.0	3.4	18.0	23.4	3.8	0	0	0.8	0.8	53.2	0.2	0.2	45.6	46.0	0.8	1.15
	M3P4	1198	7.0	7.0	26.8	19.6	2.0	0.2	0.4	0.4	0.2	63.6	0.2	0	36.0	36.6	0.2	1.76
	F5P4	1245	0.4	0.2	3.0	7.4	10.2	0	0.4	0.2	0.8	22.6	0.2	0.6	74.0	74.8	2.6	0.30
	M1P5	1324	2.4	2.8	12.4	37.4	4.8	0	0.2	0	0	60.2	0	0	38.6	38.6	1.2	1.56
	M1P3	1477	1.4	2.4	14.6	20.0	3.4	0	0.4	1.4	0.4	44.0	0	0	56.0	56.0	0	0.79
	F2P4	1556	0.4	9.4	17.4	26.4	15.2	0	0	0.4	0.2	69.4	0.2	0.2	29.8	30.2	0.4	2.30
	F2P5	1576	2.4	4.0	14.4	30.0	19.0	0.2	0	0	0.4	70.4	0	0.2	29.0	29.2	0.4	2.41
	M1P4	1724	0.4	9.0	20.0	15.0	23.8	0	1.2	0.6	1.0	71.0	0	0.2	27.0	27.2	1.8	2.61
Ra	F3R4	490	7.0	13.0	46.0	7.0	0	0	0	0	2.0	75.0	0	0	23.0	23.0	2.0	3.3
	M1R5	908	0.4	3.4	8.5	30.9	4.9	0.8	1.3	1.4	0.7	52.3	0.1	2.1	45.0	47.2	0.5	1.11
	F8R5	968	2.2	5.8	20.4	20.8	19.2	0	0.2	0.4	0.4	69.4	0	0.2	29.8	30.0	0.6	2.31
	F6R5	1015	3.2	3.8	19.4	29.6	14.4	0	0.2	0	0	70.6	0	0	29.2	29.2	0.2	2.42
	M4R5	1091	0.7	1.1	13.7	17.4	7.0	0	0.2	0	0	40.7	0	0	58.4	58.4	0.9	0.70
	M2R5	1380	0	8.4	16.8	17.6	15.2	0	0	0.8	0.8	59.6	0	0.4	36.4	36.8	3.6	1.62
	M1R4	1606	0.6	8.0	24.2	16.6	40.0	0	0.8	0.2	0.4	90.8	0	0	4.8	4.8	4.4	18.9
RdTh	M2T5**	97	7.8	1.2	1.8	7.2	1.4	0	1.2	1.4	0.2	20.2	0.4	1.8	67.8	70.0	9.8	0.28
	M1T5**	212	4.8	3.4	25.8	39.9	4.1	0	0.4	0.9	9.4	88.7	0.4	0.4	0.3	1.1	10.4	80.6
	F3T3	547	2.0	4.6	11.8	21.6	2.2	0	0.4	1.6	1.0	45.2	0	0	54.0	54.0	0.8	0.83
	M1T4	645	0.4	8.4	26.8	41.5	0.6	0.3	0.5	1.4	2.3	81.2	0.5	2.0	13.6	16.1	2.7	5.04
	F3T4	763	1.6	7.2	33.2	16.4	0.6	0	0.2	0.4	0.6	60.2	0.2	1.2	28.4	29.4	10.0	2.02
	M2T4	832	0	5.8	18.8	24.2	31.6	0	0.2	0.6	0.4	81.6	0	0	18.4	18.4	0	4.43
	M1T3	988	0.4	4.6	12.8	8.0	1.0	0	0.6	0	0	26.8	0	0	72.6	72.6	0.6	0.37
	M1T2	1282	0.8	9.4	24.8	20.6	23.4	0.6	1.2	0.6	1.0	82.4	0.6	0.2	14.6	15.4	2.2	5.35
MsTh	F1M5**	232	1.5	10.4	14.3	48.0	1.7	0.2	0.1	0.9	3.7	80.9	0.9	0.7	16.3	17.8	1.6	4.54
	F2M4	777	2.0	10.4	17.8	34.0	15.8	0	0.8	0.6	0.6	72.2	0	0	17.4	17.4	8.6	4.71
	F2M5	780	0.4	4.0	11.0	14.0	13.6	0.6	0.6	0.2	1.4	45.8	0	0.4	52.6	53.0	1.2	0.86
	F1M4	841	1.0	4.8	13.0	31.8	5.4	0	0.4	0.2	0.2	46.6	0	0.6	42.2	42.8	0.6	1.30
	F1M3	918	2.0	6.4	14.0	24.6	8.0	0	0.2	0.6	0.2	56.0	0	0	43.0	43.0	1.0	1.30
Sr	F1S5	960	0.2	4.8	11.6	11.6	10.2	0	2.0	0.2	0.2	40.8	0	0.6	56.0	56.6	2.6	0.72

* From femurs and reported as % of total cells counted (500 - 1000 Cells)

** Values obtained from humeris in M2T5, M1T5 and F1M5.

in blood eosinophils. In most of the dogs there is a greater percentage of the early forms of pmns, -promyelocytes, myelocytes and metamyelocytes- than is seen normally (shift to the left) and also an atypical development with premature segmentation, atypical granulation etc. The developing of red cells do not show this shift to the left seen in the white cell series.

MICRORADIOGRAPHIC STUDIES OF CORTICAL BONE OF CHRONIC TOXICITY DOGS

Webster S. S. Jee and James S. Arnold*

Microradiograms of bone sections from seven beagles have been examined. Three animals had radium burdens, two had radiothorium burdens, and two had plutonium burdens. Cross sections of the humeral shaft (25-75μc) were prepared by the method of the authors (1). The microradiograms were taken with a 6 Kv Machlett x-ray tube using Lipmann film in an apparatus similar to Engstrom and Wegstedt (2).

The microradiographic appearance of the 4-level radiothorium bones is characterized by abnormal, highly calcified Haversian systems, hypercalcified plugs of central canals of Haversian systems and large areas of resorption (fig. 1). The abnormal hypercalcified Haversian systems exhibited loss of the orderly arrangement of concentric lamellae and lacunae.

The appearance of 5-level radium bones is characterized by few highly calcified Haversian systems, hypercalcified plugs in central canals and large areas of resorption (fig. 2). The 4-level radium bones exhibited Haversian systems with a border of highly calcified bone and large areas of resorption (Figs. 3 and 4). Recently, these identical changes were described in a microradiographic study by Rowland, Jowsey and Marshall (3) in cortical bones of eleven radium patients.

The hypercalcified Haversian systems and highly calcified plugs of central canals are detectable as basophilic staining masses in celloidin sections stained with hematoxylin and eosin.

Correlation of these abnormal sites with isotopic distribution and dose will be studied and reported later.

* Present address: U. S. Naval Radiological Defense Laboratories, San Francisco, California.

References

(1) Arnold, J. S. and Jee, W. S. S., Preparing bone sections for radioautography. Stain Technol., 29: 49-54, 1954.

(2) Engstrom, A. and Wegstedt, L. Equipment for microradiography with soft roentgen rays. Acta radiol., 35:345-55, 1951.

(3) Rowland, R. E., Jowsey, J. and Marshall, J. H. The microradiographic appearance of radium damaged bones. Abstracts of papers presented before the Radiation Research Soc. Rochester, May 13-15, p55, 1957.

Figure 1. A microradiogram of the humeral shaft from a beagle which received a burden of 0.88 μc/kg of radiothorium and was sacrificed at 645 days (M1T4). Note the large atypical hypercalcified area and the frequent areas of resorption (x300).

Figure 2. A microradiogram of the humeral shaft from a beagle which received a burden of 10.5 μc/kg of radium and was sacrificed at 908 days (M1R5). Note the arrows pointing to hypercalcified plugs in the central canals of three Haversian systems. Also note large areas of resorption (x100).

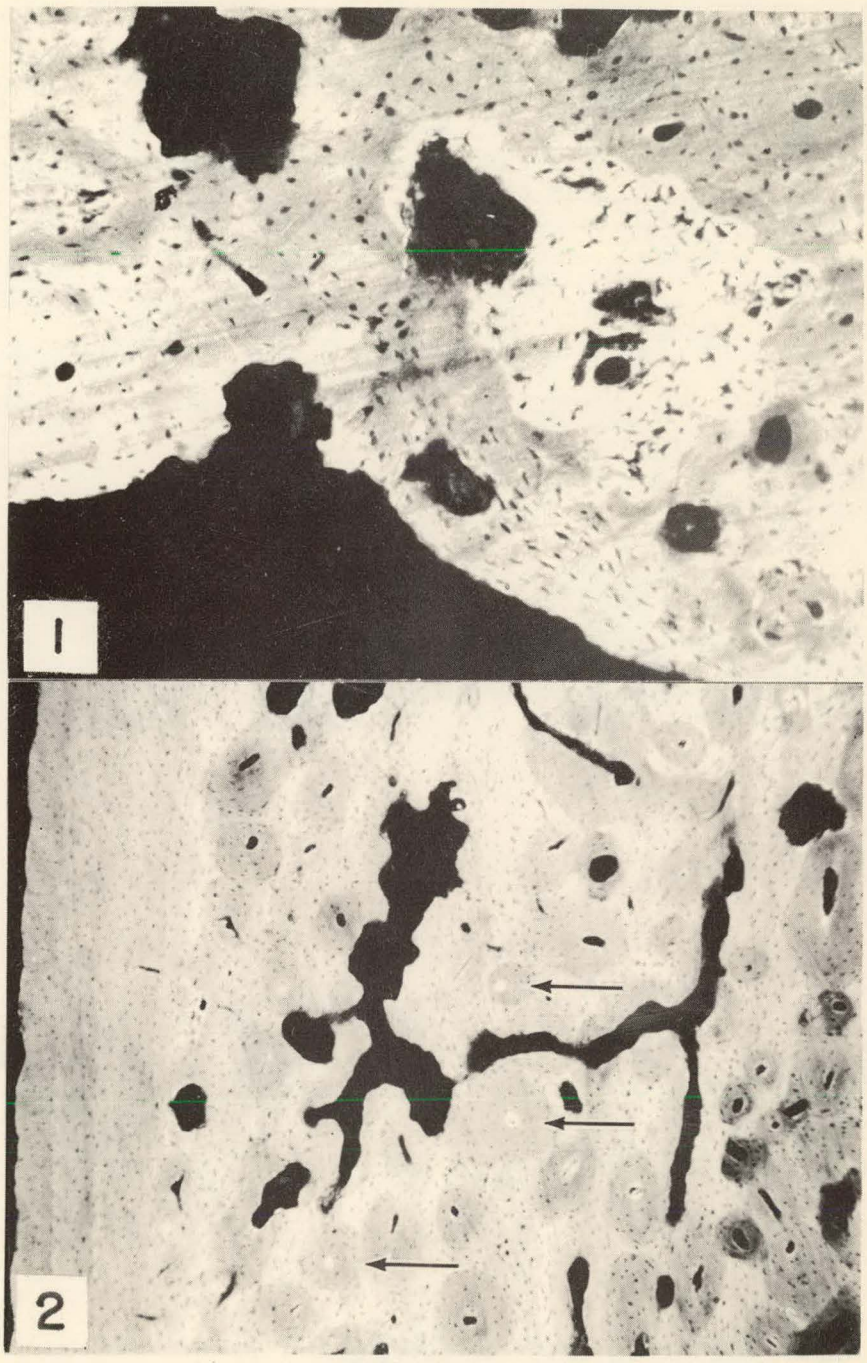
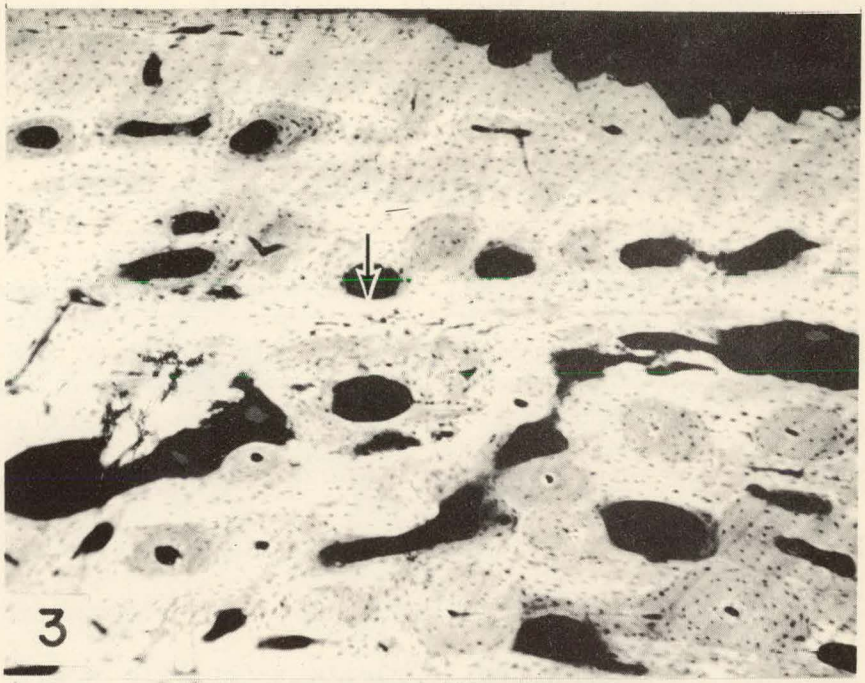
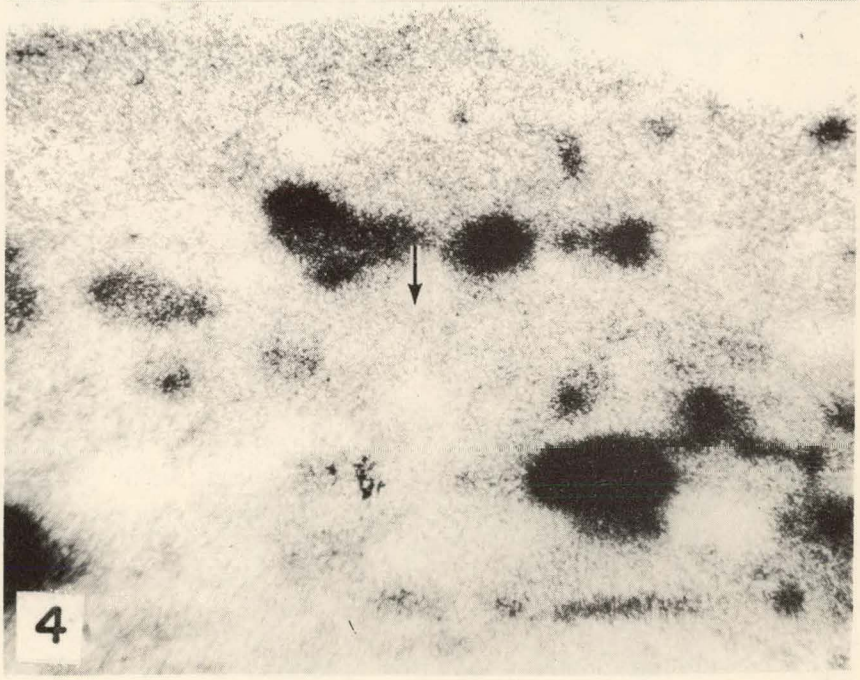


Figure 3. A nicroradiogram of the humeral shaft from a beagle which received a burden of 3.33 μc/kg of radium and was sacrificed at 490 days (F3R4). The arrow points to an abnormal Haversian system with a collar of hypercalcified bone. Note large areas of resorption and the absence of hypercalcified plugs in central canals (x100).

Figure 4. Contact radioautogram of the corresponding area in figure 3. Note the arrow pointing to the site of the abnormal Haversian system where there is no localization of radium (x100).


3


4

THE FAILURE OF PLUTONIUM TO DEPOSIT IN THE OSTEOID OF RACHITIC RATS

W. S. S. Jee, C. R. Mott*, J. S. Arnold** and R. Mical

Abstract: A detailed radioautographic study of rachitic rats injected with plutonium demonstrates that plutonium passed through the osteoid and deposited on the underlying calcified bone surfaces.

Early investigations on the behavior of plutonium^{239} demonstrated that it deposits rapidly on bone surfaces (1,2,3,4). In one of these early papers by Copp et.al. (3), these investigators radioautographically localized the plutonium binding to the uncalcified organic bone matrix. Using phosphorous deficient rachitic rats, they stated "plutonium was actively laid down in the uncalcified organic bone matrix below the epiphyseal cartilage and in the endosteum and periosteum". However, Foreman (5) recently demonstrated that the mineral component of bone in various forms concentrates plutonium ions in vitro. With these opposing results in mind, we repeated the experiment of Copp et. al. using standard rachitic rats and more refined radioautographic techniques.

Five three weeks old female Sprague-Dawley rats weighing 45 grams were weaned to the standard rachitogenic diet (6) as follows:

Ground yellow corn	76 per cent
Wheat gluten	20 per cent
Calcium carbonate (USP)	3 per cent
Sodium chloride (USP)	1 per cent

At 6 weeks of age, the 5 rachitic rats weighing 90 gm. along with 5 control rats of identical age weighing 150 gm. were injected intraperitoneally with 1 μc/kg of Pu^{239} as the citrate (7). All rats were sacrificed after 24 hours. The femurs and tibiae were split, fixed in acetone, embedded in plasticized nitrocellulose and sectioned (undecalcified) at 8 microns. Detailed radioautograms were prepared using strip film and stained with hematoxylin and eosin (8). Adjacent sections,

* Late assistant Professor of Biology, Department of Biology, University of Utah.
** Present address: U. S. Naval Radiological Defense Laboratory, San Francisco, California.

serial when possible, were stained by the von Kossa technique to demonstrate mineralized bone.

In the control rats the plutonium localized selectively on calcified bone surfaces, e.g. beneath the endosteum, periosteum and about vascular channels. In rachitic rats, the plutonium penetrated the osteoid layer and deposited on the underlying calcified bone surfaces as shown in fig. 1 which is a detailed radioautogram of the femoral shaft of a rachitic rat. The section shown in fig. 2 is adjacent to fig. 1 and is stained with the von Kossa technique to demonstrate mineralized bone. Note that the bone without the von Kossa reaction (blackening) is the broad layer of eosinophilic staining uncalcified organic matrix, the osteoid. The primary spongiosa of the femoral metaphysis in rachitic rats exhibited a broad plate of osteoid with isolated islands of calcified bone containing plutonium. In the controls, the primary spongiosa contained fine trabeculae with their surfaces lined with plutonium.

Our results and the previous findings by Foreman (4) strongly suggest that the mineral, rather than the organic matrix, is the site where plutonium binds. However, experiments performed by the authors (9) demonstrated that about 50-60% of the plutonium which deposited in vivo was not removed from acetone fixed bone sections by prolonged and repeated decalcification in 1% nitric acid. Radioautographs of these decalcified sections showed that about half of the remaining plutonium was in the organic component and the other half showed the original in vivo pattern. A possible explanation of the above observation is that when plutonium is released from dissolved mineral, it is absorbed by the adjacent organic material.

References

(1) Hamilton, J. H., The metabolism of the fission products and the heaviest elements. Radiology, 49:325-343, 1947.

(2) Scott, K. G., Axelrod, D. J., Fisher, H., Crowley, J. F. and Hamilton, J. G., The metabolism of plutonium in rats following intramuscular injection.

J. Biol. Chem. 176: 283-293, 1948.

(3) Copp, D. H., Axelrod, D. J. and Hamilton, J. G., The deposition of radioactive metals in bone as a potential health hazard. Amer. J. Roentgenol. 58:10-16,1947.

(4) Arnold, J. S. and Jee, W. S. S., Bone growth and osteoclastic activity as indicated by radioautographic distribution of plutonium, Amer. J. Anat. in press.

(5) Foreman, H., personal communication.

(6) Hawk, P. B., Oser, B. L., and Summeson, Assay of Vitamin A and D in Practical Physiological Chemistry, the Blakiston Co., Inc. New York pp 1261, 1954.

(7) Stover, B. J., Summary of plutonium studies in Second Annual Conference on Radium, Plutonium and Mesothorium pp 18-31, 1954.

(8) Arnold, J. S. and Jee, W. S. S., Embedding and sectioning undecalcified bone and its application to radioautography. Stain Tech. 29:225-229, 1954.

(9) Arnold, J. S. and Jee, W. S. S., unpublished observations.

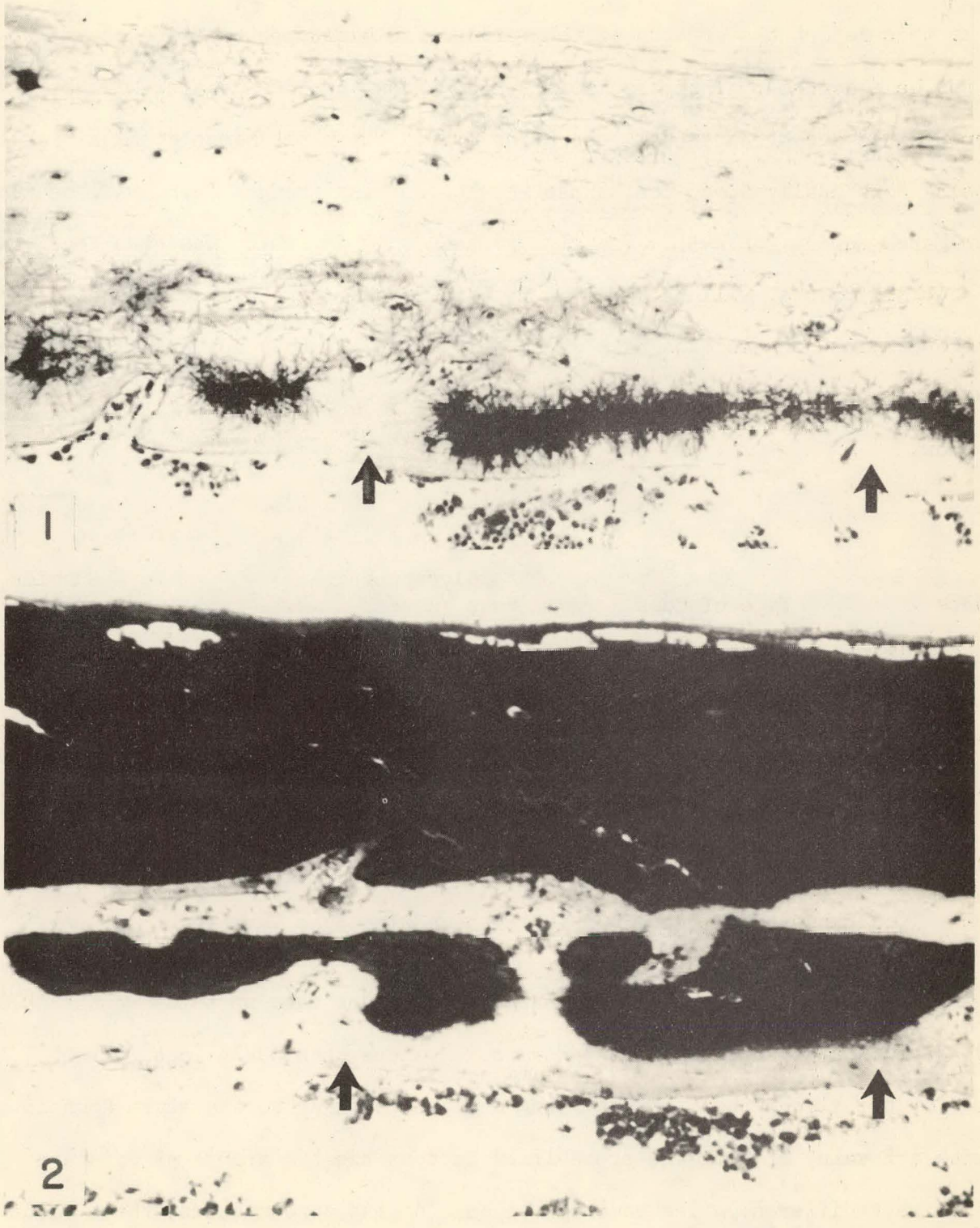
THIS PAGE

WAS INTENTIONALLY

LEFT BLANK

Figure 1. A detailed radioautogram of the femoral shaft from a rat weaned to a rachitogenic diet at three weeks, injected intraperitoneally at six weeks with 1 μc Pu^{239}/kg, and sacrificed 24 hours later. Note the deposits of Pu^{239} on the surface of the calcified bone. The uncalcified osteoid matrix is indicated by arrows (x550).

Figure 2. An alternate undecalcified bone section of the radioautogram in figure 1. The section is stained with hematoxylin, eosin and silver nitrate to demonstrate mineralized bone. Note the pale eosinophilic osteoid layer (indicated by arrows) adjacent to the endosteum containing irregular islands of calcified bone (x550).


1
2

BIOCHEMISTRY REPORT

H. C. Goldthorpe and Swanson Bennett

In this report the effects of the various radioisotopes on blood chemistry will not be discussed. There is no reason to think that any changes will have occurred since our March annual report for 1957. The basic blood chemical results are being continually added too, those results and any changes found will be reported in our March report for 1958. The glycoprotein program, which has been accelerated this past six months, will be taken up in this report. The number of analysis of glycoprotein and sero-mucoids have reached 165 each. Many young animals have been added. The addition of these young animals has changed the mean values as Table I will show.

Table I*

Date	Type of dogs	Glycoprotein Mean Values	Glycoprotein S.D.	Sero-Mucoids Mean Values	Sero-Mucoids S.D.
March 31, 1957	68 adult dogs & 7 young 60 day dogs	100.3	±14.2	19.3	±6.5
Sept. 30, 1957	103 adult dogs	99.6	±15.8	18.0	±7.5
Sept. 30, 1957	103 adult dogs & 62 young dogs	94.4	±18.9	17.0	±7.1
Sept. 30, 1957	62 young dogs	85.8	±20.5	15.3	±6.2

* Under 19 months young dogs, over 19 months adult dogs.

Analysis of the table shows definately that young dogs up to 19 months (570 days) have lower glycoprotein and sero-mucoid values than adult dogs. The t values for the glycoprotein of the young dogs as compared to the adult dogs is 4.5 with a P value of 0.01 the probability is that the two groups of dogs are significantly different. The same can be said of the sero-mucoids, the t value being 2.3 with a P value of 0.02. In our March report having only a few values to report for young dogs we could not be sure if the sero-mucoid values for young dogs were lower than adult dogs, but adding to the number of young dogs studied

we can now be sure that the values for young dogs are lower than for adult dogs. The sero-mucoids make up about the same percentage of the glycoproteins in both sets of dogs namely, 18.0 per cent in normal animals.

The graph shown in the March report of a mixed group of dogs can be enlarged now by adding young dogs. It also shows that the values of both glycoprotein and sero-mucoid are lower in youth, rise to adult values around 19 months of age and stay at a fairly level value through adult life. We do not have enough values for old dogs to determine if the values remain constant, rise or fall in that group. Values for individual dogs are shown in Table II. These are young dogs, male and female. The average values of the six dogs are shown on Table III. These tables show the rise in glycoprotein and sero-mucoid values of serum from youth to adult life.

Table II

Individual Dogs

Dog	Days	Glyco	Sero
423M	60	84	17.8
423M	445	128	18.2
425M	60	93	20.9
425M	445	131	18.5
427M	60	87	14.4
427M	446	123	19.2
429M	60	85.5	13.1
429M	451	112.0	37.7
424F	60	88.5	23.4
424F	445	136	18.5
426F	60	84.0	21.8
426F	445	133	19.2

Table III

Days	Dog	Glyco	Sero
60	6	87	18.6
445	6	127.1	21.9

Individual values for adult dogs. We have gathered together values for individual dogs, made a table for female dogs and one for male dogs.

Table IV

Nine Adult Female Dogs

Dog	Days	Glyco	Sero
F2PO	1648	96.9	20.3
F2PO	1860	100.5	15.7
F1MO	1668	94.5	13.1
F1MO	1840	96.9	20.3
F6PO	1227	99.6	18.5
F6PO	1505	136.0	14.4
F3RO	1520	108.5	24.8
F3RO	1689	123.9	34.1
F9RO	1272	100.2	18.5
F9RO	1424	*76.0	10.8
FAC13	1641	99	18.7
FAC13	2106	110	17.5
FAC14	1671	99	22.8
FAC14	2064	113	21.8
FAC15	1488	93	16.3
FAC15	1976	105	16.8
FAC17	1238	*110	27.6
FAC17	1847	107	23.0

* Two exceptions show a decrease.

Table V

Ten Adult Male Dogs

Dog	Days	Glyco	Sero
MAC6	3299	109	23.4
MAC6	3664	65	9.1
MAC7	2292	136	24.2
MAC7	2649	74.5	17.9
MAC8	1984	99	11.5
MAC8	2340	56	9.3
M3PO	1653	112.5	13.1
M3PO	1975	102.	19.0
M1RO	1738	115.5	16.6
M1RO	2017	*117.0	-
M4RO	1366	125.7	28.0
M4RO	1522	83.0	12.8
M5RO	923	108.2	42.8
M5RO	1550	92.8	20.2
M10RO	994	109.4	22.4
M10RO	1254	*113.	13.2
M8PO	1197	114.1	32.8
M8PO	1316	*120.1	18.3
M1TO	1297	109.5	15.1
M1TO	1492	93.0	18.6

* Three exceptions show an increase.

The female dogs with two exceptions seem to have an increase in glycoprotein values with time as the factor, the average increase being 7.5 mg/100 ml serum. Nothing can be said in regard to the seromucoids, there is a slight average decrease of 0.7 mg/100 ml serum but this is not significant. Table V for the adult male dogs shows a difference in that male dogs, with three exceptions, show a decline in the average values of 22.5 mg/100 ml for the glycoproteins and with an average decline if 7.6 mg/100 ml in the seromucoid values. Whether these findings are "real" for female and male dogs is not definite for upon making up separate tables for male and female dogs over 19 months old the differences shown for the

individual dogs does not show up to any great extent for the two groups.

Table VI

Adult dogs over 19 months old

	No. Dogs	Glyco values	S.D.	Sero values	S.D.
Male	42	97.7	±18.6	18.2	8.0
Female	58	100.8	±15.2	17.7	7.3

The females have a slightly higher glycoprotein value and a slightly lower seromucoid value as indicated by the tendency in individual dogs as shown in Tables IV and V. However, applying the t test to the values of table VI gave 0.9 for the male with a P value of 0.3 and 0.3 for the females with P value of 0.7. Neither being significant as different groups.

THE EFFECT OF ENVIRONMENTAL TEMPERATURE UPON GLYCOPROTEIN AND SEROMUCOID VALUES.

Another factor which may effect the values of these two groups of proteins is environmental temperature. We have gathered together the values found for all dogs for the months of June, July, August and the first four days of September and compared them with values from dogs whose blood was drawn during the months of November December, January and February. All were adult dogs, male and female.

Table VII

Effect of Environmental Temperature

	No. Dogs	Glyco mg/100 ml	S.D.	Sero mg/100 ml	S.D.
Summer	40	96.6	±12.1	16.6	±4.0
Winter	21	101.5	±11.3	19.8	±8.6

From table VII it appears that higher values are found both for glycoprotein and seromucoid during the winter months. Applying the t test to glycoprotein gives a value of 1.5 with a P value of > 0.1 the results therefore not being too significant while the seromucoids upon comparison give a t test value of 1.9 and a P value of < 0.1 making them significant. This will be checked again when more values for the two periods are available.

A SUMMARY OF THE Ra^{228}(MsTh) INJECTION SOLUTIONS

Betsy J. Stover and David R. Atherton

Abstract: Injection of the F7M group on June 4, 1957 completes our current Ra^{228} injection program, and a description of the chemistry and radioactivity of the various Ra^{228} injection solutions appears below.

A. F1M group.

The F1M group's injection solutions were prepared by diluting the Ra^{228} stock solution with isotonic NaCl solution which had been adjusted to pH=2 with HCl,(the same method used for the M1R and M2R groups). The determination of μc's Ra^{228} and Th^{228} injected was based on Dr. Grove's original measurements of the stock solution. μc's Th^{228} injected = 0.15 μc's Ra^{228} injected.

B. F2M and M3M groups.

For the F2M and M3M groups' injection solutions, Th^{228} was separated from Ra^{228} a month prior to the injection. For the F2M group, Th^{228} was extracted with TTA (thenoyltrifluoracetone) in benzene; for the M3M group, Th^{228} was coprecipitated with $La(OH)_3$. Citric acid, sodium citrate buffer solution, pH=3.5 and Σ citrate = 0.08M, served as the diluting fluid in both cases. The determination of μc's Ra^{228} and Th^{228} injected was based on gamma measurements made by M. A. Van Dilla which were standardized using ampoules of Ra^{228} (+Th^{228}) and Th^{228} which had been evaluated by Dr. Grove. μc's Th^{228} injected = 3×10^{-2} μc's Ra^{228} injected.

C. M4M, F5M, M6M, F7M groups.

For these groups' injection solutions, Th^{228} was separated six days prior to injection [1] by coprecipitation with $La(OH)_3$, and the diluting fluid was the citrate buffer described above. Determination of μc's Ra^{228} and Th^{228} injected was based on absolute beta counting[2] and alpha counting[1]. μc's Th^{228} injected = 6×10^{-3} μc's Ra^{228} injected.

D. Comparison with Th^{228} dose levels.

Since Th^{228} is retained to a much greater extent than Ra^{228}, the "contamination" dose of Th^{228} becomes quite significant even when the contamination is minimized. This is illustrated by the following example.

Let A(n) = "desired retained" μc/kg for dose level n,

W = weight of dog, and

k = fractional contamination (= μc's Th^{228}/μc's Ra^{228}) in injection solution.

Then the injected dose for a mesothorium dog of dose level Mn is

$$4A(n)\ W\mu c\ Ra^{228} + k4A(n)\ W\mu c\ Th^{228}.$$

Remembering that the injected dose for a radiothorium dog of dose level Tn is

$$1.11\ A(n)\ W\ \mu c\ Th^{228},$$

it is seen that dog Mn will be injected with as many μc's Th^{228} as is dog Tn when $k = 1.11/4 = 0.28$.

Consider an M5 dog for which k = 0.03. The injected dose is $4 \times 2.5 \times W\mu c\ Ra^{228} + 0.03 \times 4 \times 2.5\ W\ \mu c\ Th^{228} = 10\ W\ \mu c\ Ra^{228} + 0.3\ W\ \mu c\ Th^{228}$.

The injected dose for a T3 dog is $1.11 \times 0.27\ W\ \mu c\ Th^{228} = 0.3\ W\ \mu c\ Th^{228}$, which is equal to the contamination Th^{228} given the M5 dog.

The following table relates the injected doses of Th^{228} received by the mesothorium dogs to the injected doses of Th^{228} of the radiothorium dogs. The purpose is to provide a guide for those in the biological groups who wish to make rough comparisons of <u>early</u> effects of various isotopes.

TABLE I

Injected Th^{228} Equivalence

Dose Level	k= 0.15	k= 0.03	k= 0.006
M5	1/2 T5	T3	2/3 T2
M4	1/2 T4	T2	2/3 T1.5
M3	1/2 T3	T1.5	2/5 T1
M2	1/2 T2	2/3 T1	2/5 T0.5
M1.7	---	T0.5	1/5 T0.5
M1	1/2 T1	1/3 T0.5	1/15 T0.5

References

1. B. J. Stover et.al, Chemistry group report, Annual Progress Report, March 31, 1957, pp 135-136.

2. M. A. Van Dilla, Absolute assay of Ra^{228}, Annual Progress Report, March 31, 1957, pp. 149-150.

STUDIES OF THE STATE OF PLUTONIUM, THORIUM AND RADIUM IN BEAGLE BLOOD

Betsy J. Stover and Nancy Keller

Abstract: Sera from Pu^{239} and Th^{228}(RdTh) dogs have been dialyzed against a variety of solutions. In beagle sera Ra^{224} is almost completely diffusible while Pu^{239} and Th^{228} are almost completely bound. Citrate ion forms a more stable complex with both plutonium and thorium than do the blood proteins, but the protein complexes are more stable than several others investigated.

I. Pu^{239}

The binding of plutonium by plasma proteins was observed early in the study of plutonium metabolism. Painter, et al.(1), who studied plutonium in dogs, dialyzed heparinized plasma against 0.075M veronal buffer, pH 8.6, and serum against 0.02M maleate buffer, pH 7.4. In both cases, most of the plutonium remained in the sack after dialysis. In addition, the proteins were separated using a Tiselius electrophoresis apparatus. Most of the plutonium moved with the fraction containing β globulin and fibrinogen, some with γ globulin and fibrinogen, but very little with the albumin and α globulin fraction. Their conclusion is that most of the plutonium in the blood is bound to globulins. Also, their studies show that the complex of plutonium with the protein is more stable than possible complexes which could be formed with the buffers used.

We have dialyzed sera from our Pu^{239} dogs against eight different solutions to compare the strength of the protein complex with the strengths of diffusible plutonium complexes. These studies are summarized in Table I. The sera used were collected at times ranging from one hour to one day after injection, and stored at 2° C.

The serum sample, in Visking sausage casing, was suspended in the dialyzing solution, and the plutonium concentration of the solution was measured as a function of time. The dialysis systems were kept in the refrigerator (about 2° C.) to minimize evaporation and growth of molds and bacteria. The first dialysis listed in Table I, D-1, using isotonic saline, showed that little, if any, of the serum plutonium existed

as a diffusible complex. The F9P3 and F9P4 outside solutions showed no significant increase during the five days, while the F9P5 solution plutonium did increase. The result is borderline, and the possibility of a small leak in the dialysis bag cannot be excluded.

TABLE I

No.	Solute	Approx. Osmolarity	pH*	Serum from	Duration of dialysis	% of Pu dialyzed
D-1	NaCl	0.31	7	F9P3	24.5 hr	<2
				F9P4	117	4
				F9P5	117	7
D-2	Sodium acetate	0.16	7.5	T9P5	124	<1
				T10P5	124	<2
				F9P5	52	<2
D-3	Glycine	0.08	7.5	T10P5	124	<1
				T9P5	242	10**
				T10P5	242	3
				T11P5	242	<1
				T12P5	242	3
D-4	Sodium oxalate	0.16	7.2	T9P5	124	<1
				T10P5	124	<1
D-5	Sodium carbonate	0.16	7.4	T10P5	192	<1
				T11P5	192	<1
				T12P5	192	3
D-6	Sodium citrate	0.16	7.5	T9P5	124	91
				T10P5	124	90
				F9P3	117	84
				F9P4	117	93
				F9P5	117	90
				T9P5	121	85
				T10P5	121	85
				T11P5	121	91
				T12P5	121	87
D-7	Citric acid and sodium citrate	0.14	3.5	F9P3	117	<4
				F9P4	117	<4
				F9P5	117	<3
D-8	NaCl	0.31	2.1	F9P3	117	45
				F9P4	117	16
				F9P5	117	10
				T9P5	71	29
				T11P5	71	8
				T12P5	71	13

* pH adjusted with NaOH or HCl, whichever appropriate.
** "buggy".

Acetate (D-2), oxalate (D-4), and bicarbonate (D-5) ions all proved to be unsuccessful competitors for serum plutonium, as did glycine (D-3). The combination of glycine and serum proved an excellent culture medium. Plutonium in the outside solutions was negligible until the solutions became obviously "buggy". Then the plutonium began to diffuse out; two samples were left in the refrigerator 35 days, and at that time 77% and 57% of the plutonium appeared to be diffusible. This could result from some metabolic product of the organisms or from break-down of the proteins.

In contrast, citrate ion at pH 7.5 (D-6) forms a stronger plutonium complex than does the protein. After five days dialysis, an average of 90% of the plutonium had been converted to a diffusible complex. The material remaining in the bag was also measured, and the concentration of plutonium was slightly higher than that of the outside solution. An average of 94% of the initial plutonium was accounted for.

Combining the results from the five different sera, a half-period of about nine hours for the appearance of plutonium in the outside solution was calculated. The plutonium concentration of the outside solution, C_t, is given by the following equation.

$$C_t = \frac{0.9\ P_o V}{V+V'}\ (1- e^{-0.077t})$$

where P_o is the initial serum concentration, V and V' the serum and citrate solution volumes, respectively, and t is in hours. At pH 7.5 almost all the citrate is present as Cit^{-3}, and $PuCit^{+}$ may be the complex formed.

A citrate buffer of pH 3.5 (D-7) was also tried and the plutonium remained with the protein. This buffer is the one used to prepare the injection solutions and the citrate is present mainly as H_2Cit^{-}. Dialyses of Pu^{239} injection solutions against this buffer at room temperature have shown the plutonium to be completely diffusible, and to appear in the outside fluid with a half-period of about two hours. This may indicate that the strengths of the plutonium complexes decrease in this order:

$$Cit^{-3} > \text{Protein} > H_2Cit^{-}.$$

Since the isoelectric points of the serum proteins lie between pH 3.5 and 7.5, the D-7 results may merely be the effect of the decreased pH on the protein. Sodium chloride of pH 2 (D-8) was also tried to see if H^{+} would displace plutonium from

the protein. A variable but significant amount of plutonium was dialyzed from each serum sample. At the end of the dialyses the pH of the saline had increased to about 3. All of the plutonium could not be accounted for in the "inside plus outside" solutions indicating that some had been displaced from the protein and then adsorbed on various surfaces.

In conclusion, an exploratory study of the binding of plutonium by serum proteins shows that at pH 7 to 7.5, citrate forms a stronger Pu complex than the proteins, but acetate, glycine, oxalate, and carbonate do not. At low pH, H^+ may displace Pu.

II. Th^{228} and Ra^{224}

One set of dialysis experiments has been done using serum drawn from T6T4 and T7T3 at one hour after injection and four of the solutions used in the plutonium studies. These dialyses were also done at 2° C.; the outside solutions were sampled daily for five days and analyzed for both Th^{228} and Ra^{224}.

The sampling schedule proved to be a poor guess for the results from the second day on were essentially the same. The dialyses are summarized in Table II. As expected, the Th^{228} results are quite similar to those for Pu^{239}. (Other studies indicate that Th^{228} also is bound by the blood proteins.) Citrate at pH 7.5 forms a stronger complex with thorium than the serum proteins do. The first day samples indicate that the half-period for the appearance of thorium in the outside solution is not far different from the 9 hours observed for plutonium.

TABLE II

	Solute	Approx. Osmolarity	pH	Serum from	% Th dialyzed	% Ra dialyzed
1	Citric acid and sodium citrate	0.14	3.5	T6T4	10	~100
				T7T3	13	~100
2	Sodium citrate	0.16	7.5	T6T4	~100	-
				T7T3	~100	-
3	Sodium chloride	0.31	7	T6T4	~ 2	~100
				T7T3	13	~100
4	Sodium chloride	0.31	2	T6T4	15	~100
				T7T3	~ 6	~100

The results with the two NaCl solutions are also similar to the plutonium results in that small variable amounts of thorium passed through the membrane. In the experiments with pH 3.5 citrate buffer, plutonium and thorium do differ since some of the serum thorium is dialyzed. While H_2Cit^- is the main form present at pH 3.5, there is also a significant amount of $HCit^=$ (and H_3Cit), and perhaps the $HCit^=$ ion is removing thorium from the proteins. Comparison of plasma concentrations of Th^{228} and Pu^{239} during the first day after injection suggests that the plutonium protein complex is stronger than the thorium complex.

Ra^{224} diffused rapidly into the pH 3.5 citrate and the two NaCl solutions in agreement with the almost complete diffusibility observed at Rochester[2] for radium in rat blood. With pH 7.5 citrate the results are not definitive since Th^{228} diffused out, too. However, the one day samples had more Ra^{224} than would be expected from the Th^{228}, and it is probable that radium is almost completely diffusible in this system, too.

In conclusion, a few dialysis studies give results for Th^{228} similar to those for Pu^{239}, and show that Ra^{224} is almost completely diffusible.

References

1. Clinical physiology of dogs injected with plutonium, E. Painter, E. Russell, C. L. Prosser, M. N. Swift, W. Kisieleski, G. Sacher. AECD-2042, June 1946.
2. T. Putnam, Ph.D. thesis, University of Rochester.

LACK OF EFFECT OF DOSE LEVEL ON Pu^{239} METABOLISM IN THE BEAGLE

Betsy J. Stover, D. R. Atherton, N. Keller, and D. S. Buster

Abstract: The Pu^{239} plasma concentration and excretion rate data through four years after injection have been examined for dose level effect. There appears to be no significant difference in retention at four years among the various dose levels.

A. General

Two kinds of dose effects are possible in the metabolism of inorganic radioactive isotopes. The first can be considered a chemical effect which results from a limited number of sites available to bind the isotope. When the number of inorganic ions introduced is less than the number of sites, the fractional retention is constant; when the sites are saturated, the fractional retention decreases. If a chemical effect exists, it will be observed early. An excellent example is Schubert's [1] study comparing carrier-free Pb^{210} and Pb^{210} with carrier Pb. At 25 minutes after intravenous injection of carrier free Pb^{210} into rats, essentially all of the Pb^{210} in the blood was in the cells. At the same time after injection of Pb^{210} with carrier Pb, only 20% of the blood Pb^{210} was in the cells and 80% in the plasma, showing a saturation of the cellular binding sites.

The second kind of dose level effect can be considered a biological one which results from damage occurring in proportion to the amount of irradiation. This effect is more easily observed when a short-lived daughter isotope is involved. An example is the decrease in Ra^{224}(ThX) excretion with increase in dose level in our Th^{228}(RdTh) dogs.[2] However, an effect on parent retention was observed by Norris et al,[3] who observed that Ra^{226} retention in rats increases with injected dose for injected doses greater than 10μc/kg.

B. Plasma concentration

The Pu^{239} concentration in plasma of P4 and P5 dogs has now been measured to 4.5 years after injection. The data for the period from 21 days to 4.5 years appear

in Fig. 1, and the equation, $P_2 = 7.33 \times 10^{-4}\ t^{-0.293}$, does not differ significantly from the last one reported.(1) Each point in Fig. 1 is the average of two or more measurements, except for the last two. A total of 80 samples from 24 dogs (P5 and P4) were measured. Since the injection weights of the dogs ranged from 6.5 to 11.4 kg, some of the scatter of the points may result from differences in total plasma volumes.

In Fig. 2, the P5 and P4 data are treated separately. Most of the P4 points are lower than the P5 points, and the equations are, for P5, $P_2' = 9.95 \times 10^{-4}\ t^{-0.327}$ and, for P4, $P_2'' = 4.26 \times 10^{-4}\ t^{-0.235}$.

Since the P4 points are quite consistently lower, this may be a dose level effect. On the otherhand, there were only 32 P4 samples compared with 48 P5's, P4's are harder to measure, and the decrease with time is so slow that scatter is accentuated.

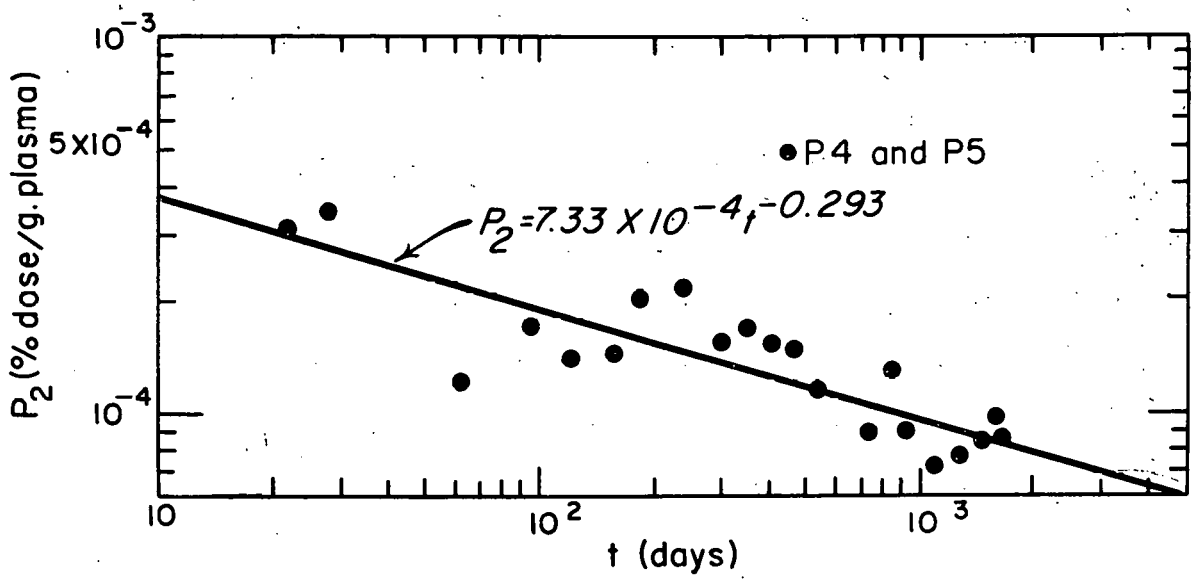
C. Excretion

The lack of effect of dose level on early Pu^{239} excretion has been reported before.(5) To review, (see also Table II) the total amount excreted during the first 22 days after injection was measured for 19 dogs, 3 each at dose levels P1, P2, P3, and P4, six at P5, and one at twice the P5 level; there was no significant correlation between dose level and total amount excreted.

Excretion measurements from 23 days to 4 years after injection have been made for 12 dogs, 3 each at dose levels P5, P4, P3, and P2, (except at 4 years, when measurements have been completed for only seven dogs to date). During the period 23 to 42 days some measurements on P1 dogs and some additional measurements on P5 dogs were made. These data have been separated according to dose level, and equations for X_2 and R have been calculated and appear in Table I. Inspection of the power functions which describe the excretion rates shows that both the coefficient and exponent increase as dose level decreases from P5 to P3. P2 does not follow this trend.

CONCENTRATION OF Pu^{239} IN PLASMA $t \geq 21$ days

(JULY 1957)



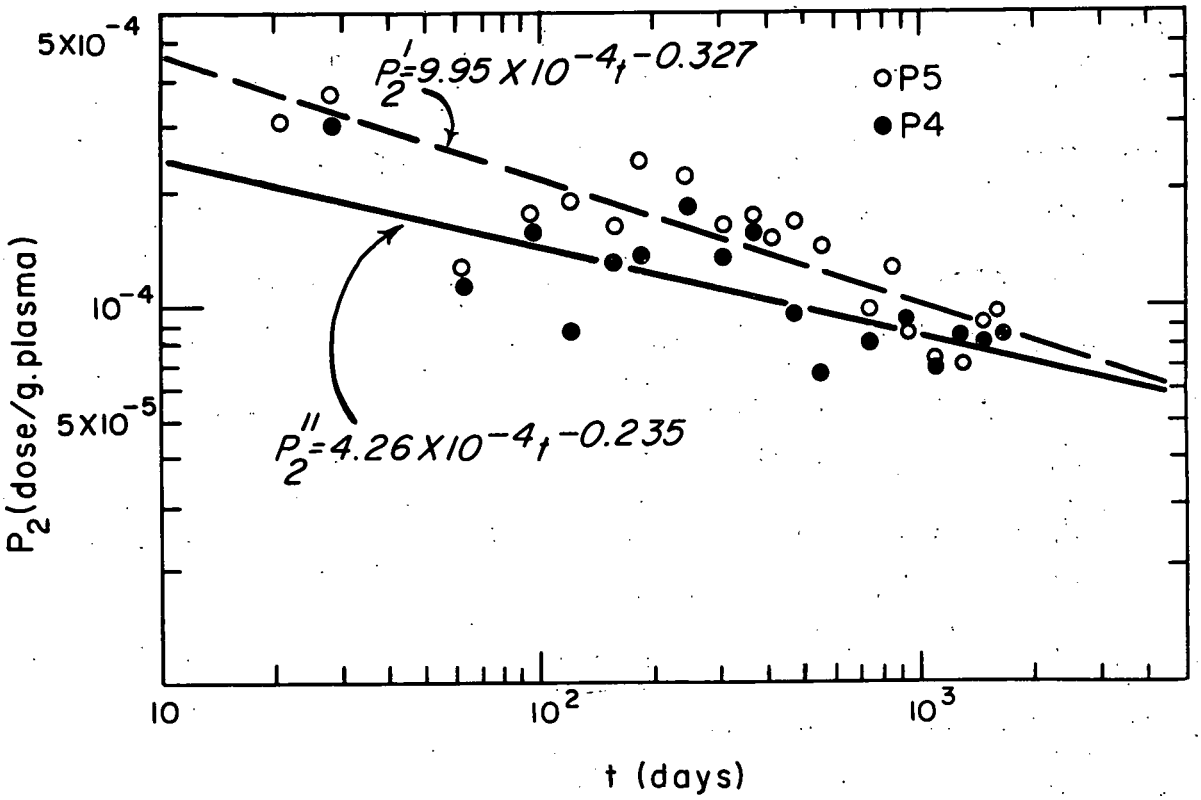


Table I

Equations for X_2 and R

	X_2	R ($t \geq 22$ ds)
All	$0.193\ t^{-0.463}$	$89.8 - 0.359\ t^{0.537}$
P5	$0.0747\ t^{-0.325}$	$90.1 - 0.111\ t^{0.675}$
P4	$0.198\ t^{-0.459}$	$91.3 - 0.366\ t^{0.541}$
P3	$0.268\ t^{-0.516}$	$89.1 - 0.554\ t^{0.484}$
P2	$0.134\ t^{-0.397}$	$88.1 - 0.222\ t^{0.603}$

The average total excretion during the first 22 days is reviewed in Table II. Also included are the cumulative excretion for the interval 22 days to 4 years and the retention at 4 years calculated from the equations of Table I. These calculations show that, while the excretion equations for the various dose levels differ markedly≠, all the excretion rates are so low and decrease so slowly with time that the cumulative excretions do not differ significantly.

Table II

Pu Excretion by Dose Level

	Cumulative Excretion 0-22 ds.	Retention at 22 ds	Cumulative Excretion 22 ds to 4 yrs	Retention at 4 yrs
All	12.1%*	87.9%	16.1%	71.8%
P5	10.8	89.2	14.3	74.9
P4	10.6	89.4	16.9	72.5
P3	13.4	86.6	16.4	70.2
P2	13.3	86.7	16.5	70.2
P1	14.2	85.8	-	-

* $\sigma = 2.1$

≠ Compare P3 and P5. The coefficient for P3 is 3.5 times that for P5 and the magnitude of the exponent is 1.5 times greater.

D. Conclusion

Examination of plasma concentration and excretion rate data for dose level effect shows that the plasma concentration is slightly higher and the excretion rate decreases more slowly for P5 dogs. However, the effect on the retention at 4 years is not significant. At this time, 4 years appears to be about the average length of time P5 and P4 dogs survive after injection. Hence the possible dose level effect need not be considered in calculating the total amounts of energy delivered to the Pu^{239} dogs. (The division of this energy between skeleton and soft tissues needs further study, since most of our tissue and bone measurements have been made on P5 dogs.)

References

1. J. Schubert and M. R. White, Effect of sodium and zirconium citrates on distribution and excretion of injected radiolead, J. Lab. and Clin. Medicine, 39, 260-266 (1952).
2. B. J. Stover et al, Chemistry group report, Annual Progress Report, March 31, 1957, pp. 141-143.
3. W. P. Norris et al, Studies on Ra metabolism in rats. ANL-5288, pp. 79-82 (1954).
4. B. J. Stover et al, Annual Progress Report, March 31, 1957, p 140.
5. B. J. Stover et al, Excretion of plutonium by the beagle, Quarterly Progress Report, December 31, 1953, pp 4-6.

CONCENTRATION OF Sr^{90} IN PLASMA

Betsy J. Stover and David R. Atherton

Measurements of Sr^{90} in plasma have now been extended to two years after injection. The early samples were measured directly using a well-type plastic scintillator(1). Since 1 ml was the maximum volume permitted by the direct method, the Sr^{90} in later samples was concentrated by co-precipitation with CaC_2O_4. The same beta detector was used for these samples (with an appropriate standard). Precautions were taken to insure Sr^{90} - Y^{90} equilibrium.

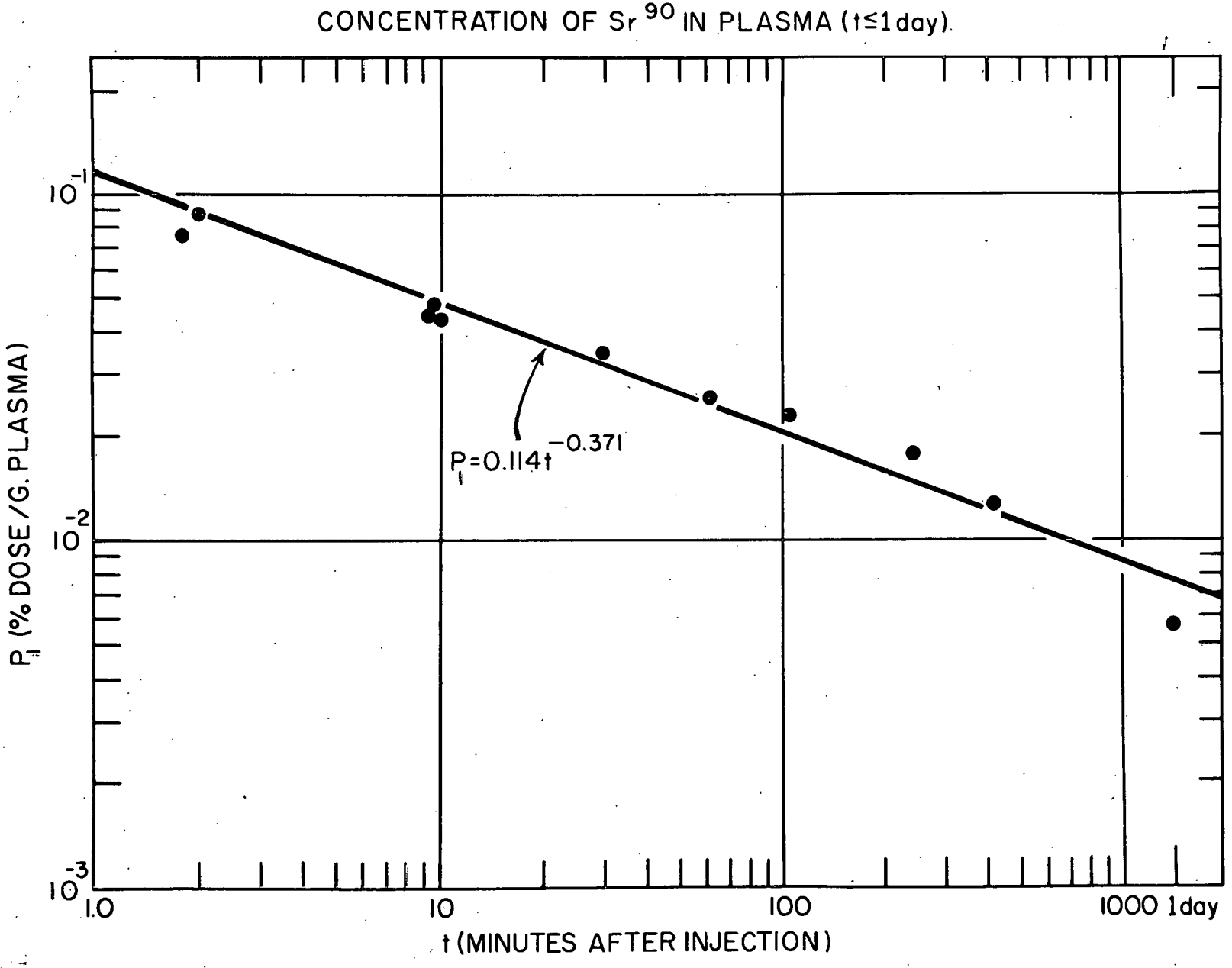
The data have been divided into two time groups, $t \leq 1$ day and $t \geq 1$ day, and a power function has been fitted to each group. The results are shown in Figs. 1 and 2. Since the radium data(2) cover the same periods and were treated in the same way, the equations can be compared without qualifications. The four power functions are listed in Table I.

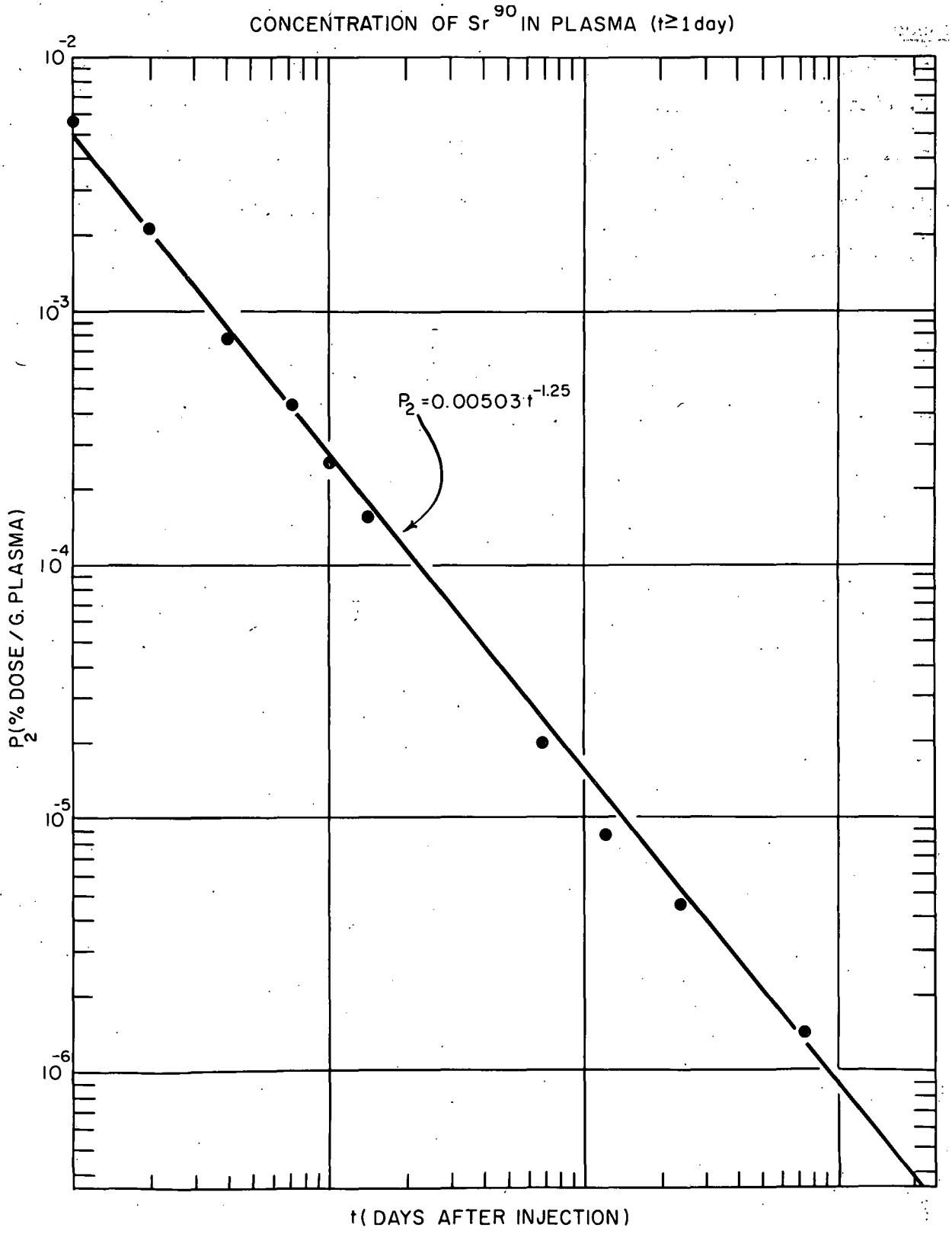
Table I

Interval	Sr	Ra	Units
$t \leq 1$ day	$P_1 = 0.114\ t^{-0.371}$	$P_1' = 0.0918\ t^{-0.454}$	% dose/g. pl., mins.
$t \geq 1$ day	$P_2 = 5.03 \times 10^{-3} t^{-1.25}$	$P_2' = 2.64 \times 10^{-3} t^{-1.24}$	% dose/g. pl., days

The calculated one minute value for strontium is about 20% higher than that for radium. Throughout the first day the strontium concentration decreases more slowly than radium, and at one day strontium is about twice radium. Thereafter, this ratio is maintained and the plasma concentrations of the two decrease at the same rate. Since $P_2 \propto \frac{dR}{dt}$, these results indicate that $\frac{\text{Sr Retention}}{\text{Ra Retention}} = \text{const.} \leq 1$

The concentration ratio of about two may well result from greater binding of strontium by plasma proteins. Assuming that all plasma radium is diffusible (see p. 81) and that the concentration of diffusible strontium equals that of diffusible radium, the "bound" fraction of strontium is 0.48. Strontium dialysis studies are


CONCENTRATION OF Sr 90 IN PLASMA (t≤1day)
P_1 (% DOSE / G. PLASMA)
10^{-1}
10^{-2}
10^{-3}
$P_1 = 0.114 t^{-0.371}$
1.0
10
100
1000
1day
t (MINUTES AFTER INJECTION)


CONCENTRATION OF Sr 90 IN PLASMA (t≥1 day)
10-2
10-3
10-4
10-5
10-6
P2 =0.00503·t-1.25
P2(% DOSE / G. PLASMA)
t(DAYS AFTER INJECTION)

planned to check on this. (Strontium excretion studies are under way.)

References

1. B. J. Stover and D. R. Atherton, Sr^{90} plasma concentration - a preliminary report. Annual Progress Report, March 31, 1956, pp. 107-108.

2. B. J. Stover et al, Chemistry group report, Annual Progress Report, March 31, 1957, p. 140.

ABSORPTION OF RADIUM AND THORIUM THROUGH THE GUT AS A FUNCTION OF CHEMICAL FORM, IV.

D. R. Atherton, B. J. Stover, and M. A. Van Dilla

Abstract: Analyses of the guinea pigs fed "home-made dial paint" show a greater relative absorption of radium than thorium. A summary of the three studies, (rat, monkey, and guinea pig,) shows considerable variation in the relative absorption, which appears to be related both to the chemical state of the radium and thorium and to the condition of the animals.

The studies on absorption of radium and thorium through the gut, previously reported[1, 2, 3], have been concluded with a study in which "Dial Paint"[2] was fed to guinea pigs. Four guinea pigs were used in this study. Each was placed in a separate cage following administration by catheter of a capsule containing 8 μc Ra^{226} and 24 μc Th^{228} (the same preparation as was given to the monkeys[2]). Two hours after administration of the paint the animals were measured in K-9[4] and it was determined that the activity was inside the animals. Measurements were made at intervals over the eleven days of the experiment, and at the end of the experiment the excreta contained over 98% of the administered dose.

On the eleventh day of the experiment the animals were killed. Following the routine described previously[2], three samples of each animal , skeleton, gut and liver-spleen,were taken for analysis. The samples were ashed, dissolved in HNO_3, and measurements were made by Pogo[5], and thoron techniques[6]. The results appear in Table I. A summary of all the gut absorption experiments appears in Table II.

Table I

Skeleton	Ra^{226} % Dose	Th^{228} % Dose	Ra^{226}/Th^{228}
1	0.33	1.7×10^{-3}	180
2	0.24	0.76×10^{-3}	320
3	0.42	1.7×10^{-3}	250
4	0.64	2.3×10^{-3}	310
Average	0.41	1.6×10^{-3}	260
Gut			
1	0.15	0.16	0.94
2	0.024	0.032	0.75
3	0.031	0.030	1.03
4	0.36	0.39	0.92
Average	0.14	0.15	0.93
Liver and Spleen			
1		1.6×10^{-4}	
2		1.1×10^{-4}	
3	3.6×10^{-4}	0.92×10^{-4}	3.9

Table II

Skeletal Retention at 10 Days

	Chloride			Citrate			Sulfate		
	Ra	Th	Ra/Th	Ra	Th	Ra/Th	Ra	Th	Ra/Th
Rat	8.7	0.6	14	16.3	0.069	240	0.0017	0.024	0.071
Monkey							0.017	0.0059	28
Guinea Pig							0.41	0.0016	260

One of the goals of the series of studies involving the monkey and guinea pigs was to observe whether or not a species difference could be demonstrated. Certainly there was a marked difference in the absorbed material; the guinea pig/monkey ratio was 2.5 for thorium and 24 for radium. It is felt that this cannot properly be assigned to a species difference because of the fact that, while the guinea pigs appeared to continue in their usual phlegmatically contented mode of life, the monkeys were in a state of continual fright which seemed to be reflected in a skimpy appetite and what appeared to be a mild diarrhea. If these animals did indeed have a diarrhea it could very well account for their absorbing less of the activity than did the guinea pigs.

As in the monkey experiment, the guinea pig experiment shows considerable scatter in the Ra/Th ratio of skeletal burden, 180 to 320 with an average value of 260, but certainly it is evident that with the "clean chemistry dial paint" the radium is much more readily absorbed, even though radium is absorbed to less than 1%.

It is of passing interest to note that the gut samples, while having a spread of a factor of 10, exhibit Ra/Th ratios of very close to 1.0 (0.75-1.03) indicating that this material is residual paint that has been delayed in making its exit.

As was observed in the monkeys, the guinea pig liver-spleen samples are very low compared with the skeleton.

The results of the guinea pig experiment appear to substantiate our previous observations that chemical form is very critical; that absorption of thorium dominates only when it is in a relatively more soluble form than is the radium.

References

1. 2nd Annual Conference, June 1954, pp. 65-67.
2. Annual Progress Report, March 31, 1955, pp. 14-16.
3. Annual Progress Report, March 31, 1956, pp. 79-81.
4. K-9; A large 4Π Gamma-Ray Detector, M. A. Van Dilla, R. L. Schuch and E. S. Anderson, Nucleonics 12 No. 9, 22-27 (1954).
5. Annual Progress Report, March 31, 1956, p. 77.
6. Ibid., p. 82 .

ISOTOPE BURDEN AND RADIATION DOSE RATE IN INJECTED BEAGLES

C. W. Mays, B. J. Stover, D. R. Atherton, and M. A. Van Dilla

Abstract: Normalized body burden as a function of time after injection is presented by isotope for the average toxicity beagle injected between 16 and 18 months of age. Dose rate to the bone has been computed for a 1-level dog in each injection group.

INTRODUCTION: Retention data from previous progress reports has been sifted and brought up to date. Relations represent the best estimates as of September 1957 and not final measurements. This information should be useful in relating radiation effects to dose.

DEFINITION:

$$\text{Normalized Body Burden} = \frac{\mu c \text{ OF RETAINED ISOTOPE AT TIME } (t)}{\mu c \text{ OF PARENT AT INJECTION } (t=0)}$$

GENERAL: The isotopes we inject locate mostly in the skeleton. Body burden changes with time after injection due to natural radioactive decay and biologic excretion.

PLUTONIUM: As plutonium is not readily excreted, dosage rate decreases only slightly after injection. About 26% of the body burden is in the liver and about 73% in the skeleton, and in the dog, these percentages do not seem to change greatly with time after injection.

RADIUM: Radium is rapidly excreted at first, but more and more slowly later on. Due to its chemical similarity to calcium nearly all the body burden is in the inorganic (mineral) part of bone.

Radon is the only daughter of radium which is appreciably excreted. Being a chemically inert gas, most radon atoms are able to diffuse into the blood, where they are carried to the lungs and exhaled before decaying in the body. Increasing fractional radon retention combines with a decreasing radium burden to give a constant radon burden through the lifetime of our dogs.

Lead 210 activity builds up slowly due to its 20 year half life and contributes

very little damage the first few years after injection. Excretion of lead 210 is believed to be small.

RADIOTHORIUM: The skeletal burden of thorium stays at about 73% of the injection dose (corrected for decay) while the thorium excreted seems to come from soft tissue fraction. The 1.9 year half life of thorium is much more effective in decreasing body burden than is the small biologic excretion.

The daughters of radiothroium are not readily excreted and the amount excreted decreases with time.

MESOTHORIUM: As mesothorium (Ra^{228}) is an isotope of radium, it locates in the mineral part of bone, is excreted rapidly at first, and then more slowly later on.

Most of the radiation dosage comes not from beta-emitting mesothorium, but from its alpha-emitting daughters in the radiothorium decay series. Ingrown radiothorium is not appreciably excreted, and the excretion of its daughters is even less than the small excretion of the daughters of injected radiothorium.

The lag in buildup of ingrown radiothorium causes the dose rate to increase for the first few years after injection in contrast to the decreasing dose rates in all other injection series.

STRONTIUM^{90}: Due to its chemical similarity to calcium and radium, strontium locates in mineral bone. Data to date indicates that strontium retention is slightly less than radium retention in dogs. Ingrown yttrium seems about 100% retained.

. . . . . .

Fractional body burden vs. time is graphically presented. A simplified decay scheme is included on each graph with average alpha (α) and beta (β) energies given in million electron volts (MEV) for each transition. Energy of the recoil nucleus is included with alpha energy. Average beta energy is about 1/3 maximum beta energy.

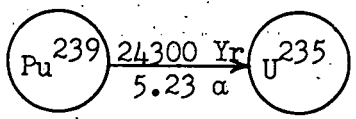
NORMALIZED BODY BURDEN EQUATIONS ARE LISTED FOR AVERAGE DOSE LEVEL AND 16-18 MO. INJECTION AGE

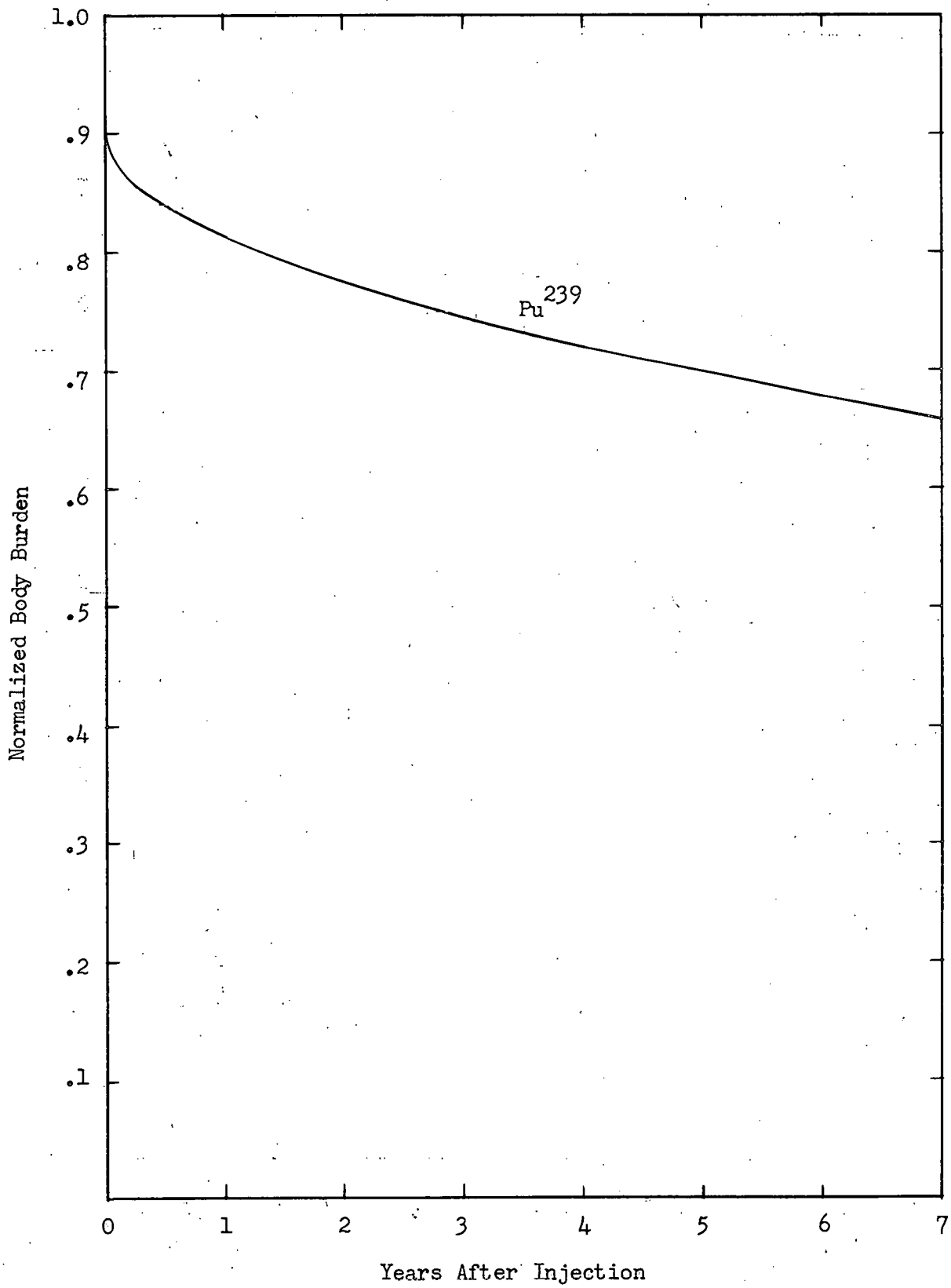
Isotope	Equation	Source
PLUTONIUM		
Pu^{239}	$= .90 - .0036\ t^{+.54}$	File, B.J. Stover
RADIUM		
Ra^{226}	$= .8\ t^{-.2}$	P.R. March 56, pg 74
Rn^{222}	$= .045$	P.R. Sept. 57, pg 119
Pb^{210}	$= .045\ (1-e^{-.000095t})$	Calculated assuming no Pb^{210} excretion.
RADIOTHORIUM		
Th^{228}	$= .93\ t^{-.023}e^{-.001t}$	P.R. Sept. 55, pg 38
Ra^{224}	$= (.93t^{-.023} - .47\ t^{-.31})e^{-.001t}$	P.R. March 57, pg 141
Tn^{220}	$= (.93\ t^{-.023} - .47t^{-.31} - .06t^{-.15})e^{-.001t}$	P.R. Sept. 57, pg 113
MESOTHORIUM		
Ra^{228}	$= .8\ t^{-.2}e^{-.0284t}$	Ra^{226} Retention equation
Th^{228}	$= .0008\ e^{-.001t}\int_{.06}^{t} t^{-.2}e^{+.000716t}dt$	Calculated assuming ingrown Th^{228} not excreted
STRONTIUM		
Sr^{90}	$= .7\ t^{-.23}$	K-9 II, whole dog counter
Y^{90}	$= .7\ t^{-.23}$	P.R. March 55, pg 22

Note: t is the number of days after injection.

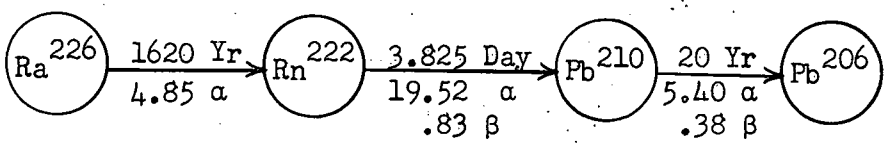
Normalized body burden depends on both biologic excretion and natural radioactive decay.

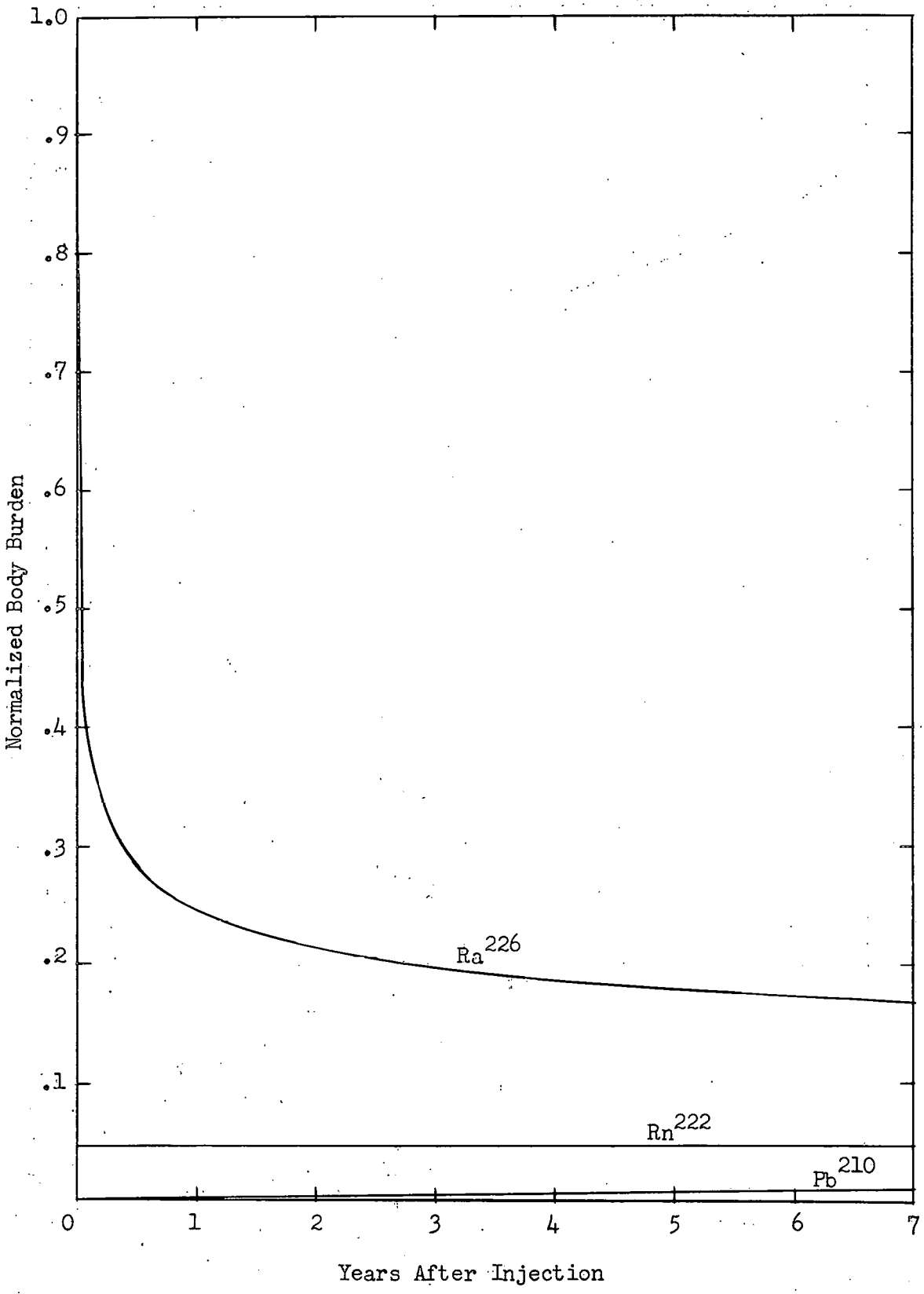
PLUTONIUM BODY BURDEN VS. TIME



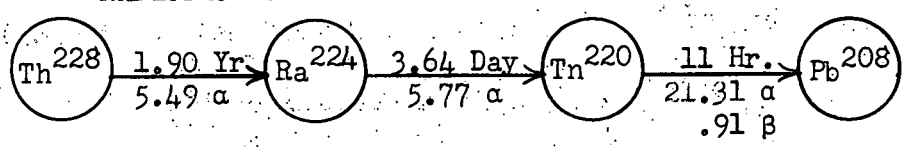


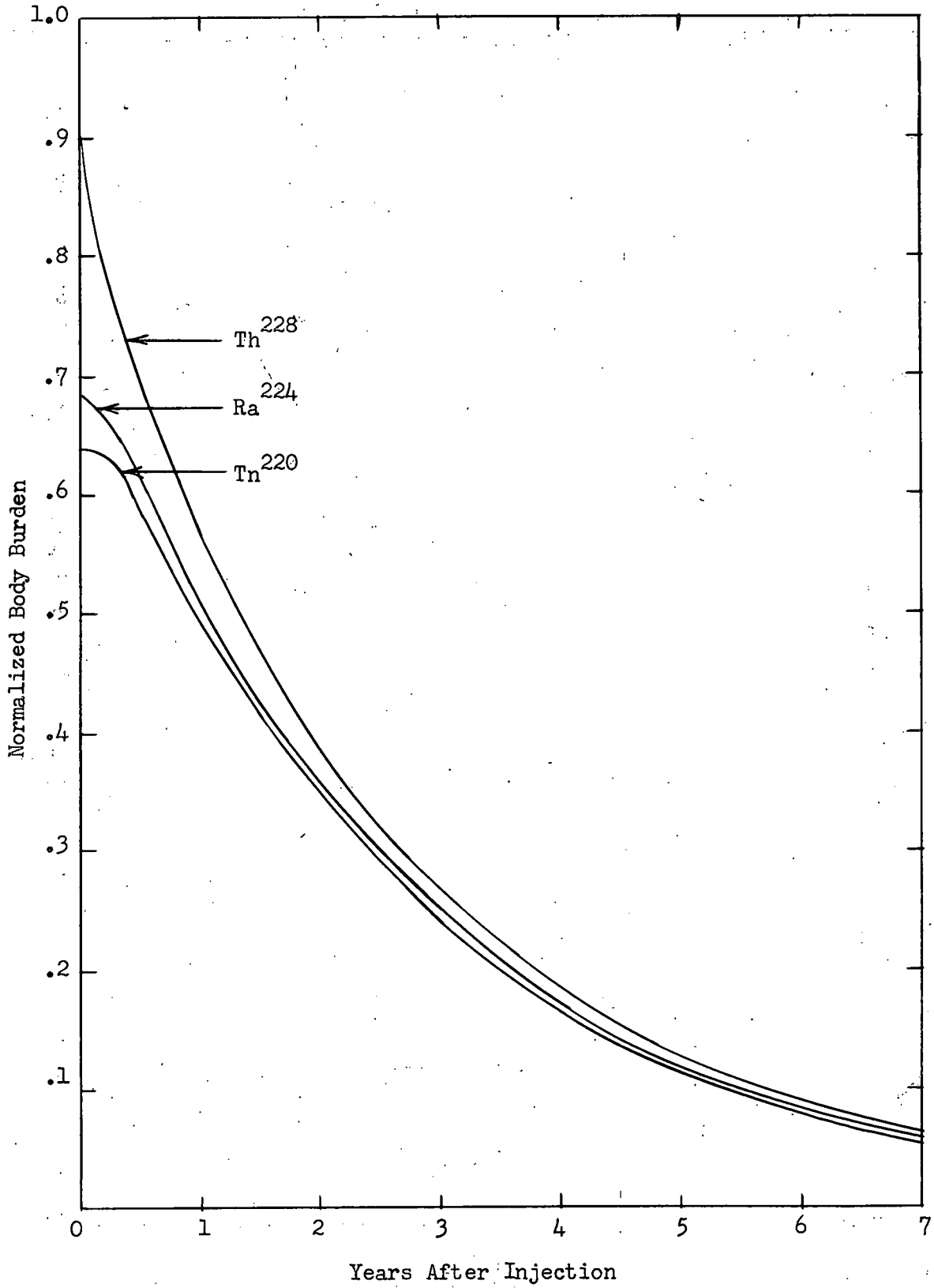
RADIUM AND DAUGHTERS BODY BURDENS VS. TIME



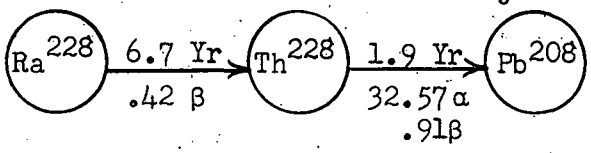


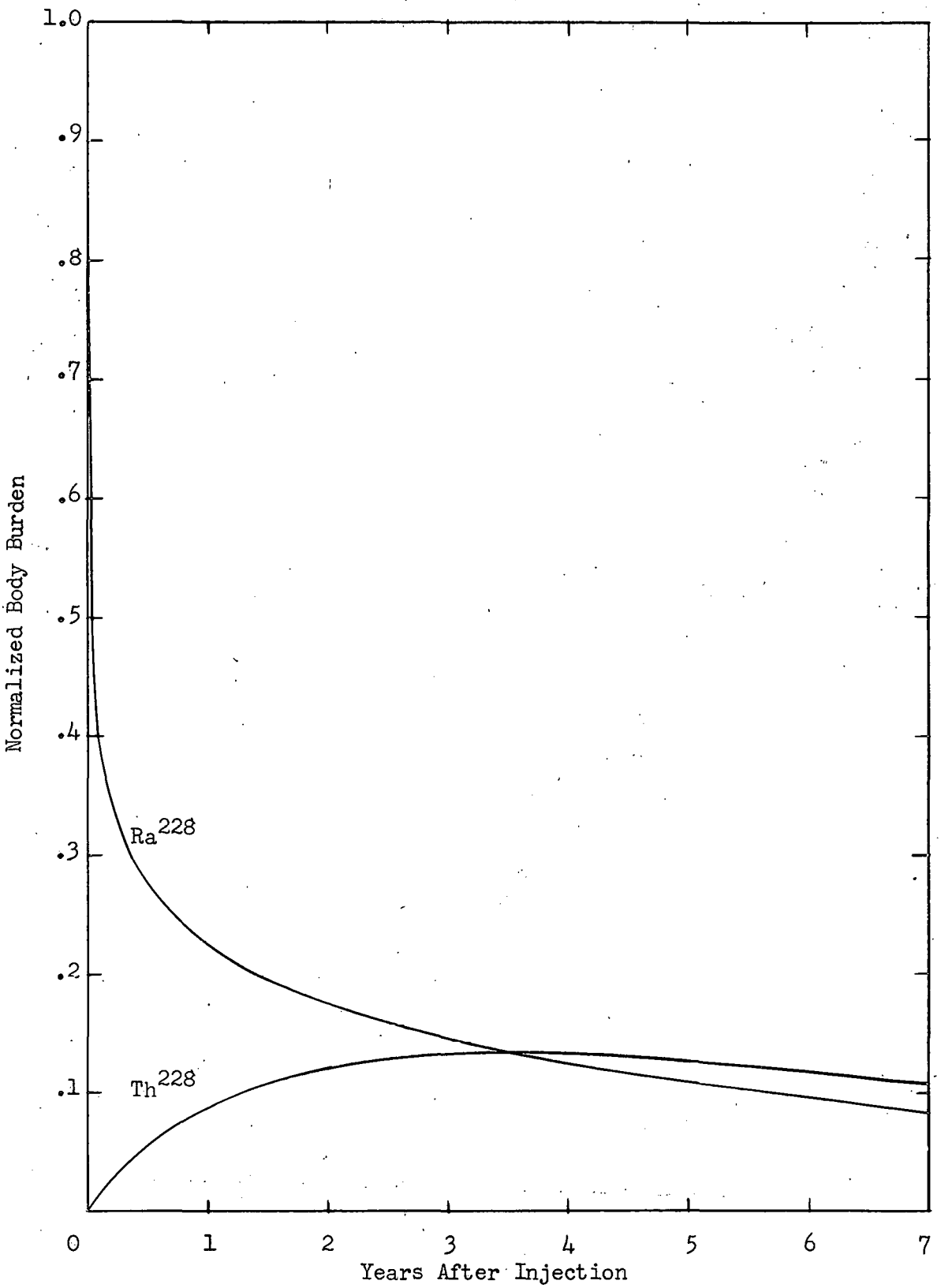
RADIOTHORIUM AND DAUGHTERS BODY BURDENS VS. TIME



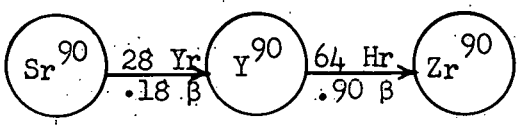


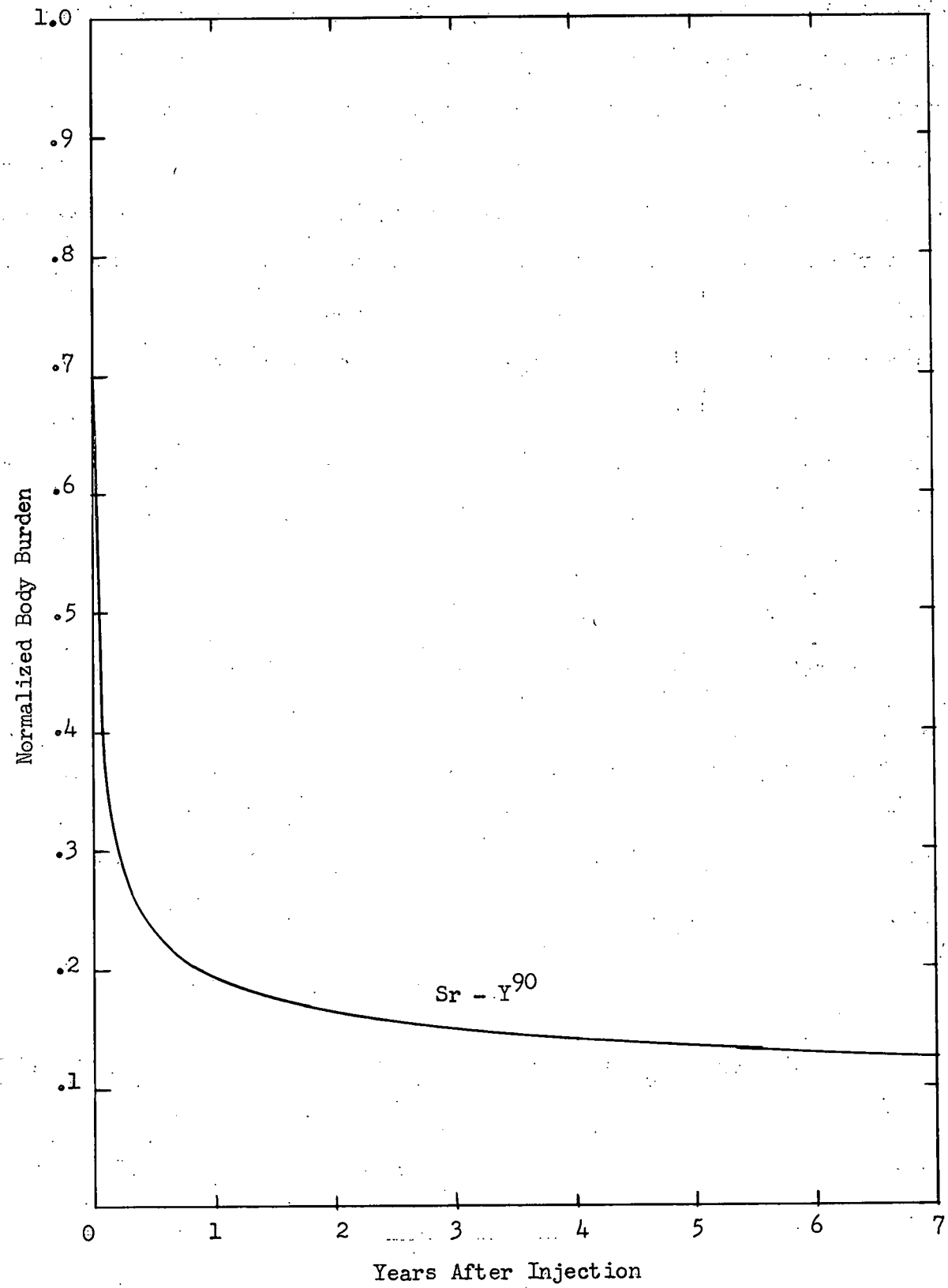
MESOTHORIUM AND DAUGHTER BODY BURDENS VS. TIME
(Assuming no Th^{228} contamination in the injection solution)





STRONTIUM AND DAUGHTER BODY BURDENS VS. TIME





CALCULATION OF DOSE RATE

If the body burden of each isotope in a decay series is knowm, the average dose rate to the bone in rads per year for the 1-level dogs in each injection group can be computed. (1 rad corresponds to the absorption from ionizing radiation of 100 ergs of energy per gram of material.) Only alpha plus recoil nucleus plus beta dose has been computed, as in comparison, gamma dose is negligible. Bone is assumed to make up 1/10 of body weight. To find the dose rate for the 5, 4, 3, 2, 1.7, 1.5, and 0.5 levels multiply the 1-level dose rate by 162, 54, 18, 6, 3, 2, and 1/3 respectively.

In plutonium, 73% of the body burden is assumed to be in the skeleton. 1-level plutonium dogs are injected with 0.0159 μc/kg body weight.

In radium and daughters, 100% of the body burdens are assumed to be in the skeleton. 1-level radium dogs are injected with 0.0572 μc/kg body weight.

In radiothorium, skeletal burden of Th^{228} is assumed to be 73% of the injected dose corrected for decay. All the Ra^{224} burden is assumed to be in the skeleton. Skeletal loss of Tn^{220} is assumed to be twice the exhaled Thoron. 1-level radiothorium dogs are injected with 0.0159 μc/kg body weight.

In mesothorium, 90% of the energy of ingrown Th^{228} and daughters is assumed delivered to the skeleton. 1-level mesothorium dogs are injected with 0.057 μc/kg body weight. Dose rate has been calculated for 15% , 3%, and 0.6% RdTh contamination in the injection solution, and is shown on a separate graph.

In strontium, 100% of the body burdens are assumed to be in the skeleton. 1-level strontium dogs are injected with 0.572 μc/kg body weight.

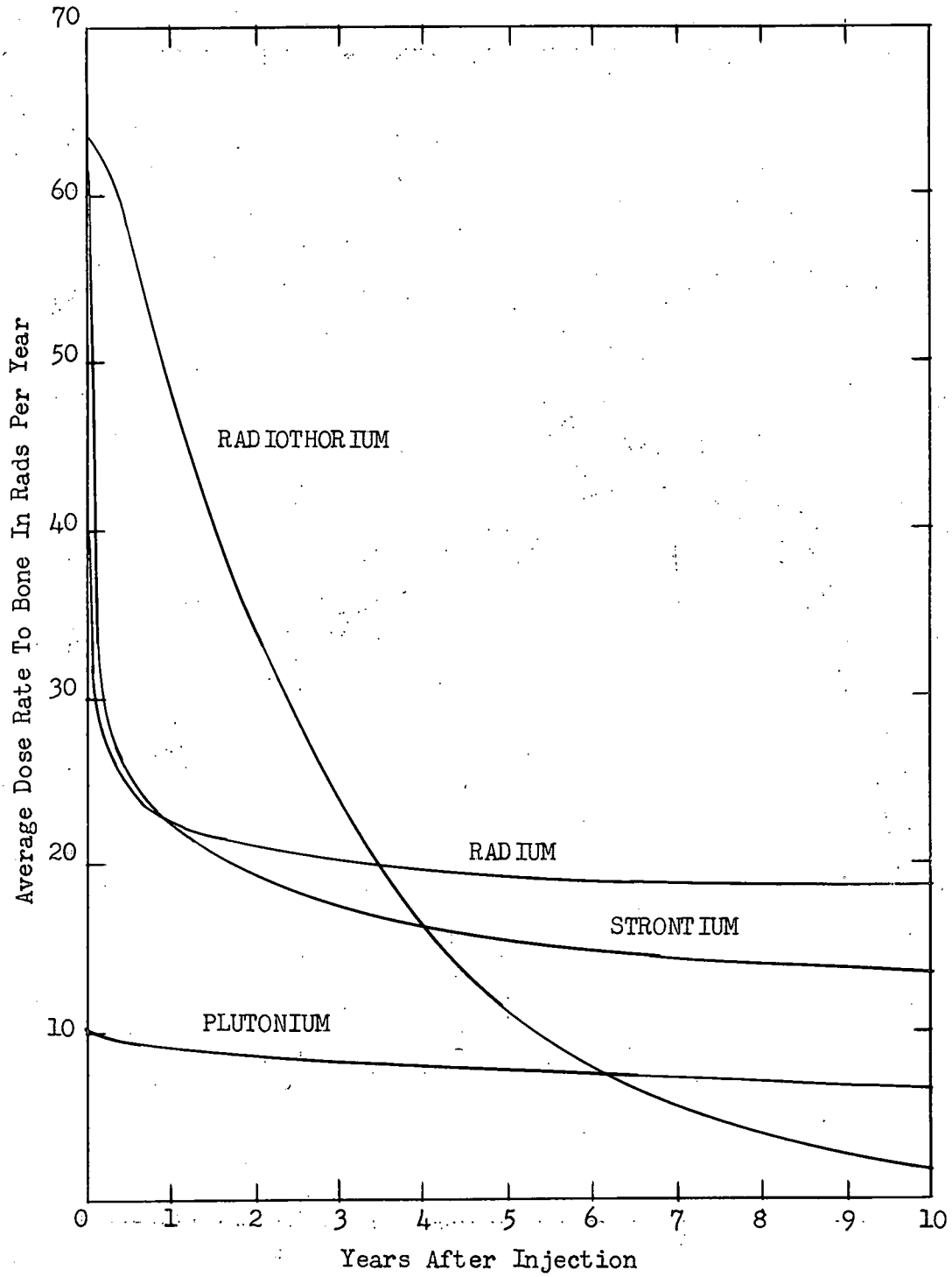
SAMPLE COMPUTATION:

$$\text{DOSE RATE} = \left[\text{INJECTED } \frac{\mu c}{KG}\right]\left[\text{SKELETAL BURDEN}\right]\left[\text{DISINTEGRATION MEV}\right]\left[187\ \frac{\text{RAD}}{\text{YEAR}}\right]$$

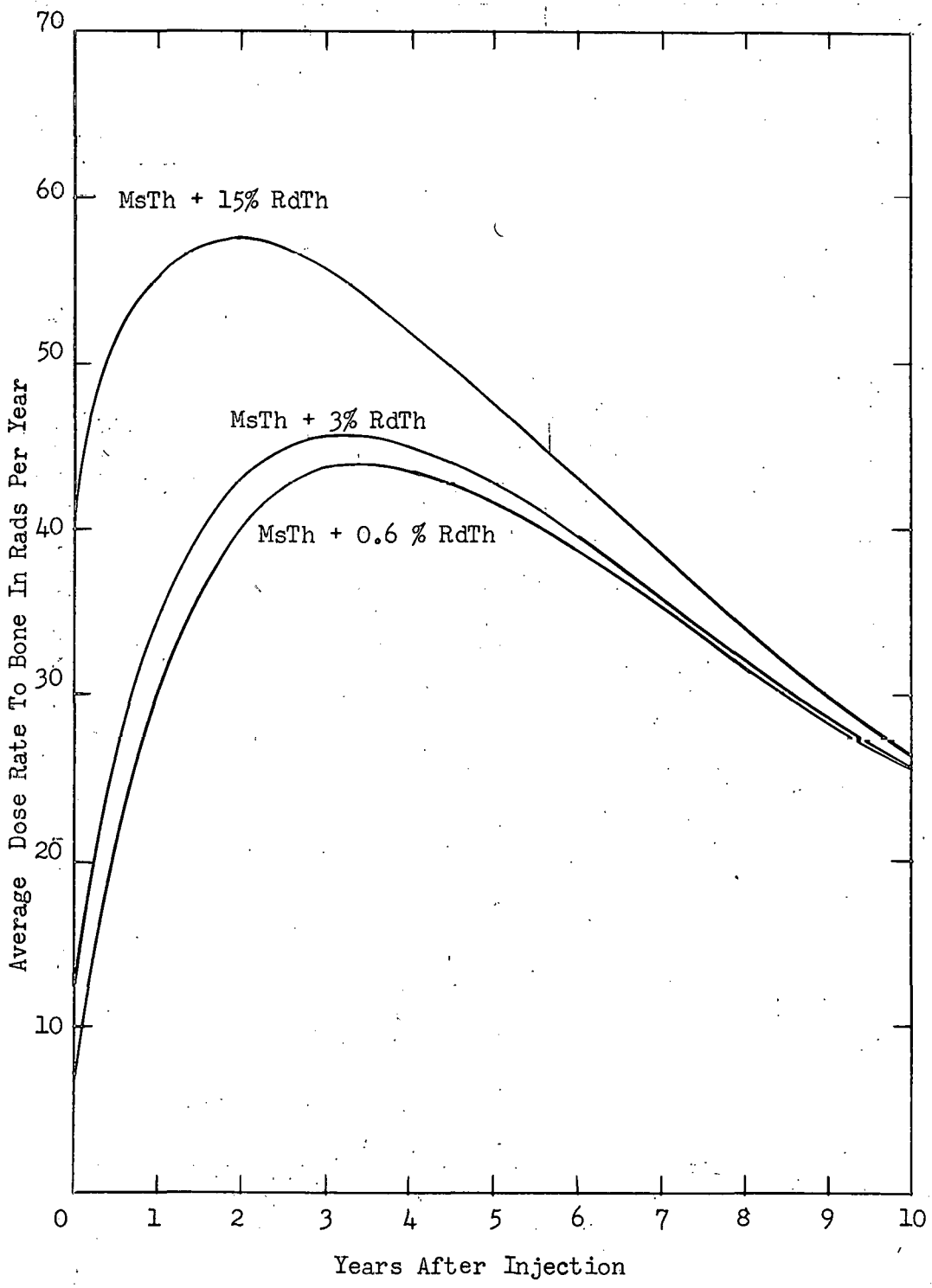
for a 1-level plutonium dog one year after injection

$$\text{DOSE RATE} = \left[.0159\right]\left[.73\quad(.90 - .0036 \times 365^{.54})\right]\left[5.23\right]\left[187\ \frac{\text{RAD}}{\text{YEAR}}\right] = 9.2\ \frac{\text{RADS}}{\text{YEAR}}$$

DOSE RATES TO 1-LEVEL BEAGLES



DOSE RATES TO 1-LEVEL MsTh BEAGLES



K-9 BARK II

D. H. Taysum, G. D. Westenskow, C. W. Mays

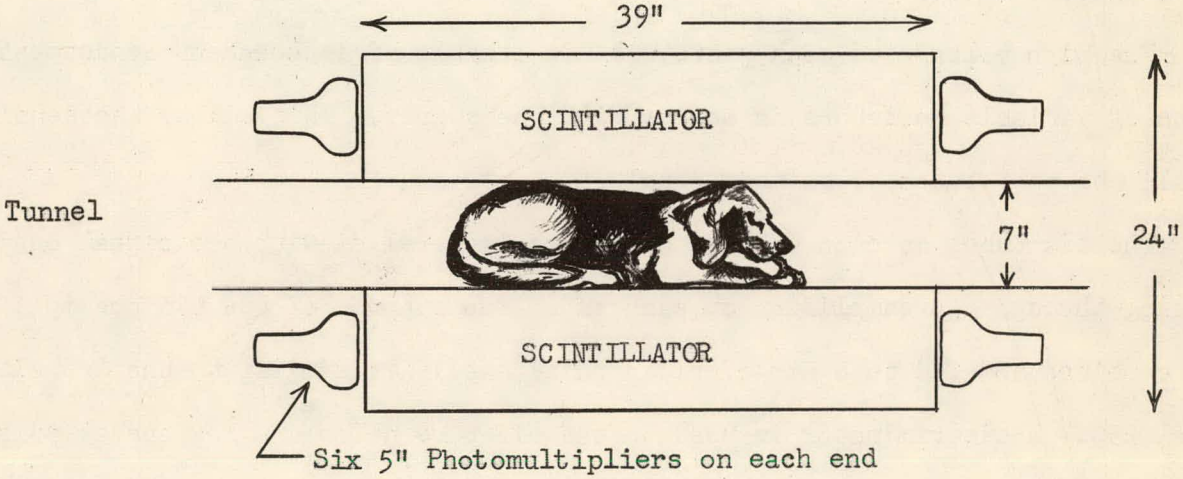
M. A. Van Dilla, L. W. Blake and A. G. Liddell

Abstract: A whole body gamma scintillation detector designed to admit a living dog has been completed.

The new detector is four times more sensitive than the earlier model (K-9) it replaces.

Natural potassium 40 and fallout cesium 137 are being measured in dogs and humans.

Design: The design of K-9 Bark II is similar to the design of K-9[1] the previous model and the Los Alamos human counter[2].



K-9 Bark II is an annular cylinder 39 inches long, 24 inches in diameter with a 7 inch diameter tunnel through the long axis.

The liquid scintillator is contained inside a steel shell 3/16 inches thick with end plates 3/8 inches thick in which are welded threaded inserts which receive the plastic viewing ports and light tight compartments.

The seven inch diameter tunnel has a wall thickness of 0.032 inches. It passes through the center of the end plates and traverses the length of the counter.

The tunnel projects 10 inches beyond each end to facilitate easy entrance of materials to be counted.

This arrangement provides a liquid layer 8.5 inches thick and 39 inches long.

The interior (part occupied by scintillator) was sand blasted and painted with a paint composed of Epon Resin Formulation XA-200 containing anatase TiO_2 as the reflecting pigment[3].

The plastic ports are discs of CR-39 plastic[4] 1/8 inches thick mounted in pre-treated neoprene "O" rings[5]. The photomultipliers are not yet optically coupled to the plastic ports but coupling with a transparent viscous fluid is planned.

Six 5 inch Dumont phototubes are arranged on each end and are situated in light tight compartments against CR-39 plastic ports through which scintillations are viewed.

The arrangement is such that the tubes may be removed without disturbing the scintillator liquid.

The high voltage to each phototube is capable of independent adjustment by means of variable resistors in series with the supply. This allows the sensitivity of all the phototubes to be made equal.

The six tubes at each end are connected in parallel with the signal output passing through a preamplifier at each end. The outputs of the two preamplifiers are combined and fed to a non-overload pulse amplifier similar to the Los Alamos model 250. A discriminator is used to cut off tube noise and low energy pulses. The pulses are then counted by both a scaler and precision count rate meter.

The volume of this counter is 70 gallons. Analytical reagent grade toluene with 5 gms/liter of p-terphenyl and 0.2 gms/liter of "POPOP" is the scintillator. This liquid was bubbled with 200 cubic feet of argon to sweep out dissolved oxygen. This was done to reduce the light quenching action of oxygen.

The counter is housed within a hollow steel cylinder 5 feet long and 5 feet in diameter with a 5 inch wall thickness. Five foot diameter disks of steel three inches thick close the ends.

The arrangement of the counter in its enclosure allows 3/4 of the space to be used to accomodate human subjects or space in which to carry out other measurements which require a low level of background.

The steel compartment is effective in reducing the background of a sodium iodide crystal counter by a factor of 20 for the energy range 0.25 to 1.00 M.E.V.

At this time an upper gate is being prepared for use with this counter. It should reduce that portion of background attributable to high-energy cosmic ray particles. This is expected to lower the total background appreciably.

Measurements: Dogs to be counted are anesthetized and placed in a plastic bag (open at the head end) to keep the counter clean, then slid on a cloth sling into the tunnel for gamma counting.

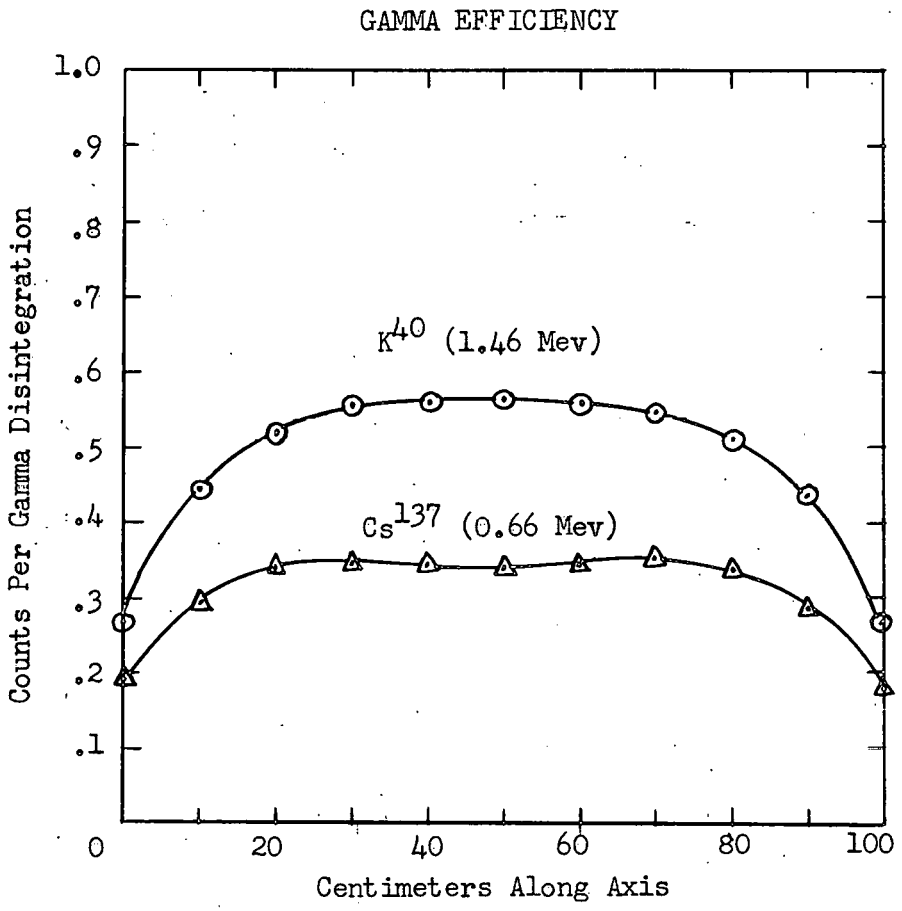
The upper limit of dog size is about 15 kg (33 lbs.) which is well above our colony average of about 10 kg.

The geometry for the counting of humans is not as good as it is for dogs, since humans must be counted outside the tank. The counter is, however, able to count potassium 40 and cesium 137 in humans to a reasonable accuracy.

The optimum counting condition to reduce the effect of purely statistical errors when the source is weak compared to background is to make the ratio S^2/B a maximum. Where S is the net count rate of the source and B is the background count rate.

However, an operating point of slightly lower sensitivity was chosen to reduce tube noise, which experience has shown to be erratic.

Efficiency: The figure on the following page shows the variation in counting efficiency with position along the axis for two unshielded gamma sources. If the average length of a dog (70 cm) is compared to the sensitivity curve shown, it will be seen that the dog is contained within the linear response region of the curve. This minimizes errors due to isotope distribution in the dog.



For a background of 70,000 c.p.m. samples giving count rates of 700 counts per minute (1/100 background) can be counted with a statistical standard deviation of ± 10% in a counting time of three minutes when using a three minute background count.

Non-statistical errors such as electronic drift tend to make the total error greater than the statistical error. However, for low level samples and short counting times the chief error is statistical.

The activity of several emitters which give a count rate of 700 counts per minute (1/100 background) are listed. The recorded counts per parent atom disintegration are also tabulated.

Emitter	Parent Activity in $\mu\mu$c for 700 c.p.m.	Counts per parent disintegration
Co^{60}	380	0.83
Ra^{226} + daughters	500	0.63
Th^{228} + daughters	710	0.45
Cs^{137}	1,000	0.31
K^{40}	5,600	0.056
Sr^{90} + *daughters	240,000	0.0013

* Deposited in bone in a 10 kg beagle.

The sensitivity of this instrument increases with increasing gamma ray energy as is shown in the preceding table and the gamma efficiency curves.

Summary:

1. An improved live dog gamma counter has been completed.

2. A 5×10^{-10} curie radium source in equilibrium with its daughters can be measured to within a statistical standard deviation of $\pm$ 10% in only three minutes of of counting time.

3. Natural K^{40} and fallout Cs^{137} burdens are being measured in uninjected dogs and humans.

References and footnotes:

1. K-9: A large 4Π Gamma-Ray Detector. Nucleonics, September 1954, Vol. 12, No. 9, Pgs. 22-27.

2. The Los Alamos Human Counter, Nucleonics, January 1956, Vol. 14, No. 1, Pgs. 26-29.

3. Glidden's Zopaque SD in Shell Chemical Companys Epon Resin Formulation XA-200.

4. CR-39: A product of Cast Optics Corporation Hackensack, New Jersey.

5. "O" rings soaked in toluene for three days before use.

MEASUREMENT OF X-RAY DOSAGE TO DOGS

C. W. Mays, D. H. Taysum, G. N. Taylor* and M. A. Van Dilla**

Abstract: The bone dose (in rads per year) from two complete x-ray examinations was found to be about 75% of the internal emitter dose in a 1-level plutonium dog. In all control dogs and the lowest toxicity level dogs in each group, the x-ray dose has been reduced to about 30% of the 1-level plutonium dose.

Introduction: All injection series plus 1/2 the control dogs have been given a series of diagnostic x-rays twice a year. These x-rays form an extremely valuable record of bone changes, but considerable concern has arisen as to whether the x-ray dose may modify the changes caused by internal emitters in low level animals.

Technique: Dosage was measured with a Victoreen r-meter (I) which had been calibrated against 25 r and 100 r chambers at various dosage rates. Correction was made for temperature and pressure.

Entrance and exit dose was measured on the dog F5R5 during a regular x-ray examination. The meter was placed on top of the dog to measure entrance dose and underneath the animal to measure exit dose. Average dose to the exposed part was taken as the average of entrance and exit dose.

Average dose to the exposed part times the fractional part of the dog exposed gives equivalent whole body dose per shot. The sum of equivalent whole body doses per shot gives the total equivalent whole body dose in roentgens. Multiplying equivalent whole body dose by the energy absorption coefficient for bone gives the average x-ray dose to the bone in rads.

(I) 1-r chamber model 227, Victoreen Instrument Company, 5806 Hough Ave. Cleveland, Ohio

* Now at Michigan State University, Department of Veterinary Pathology.
** Now at Los Alamos Scientific Laboratories.

On the suggestion of L. D. Marinelli of the Argonne National Laboratory, the effect of self-shielding due to the high x-ray absorption coefficient of bone was investigated. Due to the thinness of dog bones, average dose to the exposed part is close to the average of entrance and exit dose. In a larger animal, such as the elephant, self-shielding would materially reduce the bone dose.

Results: X-rays were taken with a Westinghouse x-ray machine (style 981625) with 1.5 mm Al filter, 100 ma, 1/5 sec, and 31 inch focal-film distance except for shot #2 where the focal-film distance was 15 inches.

Shot	Description	X-ray Peak Kilovolts	Average Roentgens to exposed Part	Fraction of Dog Exposed	Equivalent whole body Roentgens
1	Right lateral thorax	75	0.234	0.7	0.164
2	Mandible	55	0.357	0.1	0.036
3	Oblique lateral of skull	80	0.284	0.2	0.057
4	Anterior-posterior of skull	85	0.269	0.2	0.054
5	Anterior-posterior upper thorax	75	0.186	0.6	0.112
6	Anterior-posterior lower thorax	70	0.182	0.4	0.073
7	Lateral of forelegs	75	0.212	0.5	0.106
8	Lateral of hindlegs	75	0.205	0.3	0.062
9	Posterior-anterior of hindlegs	80	0.222	0.2	0.044
			TOTAL EQUIVALENT WHOLE BODY ROENTGENS		0.7 ± 0.2 (Est)

$$\text{DOSE TO BONE PER X-RAY EXAM} = \left[0.7 \text{ Roentgen}\right]\left[4.75 \frac{\text{Rads}}{\text{Roentgen}}\right]^{II} = 3.3 \text{ RADS}$$

Thus, two complete exposures per year gives an average bone dose of 6.6 rads or $\frac{6.6 \text{ rads}}{8.8 \text{ rads}} = 75\%$ of the internal emitter dose to the bone of a 1-level plutonium dog two years after injection[III]. Localized dose due to both x-rays and internal

II O. Glasser, Medical Physics Vol Two, 1950, 791. (Data of F. W. Spiers)
III Pg 103 this report.

emitters varies considerably from the average dose due to the difference between entrance and exit dose and non uniform isotope deposition.

Reduction of x-ray dose: By limiting the x-ray beam to parts of the animal directly over the film, dose is reduced to about 2/3 of its original value. In addition, one of the two complete x-ray examinations per year has been replaced with an abbreviated examination consisting of the lateral thorax, mandible, and lower foreleg in the controls, and 1-level dogs (0.5 level for Radiothorium). This reduces dose in these dogs to about 30% of the 1-level plutonium internal emitter dose to the bone. The higher level dogs still receive the two complete examinations per year.

Dose could be still further reduced by using thicker filters and high kilovoltage but at the price of loss of contrast in the films. Good contrast is needed to detect minimal changes in bone architecture. We feel that poor films defeat the purpose of taking x-rays.

Conclusions:

1. With two x-ray examinations per year, the x-ray dose is uncomfortably close to the internal emitter dose in the lowest level animals.

2. By improved technique and reduction in exposures, the x-ray dose to control and lowest level dogs has been reduced to about 30% the 1-level plutonium dose.

3. Continued effort will be directed toward further reduction of x-ray dose while maintaining high quality radiograms.

FURTHER THORON EXHALATION MEASUREMENTS

C. W. Mays and L. W. Blake

Abstract: Using the technique previously described (Annual Report March 56 pg 81), breath thoron measurements have been continued in the radiothorium dogs and started in the "pure" mesothorium series.

Exhaled thoron in radiothorium dogs: Exhaled thoron was measured by having the dog breathe into a large tank in which the thoron decay atoms were collected on a negatively-charged steel rod, whose activity was measured later. Values were adjusted to a standard ventilation rate of 3.33 $\frac{\text{liter}}{\text{min.}}$.

Total thoron produced in the body was calculated by subtracting Ra^{224} excretion from the Th^{228} retention equation, (see pg 96 this report). (After 100 days this becomes a constant 73% of the injected Th^{228} corrected for decay). Because body-produced thoron is greater than was originally assumed, and due to more accurate calibration, the thoron exhalation values previously reported (Annual Report March 1956) are too high.

Based on 76 measurements, the percent of thoron atoms formed in the body which are exhaled from 1 to 1000 days after injection may be expressed as:

2- and lower levels	$P = 8\% \, t^{-.11}$
3-level	$P = 8\% \, t^{-.17}$
4-level	$P = 8\% \, t^{-.24}$

where t is the number of days after injection.

Comments:

1. The percent of thoron exhaled is small and becomes even smaller as burden time increases. This is caused by the short half life of thoron (54 seconds), and the burial of parent atoms in locations less accessable to the circulation.

2. High level (3 & 4) dogs exhale a smaller percent of their thoron, while there is no significant difference among the 2 or lower level dogs. This effect

becomes more pronounced as burden time increases and may indicate impaired circulation in areas of radiation damage.

Exhaled thoron in mesothorium dogs: The M4M, F5M, M6M and F7M groups were chosen for measurement because of the low (0.6%) radiothorium contamination in their injection solutions. 24 dogs in dose levels 1 through 5 were measured.

Exhaled thoron produced from injected mesothorium was found by estimating (based on previous RdTh dogs) the exhaled thoron due to contamination of RdTh in the injection solution, and subtracting this from the measured total exhaled thoron.

Calculation of the fraction of thoron exhaled: Total mesothorium produced thoron activity was assumed to be 90% of the ingrown radiothorium activity. Ingrown radiothorium was computed assuming mesothorium (Ra^{228}) was retained like radium (Ra^{226}) ($MsTh = .8t^{-.2}\ e^{-.0284t}$) and that in vivo produced radiothorium is not excreted. The radiothorium build up equation, $RdTh = .0008\ e^{-.001t}\int_{.06}^{t} t^{-.2} e^{.00716t}\,dt$ was evaluated on a Datatron model 204 digital computer, the results of which appear on page 100 of this report.

Results: From 50 to 200 days after injection, 1.8 ± 0.6% of the mesothorium-produced thoron atoms formed in the body are exhaled.

This is only about 1/3 as great as the percent of thoron exhaled by low level radiothorium dogs with the same burden time. The scatter in data prevents detection of the effects of time after injection and dose level.

Conclusion: We believe that the smaller fractional thoron exhalation in mesothorium injected dogs is due to failure of ingrown radiothorium to translocate appreciably from injected radium deposition sites (mineral bone) to injected thorium deposition sites from which escape of thoron is presumably easier.

FURTHER STUDIES ON RADON RETENTION

C. W. Mays, M. A. Van Dilla, W. S. S. Jee, D. R. Atherton, J. Watkins, D. Barton, L. Blake and W. Stevens

Abstract: This is a continuation of work reported in the March, 1957 Annual Report. Conclusions remain unchanged except that fractional radon retention in mature, as well as in growing, bone formed after injection is smaller than that in pre-injection bone. Ethelyene diamine soxhlet extraction greatly increases radon retention.

Method: Fractional radon retention was measured by the method previously described pages 156-157 Annual Progress Report, March 1957.

Radon retention in post-injection bone: We have found that fractional radon retention in bone grown after injection (hereinafter called "post-injection bone") is smaller than that in pre-injection bone. In an earlier Progress Report (March 1956, pp71) the reverse was (erroneously) reported. The discrepancy was solved by exhuming the frozen skeletons of F3R4 and M1R5 and measuring radon retention in dead bone kept moist and exposed to air at room temperature. Fractional radon retention was measured by comparing radon activity in the air-exposed sample to equilibrium radon activity in the sealed sample. Due to absence of circulation, retention in dead bone is always greater than in live bone. By a rare stroke of luck the previously measured rib samples from F3R4 were discovered and remeasured in their original container.

The following data show that the previously reported retention values in post-injection bone are much too high, while the values for pre-injection bone are probably about correct.

	Part of Rib	Reported in vivo Retention	Exhumed sample Retention	Remeasured original sample Retention
F3R4	Tip (Post-inj)	29% (?)	18.0%	18.1%
F3R4	Shaft (Pre-inj)	16	20.3	22.0
M1R5	Tip (Post-inj)	36 (?)	18.5	—
M1R5	Shaft (Pre-inj)	23	23.1	—

The reason for the high values originally reported in post-injection bone seems to be the shift of the center of radiation in the containing vial due to diffusion of radon from the small porous rib tips which thawed while being serially counted. The well type counter used in the original measurement was unfortunately quite geometry dependent.

Lower fractional radon retention in post-injection bone is confirmed by the following measured values for live bone.

RIB SAMPLE	FRACTIONAL RADON RETENTION			
	T12R5	T13R5	M4R5	M2R5
Tip (Post-inj)	11.9%	12.7%	17.2%	17.0%
Hot line	15.5	15.9	20.7	22.2
Shaft (Pre-inj)	16.2	15.8	20.7	21.2

Since post-injection bone has a smaller radium burden time than bone existing at injection, these results are in line with the observed increase in whole body fractional radon retention with burden time. Hot line retention is the same as shaft retention.

Erroneous carcass measurements of T8R5 and T9R5: Using the method just described, suspected measured values of whole body radon retention for T8R5 and T9R5 (Semi-Annual Report September 1955, page 15) were proved to be in error. Representative bones were removed from the frozen skeletons and fractional radon retention was measured in these samples which were kept moist and exposed to air at room temperature.

Fractional radon retention in the exhumed bones is listed and compared with in vivo values for the humeri and carcasses made at the time of death.

FRACTIONAL RADON RETENTION

SAMPLE	T8R5	T9R5	T10R5 Control
Femur shaft	14.2%	16.8%	16.1%
Rib shaft	23.3	12.5	13.2
7th lumbar vertebra	15.6	15.1	9.3
Calvarium (top of skull)	18.9	15.2	15.8
Pelvis	14.2	12.2	10.8
Average (air exposed)	17.3	14.4	13.1
In vivo humerus	13.7	13.8	12.2
In vivo carcass	20.3 (?)	31.7 (?)	10.0

Due to lack of circulation in dead bone, true in vivo radon retention is less than dead bone radon retention. Hence, the suspected values of in vivo radon retention, being higher, are wrong.

In contrast, the humeri retention values, measured by an independent method at time of death appear to be correct, and form the best available estimate of true whole body retention for these dogs.

Bones from T10R5, whose measured whole body retention was unquestioned served as a control.

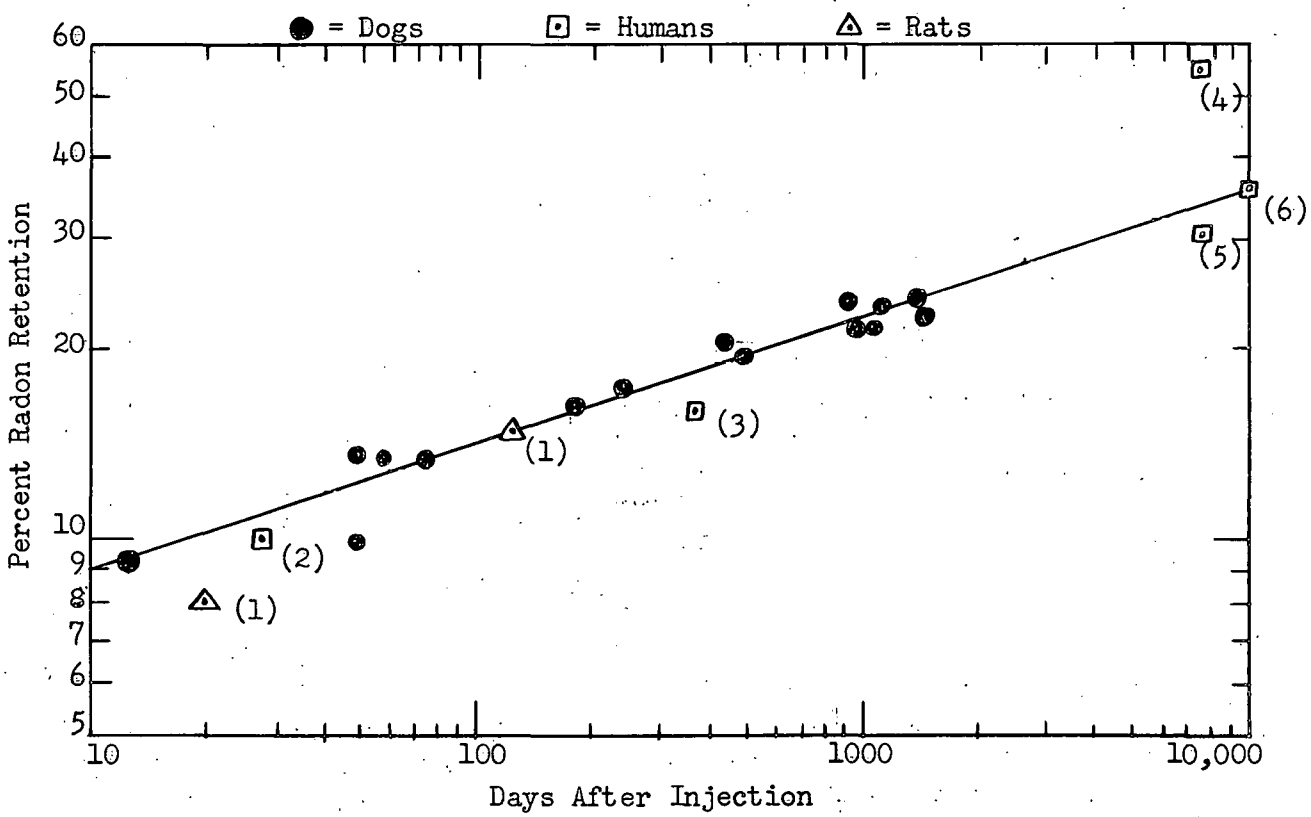
We do not know why the in vivo carcass values of T8R5 and T9R5 were so high. Both dogs were measured on the same days in K-9, a whole dog gamma counter.

Effect of time after injection: Whole body fractional radon retention values at time of death for 16 beagles* are presented in tabular form and graphically. For comparison some retention values from other authors for rats and humans are also plotted.

* K-9 measurements were made by D. H. Taysum, Physics Research Associate at this Laboratory and R. L. Floyd, now Associate Scientist at Linfield Research Institute, McMinnville, Oregon.

Dog	Injected μc Ra/kg	Months at Injection	Days at death	Fractional Radon Retention	Method
T16R5	9.70	20	12	9.2%	4-L tank
T10R5	1.98	1.7	49	10.0	K-9
T11R5	1.91	1.7	49	13.8	K-9
T8R5	1.92	10	58	13.7	Humerus value used
T9R5	1.94	75	58	13.8	Humerus value used
T14R4	3.17	17	72	13.5	NaI crystal
T13R5	9.76	13	188	16.2	4-L tank
T12R5	9.72	13	225	17.4	4-L tank
T3R5	4.76	48	428	20.7	K-9
F3R4	3.33	13	490	19.8	K-9
M1R5	10.5	16	908	24.0	K-9
F8R5	9.68	16	968	21.6	4-L tank
F6R5	10.2	16	1015	21.7	4-L tank
M4R5	10.6	14	1091	23.7	4-L tank
T2R5	4.39	30	1368	25.0	4-L tank
M2R5	10.8	15	1385	22.6	4-L tank

FRACTIONAL RADON RETENTION VS. TIME



The following relations are evident from the preceeding data:

(a) Fractional radon retention increases with time after radium injection and for beagles may be expressed as:

$F = 5.7\% \, t^{.20}$ where t is the number of days after injection.

(b) Fractional radon retention is similar in rats, dogs, and humans at the same time after injection.

(c) Fractional radon retention seems independent of dose level (from 1.91 μc/kg to 10.8 μc/kg) and age at injection (from 1.7 months to 75 months).

Radon retention in whole bones: Fractional radon retention at time of death has been measured in individual whole bones from the 8 radium dogs whose bones were counted in the 4-liter scintillator tank. The most striking fact was the similarity in fractional radon retention from bone to bone in a particular animal. In any one dog, there is a definite tendency for retention to fall in the same sequence. Average retention values are presented by bone, and correspond to a burden time of 500 days.

BONE	Average Fractional radon retention
Mandible (jaw bone)	22.0%
Calvarium (top of skull)	21.4
Pelvis	20.6
Humerus and Femur (upper leg bones)	20.3
Cervical vertebra (neck bones)	19.6
Scapula (shoulder blade)	19.3
Lumbar vertebra (lower backbone)	19.3
Hyoid bones (small bones in throat)	19.1
Thoracic vertebra (upper backbone)	18.8
Foot bones	18.6
Ribs	18.1
Sternum (breast bone)	18.0
Coccyx (tail)	17.1
Whole body	19.6

The similarity of radon retention among individual whole bones and different parts of whole bones suggests that whole body fractional radon retention could be closely estimated from a small bone sample. Our Pathologist, Dr. T. H. Cochran, has observed that small bone samples are often removed in the course of normal surgery and that we can expect radium poisoned humans to require surgery more often than non poisoned persons. Measurement of radon retention in these samples would be simpler than whole body gamma counting and breath radon measurements, and yet might be more accurate.

Low radon retention in teeth: The similarity of individual bone retention to skeletal retention does not apply to teeth where much lower retention has been found. Six canine teeth and two molar teeth have been measured from 5 dogs. In four of the canine teeth, the pulp cavity and its lining were cored out to test whether retention might be different near the pulp cavity (extremely high radium concentration) than in the remainder of the tooth (diffuse radium concentration). Results show tooth retention is only about half of skeletal retention for these dogs.

DOG	SKELETAL RETENTION	TOOTH RETENTION	DESCRIPTION
T1R5	23.0	10.9%	whole canine
T1R5	23.0	8.8	whole molar
T2R5	25.0	11.0	whole molar
F6R5	21.7	11.2	cored canine
M4R5	23.7	13.5	cored canine
M4R5	23.7	13.3	cored canine
M2R5	22.6	9.7	cored canine
M2R5	22.6	12.9	whole canine

It is ironic that teeth, having the lowest radon retention, grow in jawbones which average the highest radon retention among the whole bones.

This suggests a fundamental difference in crystal size or composition between bone and teeth. It may be that the narrowest dimension in bone crystals is about twice that in teeth. X-ray diffraction and electron microscope studies may amplify this speculation.

Retention in bone tumors: Fractional radon retention was measured in samples taken from the exterior and interior of the calcified bone tumor on the lumbar vertebrae

of M4R5. Retention in the exterior sample (17.7%) was lower than retention in the older interior sample (20.2%) which in turn was lower than retention in the lumbar vertebrae (21.7%).

In F8R5 fractional radon retention was measured in a sample from the slightly calcified, extremely rapidly growing tumor on the right foreleg. The tumor retention of 6.5% was much less than the skeletal retention of 21.6%.

These results are in line with our idea that fractional radon retention increases with the length of time after radium deposition.

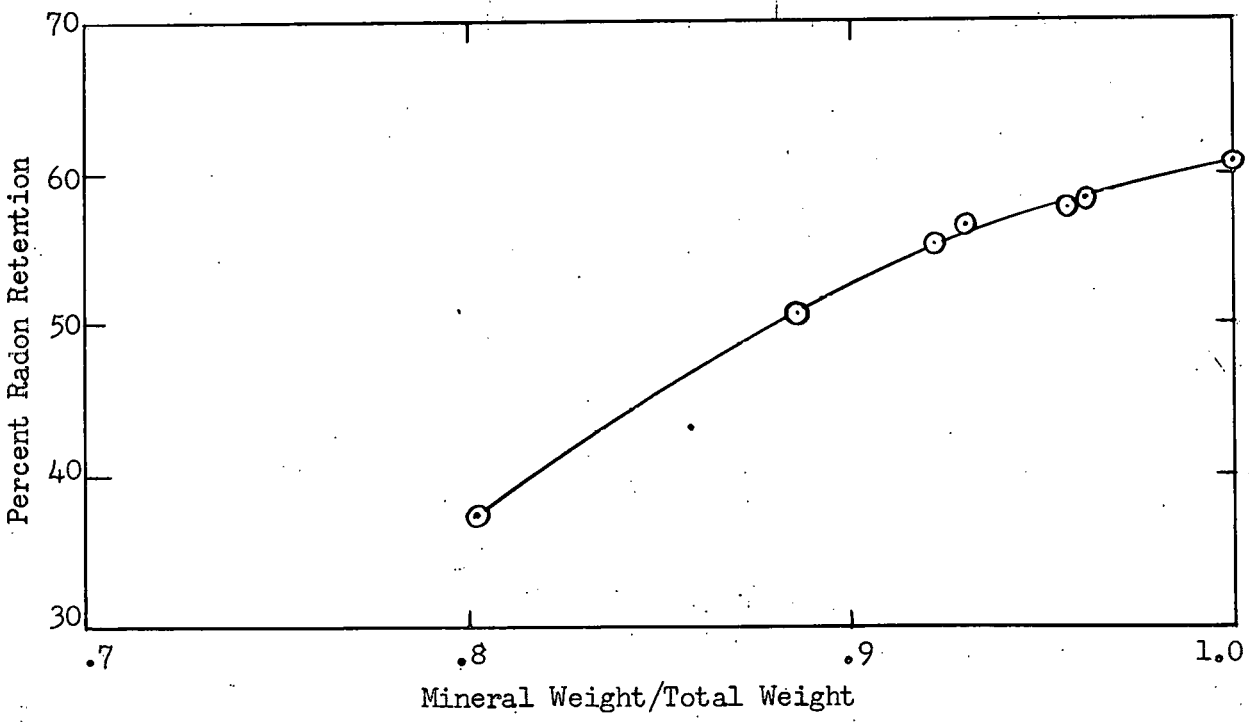
Radon retention in mineral bone: The organic fraction was removed from 10 radium bone samples from the dog T12R5 using a soxhlet extractor with 80% ethylene diamine (EDA) for 3 days. The samples were then flushed with water for 3 days and the radon allowed to equilibrate in the air exposed samples for 2-4 weeks at various temperature and humidity conditions. Mineral fraction was taken as the ratio of sample weight at 230° C (assumed 100% mineral) to the sample weights at other conditions. The samples represented cortical and trabecular bone from the long bones and vertebrae, and post and pre-injection bone from the rib. No significant differences existed between individual and average retentions in the EDA treated samples. Temperature, average mineral fraction and average fractional radon retention are presented in the same order measurements were taken.

Temperature °C.	Mineral weight / Total weight	Percent Radon Retention
56	-	56.4
25	-	52.7
-30	0.886	50.7
58	0.922	55.2
104	0.930	56.5
160	0.961	58.2
230	1.000	60.7
25	0.962	58.2
3	- unknown*	34.5
25	0.957	57.7
25	0.805	37.4

* Samples soaked in water, exact mineral fraction unknown.

RADON RETENTION VS. MINERAL FRACTION

IN EDA EXTRACTED BONE



The increase of radon retention with increasing temperature was initially a surprise as temperature alone, by increasing molecular kinetic energy normally increases diffusion and hence should produce a decrease in radon retention. Temperature however, was not acting alone but was causing air-exposed bone samples to gain or lose water from the atmosphere. This altered the mineral fraction and it was this change in mineral fraction which accounts for the change in radon retention.

The lack of a pure temperature effect was proved by changing the temperature from 230° to 25° C while keeping the samples as dry as possible in a large sealed vessel. Radon retention at a temperature of 25° C. and a mineral fraction of 0.962 was the same as retention at 160° C and the same mineral fraction. Hence, under the conditions tested, temperature acting along does not measurably alter radon retention in EDA treated bone.

However, when the water content (and hence mineral fraction) was altered while the temperature was held constant radon retention changed significantly. The sequence of runs shows that these effects are reversable.

Part of the increase of radon retention with increasing mineral fraction can be explained on the basis of more recoiling radon atoms stopping inside crystals from which diffusion is difficult, and fewer in water from which diffusion is easy, but this is not enough to completely account for the observed changes. We do not know the complete explanation for increase of radon retention with increasing mineral fraction in EDA treated bone. This relation does not necessarily exactly apply to change of radon retention with mineral fraction in vivo where the crystals are always water saturated.

Another puzzle remains. In the live animal, fractional radon retention was 17%. Before EDA extraction, retention in air exposed samples was 22%. But after extraction, retention more than doubled! It is clear that EDA Soxhlet extraction changes at least one property of mineral bone, i.e., its ability to retain radon, but we do not know whether this is caused by effective increase in crystal size

or a decreased diffusion coefficient produced by modifications in crystal structure. Our results on mineral bone agree with the more extensive work done by R. E. Rowland[7] at the Argonne National Laboratory.

SUMMARY OF RADON EXPERIMENTAL RESULTS TO DATE

Fractional radon retention:

1. Is similar for the rat, dog, and human at equal times after radium injection.
2. In dogs is independent of injected dose, age at injection, sex, state of health, rate of circulation, weight and fat content.
3. Increases with time after radium deposition.
   a) Is lower in post-injection bone.
   b) Is lower in areas of rapid bone growth.
   c) Is lower in rapidly growing bone tumors.
4. Is similar among different whole bones, tending to be slightly smaller in the smaller bones.
5. Is only half as large in teeth as in bone.
6. Is increased to about 90% in water saturated, frozen bone.
7. Is increased by EDA soxhlet extraction.
8. Increases as the mineral fraction increases.

Correlation with theory: Because of lack of exact knowledge of the true shape of bone crystals, retention equations have been worked out for a tablet, cylinder and sphere assuming radon recoil range was large compared to the smallest dimension of the crystal and that the diffusion coefficient was uniform throughout the crystal. The derivation for the tablet case has been worked out in detail previously (Annual Progress Report March 1957). The other derivations are similar.

Assuming bone crystals are long thin cylinders:

$$F = M\left(1 - \frac{2}{x}\,\frac{I_1(x)}{I_0(x)}\right)$$

Assuming bone crystals are thin tablets:

$$F = M\left[1 - \frac{\tanh(x)}{x}\right]$$

Assuming bone crystals are spheres:

$$F = M\left[1 + \frac{3}{x^2} - \frac{3\,\mathrm{Coth}(x)}{x}\right]$$

where F = fractional radon retention

$M = \dfrac{\text{mineral density within recoil range}}{\text{total density within recoil range}}$

$x = r\sqrt{\dfrac{\lambda}{D}}$

r = (half thickness of tablet / radius of cylinder / radius of sphere

λ = decay constant of radon

D = diffusion coefficient of radon in bone crystal

$I(x)$ = Hyperbolic Bessel function (tabulated in Handbook of Chemistry and Physics)

Radon retention is regarded as chiefly a function of three variables, M, r, and D. As bone ages, the mineral fraction (M) increase, crystal size (r) increases and as the crystal matures and becomes more nearly perfect the diffusion coefficient (D) probably decreases.

Each indicated change in these variables tends to increase fractional radon retention (F) in all three equations. This predicted increase in fractional radon retention with time after radium deposition is observed experimentally.

A logical question is why is there no effect of age at injection? We think the answer is that most of the radium atoms deposit in growing areas. In these areas the crystals are young regardless of the age of the animal, and these young crystals mature at about the same rate. The similarity of radon retention in rats, dogs, and humans suggests a basic species uniformity in the sizes of bone crystals and the rate at which they mature.

References

1. W. P. Norris, et al, Argonne National Laboratory Report on Biological Medicine and Biophysics Programs, July 1954 (ANL 5288).
2. F. E. Hoecker, J. Ind. Hyg. and Toxicol 26, 1944.
3. H. Schlundt, et al, Am. J. Roentgenol and Rad. Therapy 1933, 30.
4. R. D. Evans, J. Indust. Hyg and Toxicol, 25, 1943.
5. W. P. Norris, et al., Am. J. Roentgenol and Rad. Therapy, 73, No. 5, May 1955.
6. W. B. Looney, et al., Am. J. Roentgenol and Rad Therapy 73, No. 6, June 1955.
7. R. E. Rowland, Personal communication.