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BIOLOGY AND MEDICINE SEMIANNUAL REPORT

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BIOLOGY AND MEDICINE SEMIANNUAL REPORT  
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BIOLOGICAL STUDIES OF RADIOACTIVITY AND IRRADIATION

Crocker Laboratory  
University of California  
Berkeley, California

BIOLOGICAL EFFECTS OF INTERNALLY DEPOSITED RADIOISOTOPES

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Muriel E. Johnston, Nylan Jeung, Marilyn H. Williams,  
Marshall W. Parrott, George Barr, Ann Henderson, and Justine Burg

RETENTION OF CALCIUM-45 AND STRONTIUM-90 IN THE RHESUS MONKEY

Six new individuals have been introduced into the Sr<sup>90</sup> monkey colony since the preceding report.<sup>1</sup> The ages of all animals in the experiment have now been determined according to the method described by van Wagenen and Asling.<sup>2</sup> Accurate aging allows us to consider two separate groups of monkeys--adults and adolescents. The adolescent group consists of those animals under 4 years of age and the adult group of those animals 4 years or older. Table I shows the numbers or names of animals, their ages at injection, and the dates of injection.

Complete excreta collections have been made during the first 20 weeks after injection for the nine monkeys injected since January 1957, and a one-week sample has been collected every two months thereafter. Although excretion collections were complete only during the first 10 days for the four monkeys injected prior to 1957, periodic weekly specimens are being taken to determine the slope of the long-term component of the excretion curve.

Excreta samples are oven-dried and reduced to ash in a muffle furnace at 500° C. Ashing is completed by boiling with conc. HNO<sub>3</sub>. The ash is dissolved in 25 cc of 4 N HNO<sub>3</sub> and diluted to 200 ml with distilled water. A 0.5% or 1.0% aliquot (such that the final dry weight will not exceed 250 mg) is transferred to a weighted glass planchet, and dilute NH<sub>4</sub>OH is added with stirring to precipitate the insoluble phosphates. The slurry is evaporated on a hot plate at low heat and then strongly heated to sublime the volatile ammonium salts. The resulting sample is not hygroscopic and is evenly spread over the entire surface of the planchet.<sup>3</sup>

<sup>1</sup> Durbin and Asling, et. al., Strontium-90 in the Rhesus Monkey, in Biology and Medicine Quarterly Report No. UCRL-8031, 1957.

<sup>2</sup> G. van Wagenen and C. W. Asling, Rotentgenographic Estimation of Bone Age in the Rhesus Monkey (*Macaca mulatta*), Am. J. Anat. (in press).

<sup>3</sup> G. Barr, A. Method for Uniform Mounting of Samples Emitting Soft Radiations, University of California Radiation Laboratory Medical and Health Physics Quarterly Report No. UCRL-2881, p. 15, 1955.

Table I

Roster of Sr<sup>90</sup>-injected rhesus monkeys. Each animal received 35  $\mu$ C of Sr<sup>90</sup>-Y<sup>90</sup> in a single intravenous injection except where otherwise indicated.

Animal	Sex	Age at injection (years)	Group	Date injected	Remarks
Stupe	M	4.25	adult	May '54	*
Rosy	F	5.0	"	April '54	* 3 months pregnant at t=0. Has since borne 2 more offspring.
Tony	M	4.3	"	" "	* Arthritic; sacrificed at 8 months.
10(Pat)	F	3.75	adoles.	Aug. '55	* Late adolescent or young adult. Sacrificed at 3 months.
20	F	2.67	"	Jan. '57	Intraperitoneal injection
21	F	2.75	"	" "	" "
23	M	> 7	adult	" "	" "
33	F	3.17	adoles.	Feb. '58	Java monkey Ca <sup>15</sup>
34	F	2.92	"	" "	"
35	F	2.42	"	" "	"
27	M	3.58	"	Sept. '58	" late adolescent or young adult.
28	F	2.58	"	" "	"
29	F	3.08	"	" "	"

\* Complete excretion collections to day 10; samples taken sporadically thereafter.

The plated samples are counted for at least 2000 counts with a thin end-window G-M tube and a Nuclear-Chicago scaler fitted with an automatic changer. When the initial count of a group of samples is complete the group is recounted with a 64-mg/cm<sup>2</sup> Al filter to screen out the Ca<sup>45</sup> beta particles. Percent transmission of the Sr<sup>90</sup> and Y<sup>90</sup> beta particles through the absorber is determined each time samples are counted. An aliquot of each injection solution serves as the counting standard.

Beta-particle self-absorption curves (shown in Fig. 1) were prepared for both Ca<sup>45</sup> and Sr<sup>90</sup> by the dilution method, using bone ash, soft-tissue ash, urine ash, and fecal ash as mass sources. After the first few months the specific activities of both Ca<sup>45</sup> and Sr<sup>90</sup> in the excretion samples decline to less than 0.1 cpm/mg of ash, and it becomes necessary either to process the samples chemically or to apply a correction factor to account for natural K<sup>40</sup> background. The almost exclusively vegetable diet of these animals leads to approximately 20 cpm of K<sup>40</sup> in a mixed-excreta ash specimen weighing 20 mg/cm<sup>2</sup>. The counting rate due to K<sup>40</sup> (and presumably a small contribution from long-lived fallout activities) was determined in a series of uncontaminated samples of monkey urine, feces, and mixed excreta. These curves are shown in Fig. 2 for samples with and without the Al absorber. The relationship between K<sup>40</sup> count and sample thickness is not linear over all; however, most specimens fall within the range 7.5 to 20 mg/cm<sup>2</sup>, where the deviation from a straight line is not too great. The number of control samples was small, and they were scattered about the lines shown in Fig. 2. The validity of the correction for K<sup>40</sup> background activity was tested by subjecting a group of Sr<sup>90</sup>-containing excreta specimens, previously assayed by the usual method, to chemical processing. Duplicate 10 cc samples of dissolved excreta ash were made alkaline with NH<sub>4</sub>OH to precipitate the insoluble phosphates. The soluble salts--potassium, sodium cesium, etc--were separated by centrifugation, and by repeated washing of the precipitate with dilute ammonia. Results of the two assay methods are compared in Table II. Those samples for which the results of the two methods did not agree closely (generally those collected after 1000 days) will be reassayed for Sr<sup>90</sup> by the Nuclear Science and Engineering Corp. by their Y<sup>90</sup> milking process (precipitation of YPO<sub>4</sub>), and will be further processed in this laboratory by a modified Y-milking method devised by Dr. M. Nervik of the UCRL Livermore Laboratories involving extraction of Y with tributyl phosphate.

At total-sample counting rates of 35 cpm (15 cpm background and 10 cpm K<sup>40</sup>), the estimated errors are: counting  $\pm 5\%$ ; uncertainty in self-absorption,  $\pm 10\%$ ; uncertainty in K<sup>40</sup> correction,  $\pm 10\%$ ; and manipulative,  $\pm 5\%$ , for a total estimated error of 15% to 20%. At total counting rates greater than 100 cpm the estimated error declines to about 10%.

The excretion rates of Ca<sup>45</sup> and Sr<sup>90</sup> and the retention calculated from total excretion measurements are shown in Table III for a group of adolescent monkeys. These data are plotted as exponential curves in Fig. 3. The slope of the longest-term component of the Sr<sup>90</sup> curve,  $1080 \pm 250$  days, has been estimated by averaging the slopes of least-squares fits of the data from individual adolescent monkeys collected between the 250th and 530th day. This mean slope compares favorably with fits similarly obtained for data from three adult monkeys collected between the 250th and 1550th day. The

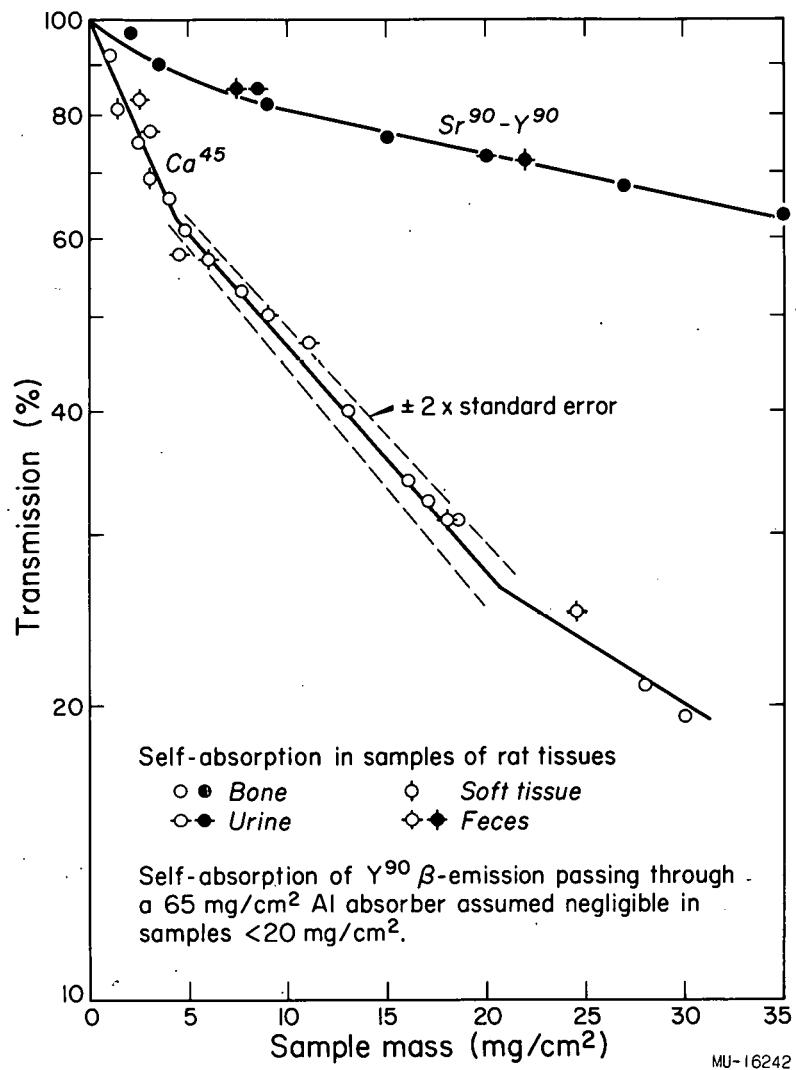
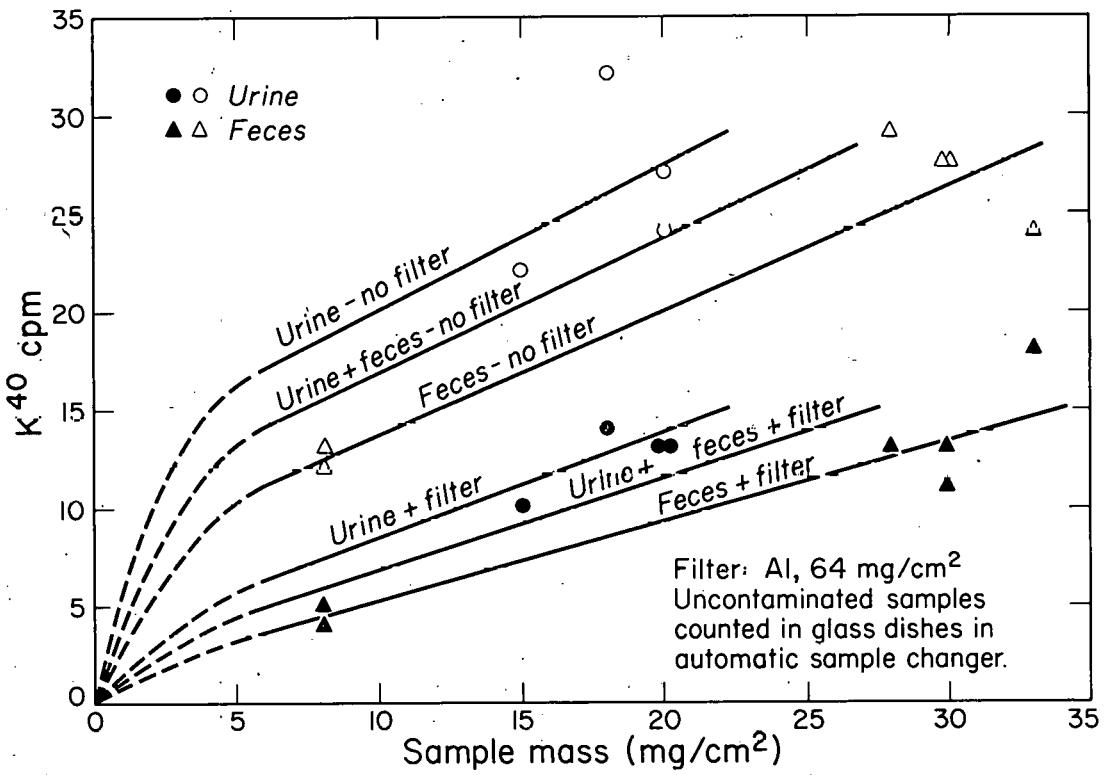


Fig. 1. Self-absorption of  $\text{Ca}^{45}$  and  $\text{Sr}^{90}$  in ash from bone, soft tissue, and excreta.



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Fig. 2. Beta-particle activity of  $K^{40}$  in unprocessed ash of monkey urine and of feces as a function of sample mass.

Table II

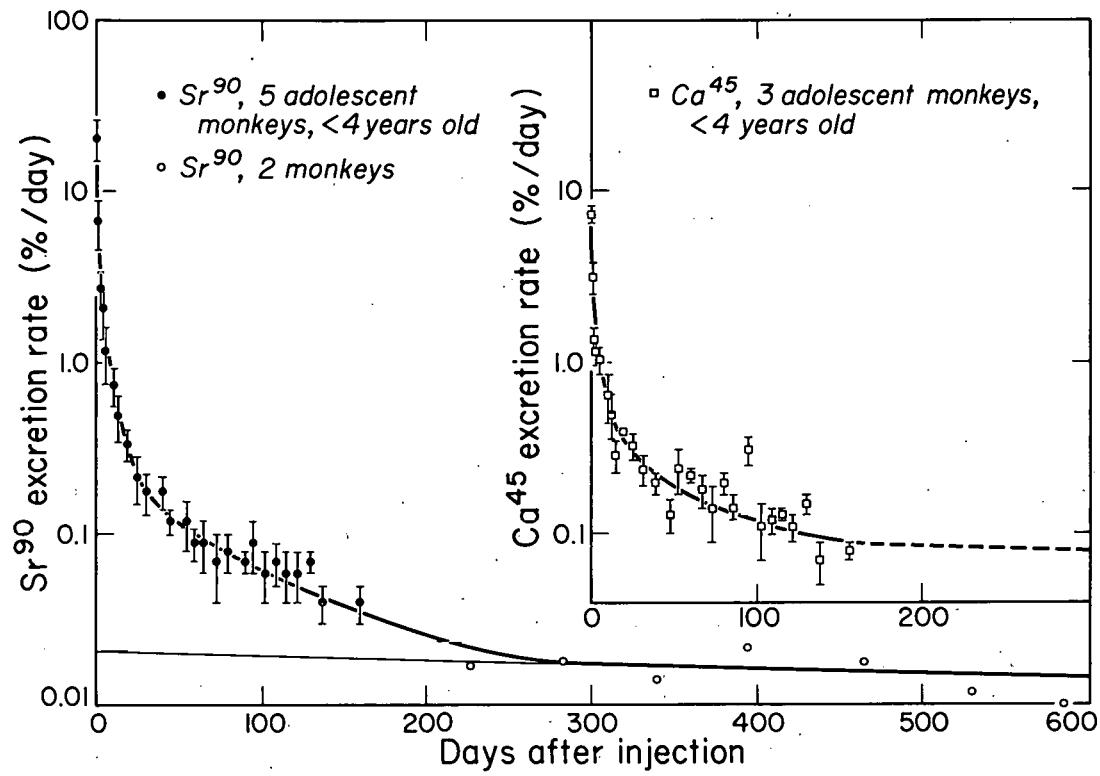
Comparison of Sr<sup>90</sup> assay of monkey excreta chemically processed to remove K<sup>40</sup> and counted without chemical processing but with a K<sup>40</sup> correction applied

	Soluble salts removed	Unprocessed excreta ash, K <sup>40</sup> correction applied		
		% dose in 1-week sample	% dose in 1-week sample	Sample weight (mg/cm <sup>2</sup> )
<u>Monkey-21</u>				
Week 57	0.170	0.156	21	203
Week 67	0.150	0.148	24	194
Week 76	0.090	0.087	17	114
<u>Monkey 23</u>				
Week 57	0.042	0.042	15	54
Week 67	0.094	0.098	16	128
Week 76	0.054	0.052	14	67
<u>Stupe</u>				
1229 days	0.067	0.054	35	84
1341 days	0.035	0.034	29	56
1479 days	0.024	0.029	33	54
1542 days	0.027	0.030	26	54
<u>Rosy</u>				
963 days	0.028	0.017	48	54
1084 days	0.017	0.012	21	38
1194 days	0.028	0.025	34	56
1362 days	0.010	0.008	24	36
1500 days	0.015	0.010	31	41

Table III

Excretion of Sr<sup>90</sup> and Ca<sup>45</sup> by adolescent rhesus monkeys, aged 29 to 38 months when injected. Means are shown in percent of administered dose  $\pm$  standard deviation.

	Sr <sup>90</sup> ; 35 $\mu$ C (5 monkeys)		Ca <sup>45</sup> ; 135 $\mu$ C (3 monkeys)	
Day	Total excretion (%/day)	Retention	Total excretion (%/day)	Retention
1	21.0 $\pm$ 5.7	79.0	7.43 $\pm$ 0.83	92.6
2	6.88 $\pm$ 2.2	72.1	3.17 $\pm$ 0.69	89.4
3	2.81 $\pm$ 0.68	69.3	1.36 $\pm$ 0.26	88.0
4	2.15 $\pm$ 0.77	67.2	1.16 $\pm$ 0.2	86.9
5	1.20 $\pm$ 0.44	64.8	1.05 $\pm$ 0.17	85.8
6			1.00 $\pm$ 0.10	84.8
7	1.07 $\pm$ 0.34	63.7	0.99 $\pm$ 0.11	83.8
8	1.13 $\pm$ 0.47	62.6	1.20 $\pm$ 0.08	82.6
9	1.42 $\pm$ 0.36	61.2	1.08 $\pm$ 0.22	81.6
10	0.76 $\pm$ 0.19	60.4	0.66 $\pm$ 0.21	80.9
11	0.61 $\pm$ 0.20	59.8	0.25 $\pm$ 0.06	80.6
12	0.75 $\pm$ 0.34	59.1	0.50 $\pm$ 0.14	80.2
13	0.50 $\pm$ 0.15	58.6	0.29 $\pm$ 0.06	79.9
14	--	--	0.29 $\pm$ 0.03	79.6
14-21	0.34 $\pm$ 0.07	55.9	0.39 $\pm$ 0.01	76.8
21-28	0.22 $\pm$ 0.07	54.3	0.33 $\pm$ 0.06	74.5
28-35	0.18 $\pm$ 0.05	53.1	0.24 $\pm$ 0.05	72.8
35-42	0.18 $\pm$ 0.04	52.0	0.20 $\pm$ 0.05	71.4
42-49	0.12 $\pm$ 0.02	51.1	0.13 $\pm$ 0.03	70.2
49-56	0.12 $\pm$ 0.04	50.3	0.24 $\pm$ 0.07	68.8
56-63	0.09 $\pm$ 0.02	49.6	0.22 $\pm$ 0.02	67.3
63-70	0.09 $\pm$ 0.03	49.0	0.18 $\pm$ 0.04	66.0
70-77	0.07 $\pm$ 0.03	--	0.14 $\pm$ 0.05	--
77-84	0.08 $\pm$ 0.02	48.0	0.20 $\pm$ 0.03	63.6
84-91	0.07 $\pm$ 0.01	--	0.14 $\pm$ 0.02	--
91-98	0.09 $\pm$ 0.03	46.9	0.31 $\pm$ 0.06	60.5
98-105	0.06 $\pm$ 0.02	--	0.11 $\pm$ 0.04	
105-112	0.07 $\pm$ 0.02	45.9	0.12 $\pm$ 0.02	58.9
112-119	0.06 $\pm$ 0.02	--	0.13 $\pm$ 0.01	--
119-126	0.06 $\pm$ 0.02	45.0	0.11 $\pm$ 0.02	57.2
126-133	0.07 $\pm$ 0.01	--	0.15 $\pm$ 0.02	--
133-140	0.04 $\pm$ 0.01	44.3	0.07 $\pm$ 0.02	55.7
161-182	0.04 $\pm$ 0.01	--	0.08 $\pm$ 0.01	--
	(2 monkeys)			
224-231	0.017			
280-287	0.018			
336-343	0.014			
392-399	0.022			
462-469	0.018			
525-532	0.012			
580-588	0.010			



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Fig. 3. Excretion rate of Sr<sup>90</sup> and Ca<sup>45</sup> in adolescent rhesus monkeys after a single parenteral injection. Standard deviations of the mean values for groups of three or more monkeys.

size of the long-term turnover compartment is highly sensitive to the age at injection, and is still in doubt for both age groups. The excretion curve and the retention curve derived from it that give the best agreement with Sr<sup>90</sup> retention calculated from total excretion collections to 140 days, for monkeys in the 28- to 40-month age group, are

$$E^a = 34.8\%/d e^{-0.693t/0.55} + 2.24\%/d e^{-0.693t/5.3} + 0.15\%/d e^{-0.693t/58} \\ + 0.025\%/d e^{-0.693t/1080},$$

$$R_e^a = \int_0^{\pm} Edt,$$

$$= 100\% - (28.6\%e^{-1.26t} + 17.2\%e^{-0.13t} + 12.6\%e^{-0.0126t} \\ + 41.6\%e^{-6.4 \times 10^{-4}t}).$$

Sr<sup>90</sup> retention was measured in the skeletal parts of one adolescent female monkey sacrificed 94 days after injection. The retention calculated from the above curve was 42.9%, compared with the measured retention of 43.2%.<sup>4</sup>

The slopes of the components of the above equations agree with those of an equation published previously for a group of monkeys of mixed ages,<sup>5</sup> both adults and adolescents; however, the amounts of Sr<sup>90</sup> present in the fastest- and slowest-turnover compartments differ. In the adult monkey the rapid-turnover compartment accounts for about 50%, and the slowest turnover compartment contains less than 25% of the injected Sr<sup>90</sup>. These findings are in general accord with those of Speckman and Norris<sup>6</sup> on the age dependence of Sr<sup>89</sup> retention in the male rat.

It is too early to establish a slope for the long-term component of the Ca<sup>45</sup> excretion curve, but a slope similar to that for Sr<sup>90</sup> may be fitted to the Ca<sup>45</sup> data as they now stand. Similar long-term turnover rates for Ca<sup>45</sup> and Sr<sup>90</sup> have been observed in the rat, and these data appear elsewhere in this report.

<sup>4</sup> Durbin, Parrott, Williams, et al., Metabolic Studies with Strontium-90 in the Rhesus Monkey (preliminary report) in The Shorter-Term Biological Hazards of a Fallout Field, a Symposium, G. M. Dunning and J. A. Hilcken, Eds., Washington D. C., Dec. 1956, pp. 173-182.

<sup>5</sup> P. W. Durbin and H. B. Jones, Estimation of the Turnover Equation of Strontium-90 for Human Bones, Second International Conference on the Peaceful Uses of Atomic Energy, Geneva, Switzerland (United Nations, New York, 1958) (in press).

<sup>6</sup> T. W. Speckman and W. P. Norris, The Retention of Strontium-89 in Rats as a Function of Animal Age at Injection, in Medical Research Division Quarterly Report, ANL-5597, pp. 77-78, July 1956.

During the first 70 days, when the Sr<sup>90</sup> excretion rate is declining rapidly, excretion data for the adolescent monkey can be fitted to a power function. After the 70th day the excretion rate deviates markedly from a power function. Sufficient time has not passed yet to tell whether this deviation is a second-power function of more positive slope or a region of curvature as indicated by Ward.<sup>7</sup>

The Ca<sup>45</sup> excretion data cannot be fitted to a power function during any appreciable interval up to 140 days postinjection. These data, however, can be represented as a series of exponentials, as shown in Fig. 3. The results thus far are too preliminary to establish similarities or differences of slope or of compartment size between the two isotopes.

#### RETENTION OF CALCIUM-45 AND STRONTIUM-90 IN THE ADULT FEMALE RAT

Tracer studies of Ca<sup>45</sup> and Sr<sup>90</sup> in rats reported previously from this laboratory have had some serious shortcomings: (a) measurements were not extended over a sufficiently long time to establish the slope of the longest-term component of the retention curve; (b) skeletal retention was measured at only a few widely spaced intervals, making the precision shape of the curve uncertain; and (c) balance studies were not performed, making analysis of errors difficult. The experiments reported herein were designed to overcome some of these objections. Female rats 110 days old were chosen as test animals because they were still young enough to allow at least 16 months of study before pulmonary disease and mammary tumors seriously reduced their number. Although the bones of female rats of this age are still growing to some extent, growth is proceeding at a relatively slow rate.

Sixty animals were given 0.5  $\mu$ C of Sr<sup>90</sup> in the Sr<sup>90</sup>-Y<sup>90</sup> equilibrium mixture, and varying amounts of high-specific-activity Ca<sup>45</sup>. The rats scheduled for sacrifice within 2 months after injection (1-, 4-, 18-, 35-, and 68-day groups) received 2  $\mu$ C Ca<sup>45</sup>; those held from 4 to 8 months received 4  $\mu$ C Ca<sup>45</sup>; and 18 long-term animals received 10  $\mu$ C Ca<sup>45</sup>. Both isotopes were diluted in neutral isotonic sodium citrate and injected intramuscularly. Immediately after injection the 42 rats in the 1- to 263-day groups were placed in metabolism cages in groups of three. Complete collections of separated urine and feces were made until the 130th day; pooled excreta were collected thereafter until all these groups were sacrificed. On the 35th day the long-term animals were placed in metabolism cages in groups of three for complete excreta collections until the 130th day. Because of previous difficulties in keeping rats in open-mesh metabolism cages for long periods of time without significant losses due to pneumonia, the long-term animals were transferred to stock cages at this time. Subsequently, they have been returned to the metabolism cages for a 1-week collection period each month. These animals are weighed twice each month, and any animal losing 20 g or more in any 2-week period is given an injection of

<sup>7</sup> A. H. Ward, Comparison of Excretion and Retention of Sr<sup>90</sup> in Monkeys and Radium in Man, Am. J. Roentgenol., Radium Therapy, Nuclear Med. 79, 530-531 (1958).

60,000 units of penicillin. Aureomycin is also given in the drinking water two days a week to control pulmonary disease. The excreta-collection schedules and the number of independent measurements of excretion rate are shown in Table IV.

Animals were sacrificed in groups of six except for three 135-day rats, which were autopsied at 68 days to provide a check on  $\text{Ca}^{45}$  excretion measurements. The very high initial  $\text{Sr}^{90}$  excretion rate and the low  $\text{Ca}^{45}$  dose in the short-term animals made  $\text{Ca}^{45}$  counting in the early urine and fecal specimens uncertain until a balance study could be made with a group that had received a larger amount of  $\text{Ca}^{45}$ .

At autopsy complete blood counts were taken and marrow smears were prepared. Both femurs, one tibia and fibula, and the cranial vault were dissected and weighed. The femur lengths were measured, and one was fixed for histological examination. The remaining bone specimens were ashed and assayed separately. The gastrointestinal tract was dissected as a unit and added to the last excretion collection. The incisor teeth were removed from the remains of the carcass for separate assay after ashing, and the soft tissue ash was washed from the carcass with water.

Radioactive assay techniques for the two isotopes were the same as described in the preceding section for monkey material. Because of the differences in diet of the two species, the  $\text{K}^{40}$  content of excreta ash was redetermined for the rat. The curve is shown in Fig. 4.

The time course of retention and the change of excretion rate of  $\text{Ca}^{45}$  and  $\text{Sr}^{90}$  in the same animals are shown in Table IV. The excretion curves obtained by plotting these data on a semilogarithmic scale are shown in Figs. 5 and 6 for  $\text{Ca}^{45}$  and  $\text{Sr}^{90}$  respectively. The equations of these curves are

$$E_{\text{Ca}} = 16\%/\text{d} e^{-0.693t/1.2} + 0.5\%/\text{d} e^{-0.693t/25} + 0.068\%/\text{d} e^{-0.693t/590},$$

and

$$E_{\text{Sr}} = 47\%/\text{d} e^{-0.693t/0.8} + 0.46\%/\text{d} e^{-0.693t/24} + 0.036\%/\text{d} e^{-0.693t/530}.$$

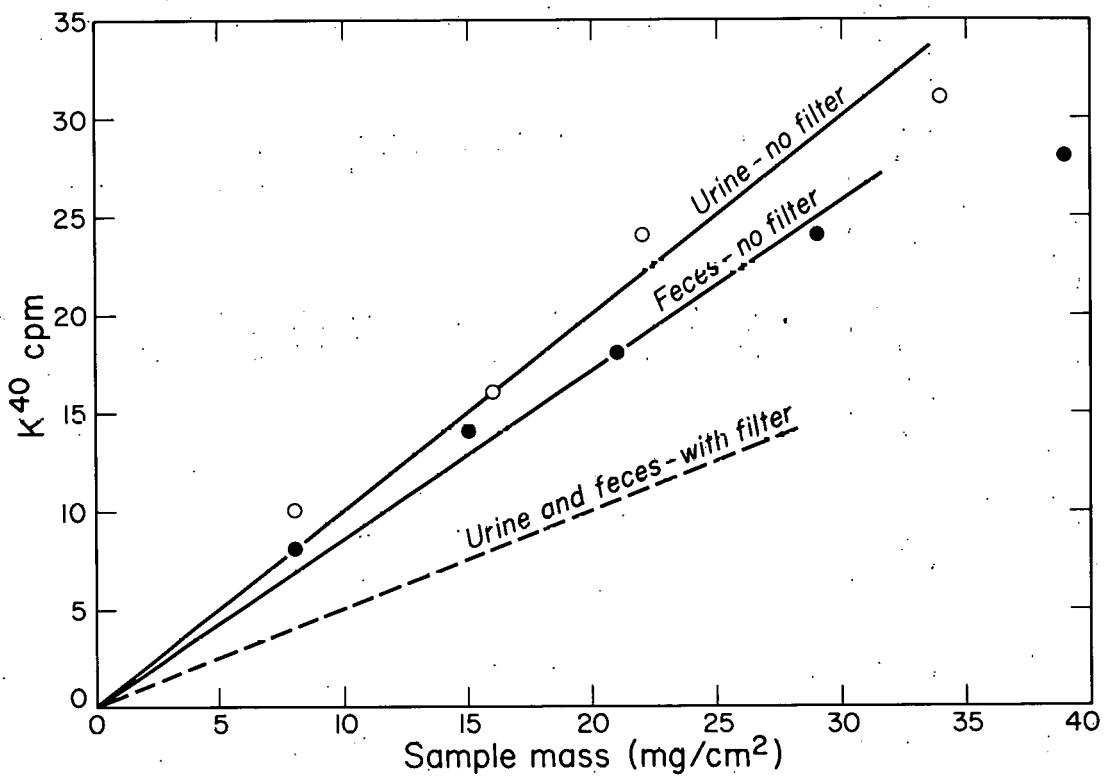
The slight deviation of the experimental points in each of these curves from the smooth curves shown during the second month is the result of the loss by attrition of those portions of the incisor teeth that were laid down shortly after injection, when the blood levels of  $\text{Ca}^{45}$  and  $\text{Sr}^{90}$  were still high. This "ground away" tooth substance appears exclusively in the feces. The amount of injected isotope represented by the area between the smooth curve and the experimental points during this interval is very close to the measured percent of injected dose in the incisors 1 to 4 days after injection.

During the first 130 days after injection, when urine and feces were being collected separately, the fecal-to-urinary ratio for  $\text{Ca}^{45}$  was 5 to 6 (except during the second month, when the labeled portions of the incisors

Table IV

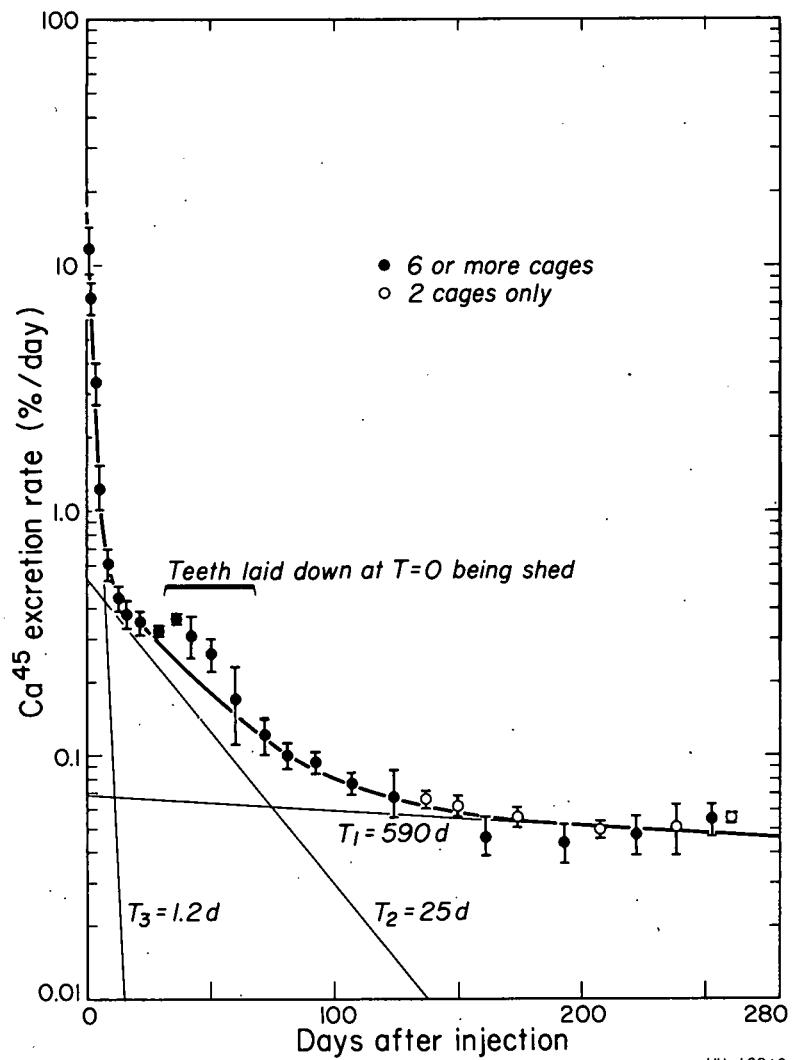
Retention of  $\text{Ca}^{45}$  and  $\text{Sr}^{90}$  by the adult female Sprague-Dawley rat. Each rat received  $0.5 \mu\text{C}$   $\text{Sr}^{90}$  and from 2 to  $10 \mu\text{C}$   $\text{Ca}^{45}$ . Data are expressed in percent of administered dose of each isotope corrected for deviation of recovery from 100%.

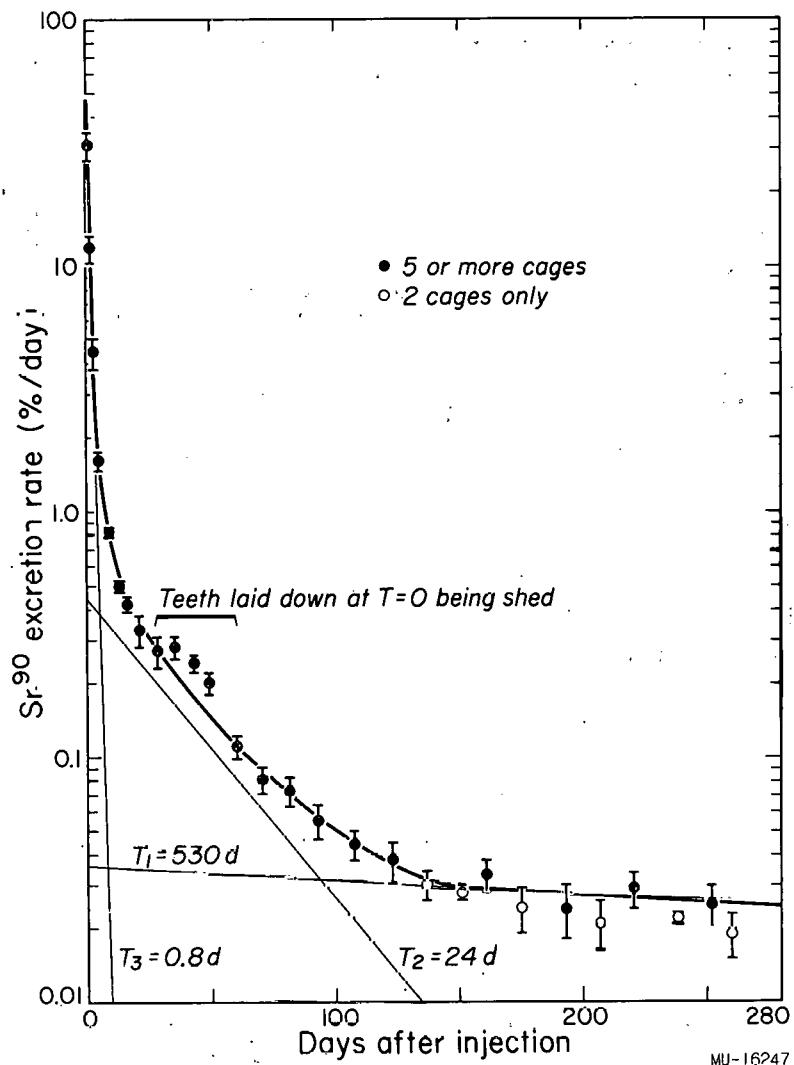
Days	Ca <sup>45</sup>				Sr <sup>90</sup>			
	No. cages	% excreted	% retained	Excretion rate (%/day $\pm$ S. D.)	No. cages	% excreted	% retained	Excretion rate (%/day $\pm$ S. D.)
0-1	14	11.8	88.2	11.8 $\pm$ 2.6	14	30.7	69.3	30.7 $\pm$ 4
1-2	12	7.41	80.8	7.41 $\pm$ 1.13	12	11.7	57.6	11.7 $\pm$ 1.37
2-4	12	6.71	74.1	3.36 $\pm$ 0.65	12	8.78	48.8	4.39 $\pm$ 0.66
4-7	8	3.78	70.3	1.26 $\pm$ 0.26	8	4.82	44.0	1.61 $\pm$ 0.14
7-11	8	2.44	67.9	0.61 $\pm$ 0.09	8	3.20	40.8	0.83 $\pm$ 0.04
11-14	8	1.33	66.5	0.44 $\pm$ 0.05	8	1.51	39.3	0.50 $\pm$ 0.02
14-18	8	1.53	65.0	0.38 $\pm$ 0.05	8	1.70	37.6	0.42 $\pm$ 0.03
18-25	8	2.47	62.5	0.35 $\pm$ 0.04	8	2.32	35.3	0.33 $\pm$ 0.05
25-32	8	2.24	60.3	0.32 $\pm$ 0.02	8	1.91	33.4	0.27 $\pm$ 0.04
32-39	6	2.49	57.8	0.36 $\pm$ 0.02	6	1.95	31.4	0.28 $\pm$ 0.03
39-46	12	2.15	55.6	0.31 $\pm$ 0.06	11	1.67	29.7	0.24 $\pm$ 0.02
46-53	12	1.86	53.8	0.26 $\pm$ 0.04	11	1.41	28.3	0.20 $\pm$ 0.02
53-67	12	2.40	51.4	0.17 $\pm$ 0.06	11	1.56	26.8	0.11 $\pm$ 0.011
67-74	8	0.84	50.6	0.12 $\pm$ 0.02	7	0.57	26.2	0.081 $\pm$ 0.011
74-88	9	1.40	49.2	0.10 $\pm$ 0.012	8	1.02	25.2	0.073 $\pm$ 0.011
88-98	9	0.96	48.2	0.094 $\pm$ 0.010	8	0.54	24.7	0.055 $\pm$ 0.009
98-116	9	1.37	46.8	0.077 $\pm$ 0.008	8	0.79	23.9	0.044 $\pm$ 0.006
116-130	9	0.94	45.9	0.067 $\pm$ 0.011	8	0.53	23.3	0.038 $\pm$ 0.007
130-144	2	0.96	44.9	0.066 $\pm$ 0.005	2	0.42	22.9	0.030 $\pm$ 0.004
144-158	2	0.86	44.0	0.062 $\pm$ 0.006	2	0.39	22.5	0.028 $\pm$ 0.001
158-165	8	0.32		0.046 $\pm$ 0.007	7	0.23		0.033 $\pm$ 0.005
165-186	2	1.22	42.5	0.056 $\pm$ 0.005	2	0.51	21.8	0.024 $\pm$ 0.005
186-200	8	0.62		0.044 $\pm$ 0.008	7	0.34		0.024 $\pm$ 0.006
200-214	2	0.69	41.2	0.050 $\pm$ 0.004	2	0.28	21.2	0.021 $\pm$ 0.005
214-228	8	0.68		0.048 $\pm$ 0.009	7	0.40		0.029 $\pm$ 0.005
228-249	2	1.08		0.051 $\pm$ 0.012	2	0.48		0.022 $\pm$ 0.001
249-256	7	0.77	38.8	0.055 $\pm$ 0.008	5	0.17	20.0	0.025 $\pm$ 0.005
256-263	2	0.38		0.053 $\pm$ 0.001	2	0.135		0.019 $\pm$ 0.004



MU-16245

Fig. 4. Beta-particle activity of  $K^{40}$  in unprocessed ash of rat urine and feces as a function of sample mass.





were being worn away, and the F:U ratio rose to 7.5). The fecal-to-urinary ratio of Sr<sup>90</sup> averaged 1.2 (rising to 2.6 during the loss of the labeled part of the incisors).

The slopes of the three components of the elimination curves are the same for both Ca<sup>45</sup> and Sr<sup>90</sup> within the errors of the experiment, providing further evidence of similar handling of these two elements once they reach the bony tissue. The differences in magnitude of the skeletal turnover compartments and the differences in fecal-to-urinary ratios can be accounted for by the more conservative treatment accorded calcium by the kidney<sup>8</sup> and by the gastrointestinal tract.<sup>9</sup>

The curves of Ca<sup>45</sup> and Sr<sup>90</sup> retention calculated by difference from the summed excretion collections are shown in Figs. 7 and 8. The retentions of these two isotopes measured in the serially sacrificed animals are shown with the curves. Except for one long-term point of measured retention for each isotope, there is good agreement between the measured retentions of the serially sacrificed animals and the retentions calculated by summing excretion over as many as 14 cage groups, agree very well. The equations of the retention curves for the two radioelements in 110-day-old female rats are

$$R_{Ca} = 26.5\% e^{-0.77t} + 18.5\% e^{-0.028t} + 55\% e^{-0.0013t},$$

and

$$R_{Sr} = 54\% e^{-0.77t} + 19.5\% e^{-0.03t} + 26.4\% e^{-0.0011t}.$$

As expected, these equations agree within experimental error with those obtained by integration of the excretion curves. Speckman and Norris measured the retention of Sr<sup>89</sup> in male rats of various ages by whole-body counting.<sup>6</sup> Their measurements ceased at 200 days postinjection because of the short half life of the Sr<sup>89</sup> isotope. The mean slope of the last portions of these retention curves (from 120 to 200 days) was  $T_b = 610 \pm 50$  days for males 40 to 440 days old. This slope is very similar to what we obtain ( $560 \pm 30$  days) from excretion measurements of 110-day-old females. The similarity in the slopes of the long-term retention component for Ca<sup>45</sup> and Sr<sup>90</sup> in mature female rats and Sr<sup>89</sup> in male rats over a broad span of ages suggests that this very slow rate process is independent of the state of activity of bone growth and gross bone turnover.

The distributions of Ca<sup>45</sup> and Sr<sup>90</sup> in the long bones, cranial vault, and incisors are shown in Tables V and VI. The half times of the retention components of these bones are presented in Table VII. The half times of the

<sup>8</sup> MacDonald, Noyes, and Lorick, Discrimination of Calcium and Strontium by the Kidney, *Am. J. Physiol.* 188, 131-136 (1957).

<sup>9</sup> Wasserman, Comar, and Nold, Influence of Amino Acids and other Organic Compounds on Gastrointestinal Absorption of Radiocalcium and Radiostrontium, *Fed. Proc.* 15, 1881 (1956), Abstract.

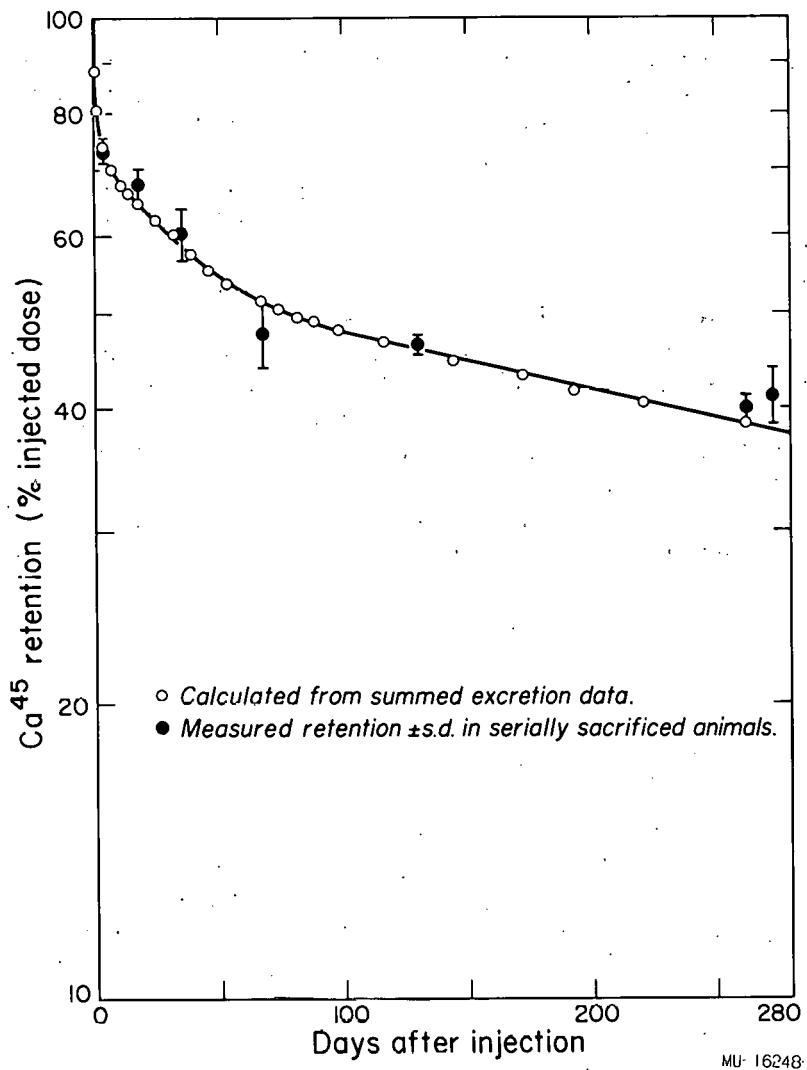


Fig. 7. Total retention of  $\text{Ca}^{45}$  by the 110-day-old female rat calculated from summed excretion measurements. Retention measured in serially sacrificed animals is shown with standard deviation.

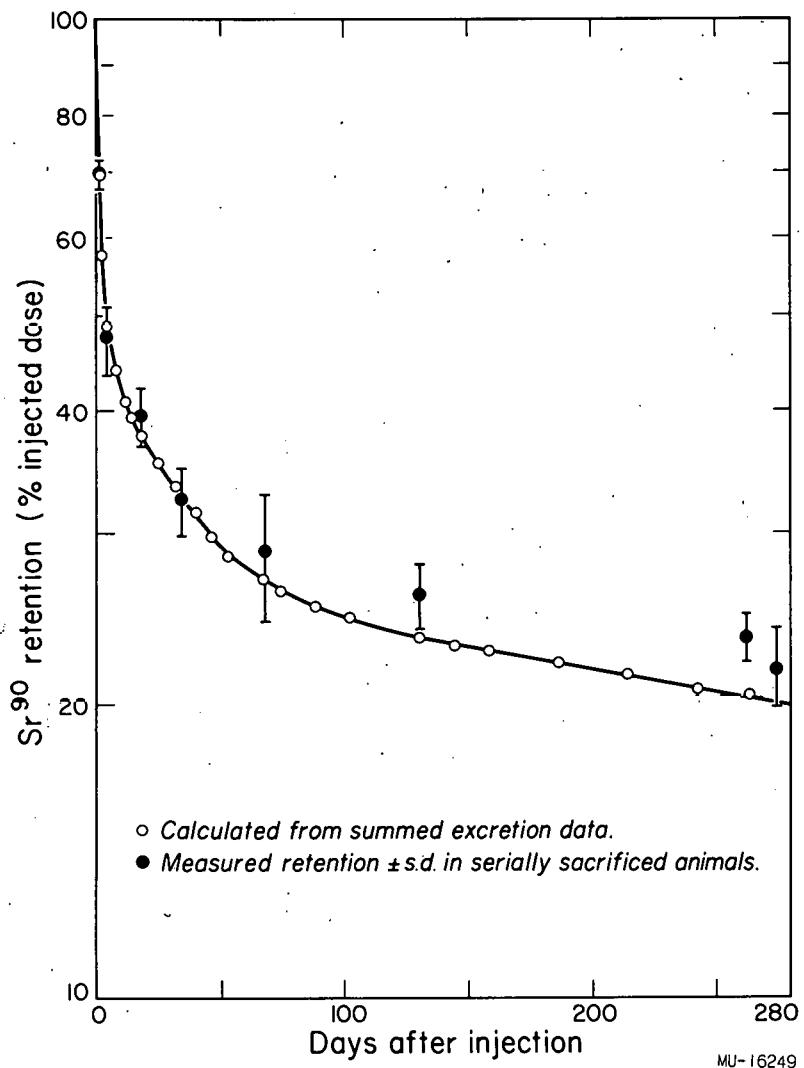


Fig. 8. Total retention of Sr<sup>90</sup> by the 110-day-old female rat calculated from summed excretion measurements. Retention measured in serially sacrificed animals is shown with standard deviation.

Table V

Distribution of  $\text{Ca}^{45}$  in representative skeletal parts of the adult female Sprague-Dawley rat. Values are expressed in percent of administered dose corrected for deviation of recovery from 100%. Means are shown  $\pm$  standard deviation. Rats 1, 4, 18, 35, and 68 days old received 2  $\mu\text{C}$   $\text{Ca}^{45}$ , and 128- and 256-day rats received 4  $\mu\text{C}$   $\text{Ca}^{45}$  in a single intramuscular injection.

	Femur		Tibia-Fibula		Cranial vault		Incisors		Skeleton		Bal-	Total	Avg.
	% dose	%/g	% dose	%/g	%/g	% dose	%/g	% dose	% dose	% dose	ance	retention	recovery
1 day (6 rats)	2.6 $\pm$ .2	2.8 $\pm$ .2	2.6 $\pm$ .1	4.0 $\pm$ .5	2.6 $\pm$ .1	2.5 $\pm$ .3	4.4 $\pm$ .6	81.2	0.93	84.6 $\pm$ 1.2		92.9	
4 days (6 rats)	2.8 $\pm$ .3	3.0 $\pm$ .3	2.5 $\pm$ .1	4.2 $\pm$ .4	2.9 $\pm$ .4	4.4 $\pm$ .5	7.6 $\pm$ .9	68.5	0.34	73.2 $\pm$ 2.1		97.4	
18 days (6 rats)	2.3 $\pm$ .2	2.3 $\pm$ .2	1.9 $\pm$ .1	2.7 $\pm$ .3	2.8 $\pm$ .5	4.8 $\pm$ .3	8.1 $\pm$ .3	63.1	< 0.1	67.9 $\pm$ 2.3		100.5	
35 days (6 rats)	2.2 $\pm$ .3	2.0 $\pm$ .4	1.6 $\pm$ .1	2.7 $\pm$ .2	3.1 $\pm$ .4	3.6 $\pm$ .5	5.7 $\pm$ .8	56.8	< 0.1	60.4 $\pm$ 3.6		105.4	
68 days (9 rats)	2.1 $\pm$ .4	2.0 $\pm$ .4	1.5 $\pm$ .3	2.1 $\pm$ .4	2.9 $\pm$ .4	8.1 $\pm$ .11	1.1 $\pm$ .1	46.9	< 0.1	47.7 $\pm$ 3.7		96.7	
130 days (3 rats)	1.8 $\pm$ .2	1.9 $\pm$ .1	1.1 $\pm$ .1	1.8 $\pm$ .2	2.4 $\pm$ .1	.36 $\pm$ .09	.49 $\pm$ .11	46.1	< 0.1	46.5 $\pm$ 1.1		104.2	
263 days (6 rats)	1.6 $\pm$ .1	1.4 $\pm$ .2	.98 $\pm$ .1	1.3 $\pm$ .1	1.7 $\pm$ .1	.22 $\pm$ .02	.27 $\pm$ .10	39.6	-	39.8 $\pm$ 1.4		107.8	
273 days <sup>a</sup> (3 rats)										41.3 $\pm$ 2.7			

<sup>a</sup>Skeletal measurements were made on three long-term rats that died of pneumonia.

Table VI

Distribution of Sr<sup>90</sup> in representative skeletal parts of the adult female Sprague-Dawley rat. Values are expressed in percent of administered dose corrected for deviation of recovery from 100%. Means are shown  $\pm$  standard deviation. Each rat received 0.5  $\mu$ C Sr<sup>90</sup> in a single intramuscular injection.

	Femur		Tibia-fibula		Cranial vault	Incisors		Skeleton	Balance	Total retention	Avg. recovery
	% dose	%/g	% dose	%/g		% dose	%/g				
1 day (6 rats)	2.3 ± .1	2.7 ± .2	2.0 ± .1	3.2 ± .3	2.4 ± .2	2.2 ± .3	3.7 ± .5	59.3	0.49	62.0 ± 2.5	104.2
4 days (6 rats)	1.8 ± .1	2.0 ± .2	1.6 ± .1	2.7 ± .1	2.0 ± .3	2.7 ± .1	4.6 ± .1	44.2	0.31	47.2 ± 3.8	105.1
18 days (6 rats)	1.4 ± .1	1.4 ± .1	1.2 ± .1	1.8 ± .2	1.7 ± .2	2.9 ± .3	4.9 ± .3	36.4	0.10	39.4 ± 2.8	104.9
35 days (6 rats)	1.2 ± .2	1.2 ± .2	.89 ± .09	1.4 ± .2	1.7 ± .2	2.0 ± .2	3.0 ± .3	30.2	0.10	32.3 ± 2.6	103.4
68 days (9 rats)	1.2 ± .2	1.1 ± .2	0.8 ± .11	1.2 ± .2	1.6 ± .2	.23 ± .1	.36 ± .13	28.4	< .10	28.6 ± 4.2	101.7
130 days (3 rats)	1.0 ± .2	.98 ± .03	.62 ± .06	.98 ± .09	1.4 ± .1	.07 ± .02	.09 ± .03	25.7	< .10	25.8 ± 2.0	103.5
263 days (6 rats)	.95 ± .06	.83 ± .06	.61 ± .07	.77 ± .06	1.1 ± .1	.05 ± .02	.06 ± .03	23.5	--	23.5 ± 1.3	100.6
273 days <sup>a</sup> (2 rats)										21.6 ± 2.4	---

<sup>a</sup>Skeletal measurements were made on three long-term rats that died of pneumonia; one inadvertently did not receive a Sr<sup>90</sup> injection.

Table VII

Turnover compartments and half times of  $\text{Ca}^{45}$  and  $\text{Sr}^{90}$  in the long bones and cranial vault of the 110-day-old female rat.

Sample		$A_1\%$	$T_1$ (days)	$A_2\%$	$T_2$ (days)	$A_3\%$	$T_3$ (days)
Femur	$\text{Ca}^{45}$	0.1%	1	0.52%	10	2.2%	550
	$\text{Sr}^{90}$	0.3%	1	0.75%	10	1.25%	580
Femur specific activity	$\text{Ca}^{45}$	--	-	0.75%/g	7	2.3%/g	400
	$\text{Sr}^{90}$	0.6%/g	1	1.0%/g	8	1.25%/g	410
Tibia and fibula	$\text{Ca}^{45}$	--	-	1.4%	16	1.3%	610
	$\text{Sr}^{90}$	0.4%	2	1.0%	14	0.77%	610
Tibia and fibula specific activity	$\text{Ca}^{45}$	0.75%/g	4	1.8%/g	17	2.1%/g	440
	$\text{Sr}^{90}$	1.0%/g	2	1.2%/g	18	1.17%/g	460
Cranial vault specific activity	$\text{Ca}^{45}$	--	-	--	-	2.9%/g	460
	$\text{Sr}^{90}$	0.5%/g	3	--	-	1.7%/g	520

$$R = A_1 e^{-0.693t/T_1} + A_2 e^{-0.693t/T_2} + A_3 e^{-0.693t/T_3}$$

longest-term components of retention of both radioelements in the separate bones were similar to those of the skeleton as a whole. The slopes of the longest-term component of the curves of specific activity vs time were steeper (half times were shorter) for both radioelements, as was expected from the continued slow growth of the long bones (the femur increased 4.4% in length from the 68th to the 263rd day postinjection).

There were not enough measurements during the first few weeks after injection to establish precisely the shapes of the retention curves of these bones; some general conclusions, however, can be drawn from the information at hand. The retention curves and specific-activity curves (percent dose per gram wet weight) for both  $\text{Ca}^{45}$  and  $\text{Sr}^{90}$  in the long bones possess at least two and probably three components:

- (a) a component with a half time of the order of 1 day,
- (b) a component with a half time varying between 7 and 18 days,
- (c) and a component with a half time similar to that of the skeleton as a whole--about 600 days.

It is tentatively suggested that these components represent

- (a) rapid physicochemical exchange at bony surfaces ( $T_b = 1$  to 2 days),
- (b) gross mineral turnover due to growth and remodeling ( $T_B = 7$  to 18 days),
- (c) and a very slow process resembling intracrystalline diffusion and exchange, as postulated by Norris et al. ( $T_B > 400$  days).<sup>10</sup>

The retention curves for the specific activity of the cranial vault possessed one long-term component similar to that found for the long bones and possibly a very short-lived component, but no component of intermediate half time attributable to bone growth. The absence of a growth component is not surprising because growth of the cranial bones is essentially complete by the 110th day of life, when the animals were injected.<sup>11</sup>

Even very small amounts of long-lived radioactive bone seekers may have profound pathological effects on the bones of rodents. The incidence of bone tumors is increased,<sup>12</sup> and bone growth is markedly altered.<sup>13</sup> It was, therefore, of interest to ascertain whether the radiation accumulated in the bones of the long-term animals might be so altering them as to render these experiments invalid as "tracer studies." Several of the blood variables were measured in a search for possible changes in the bone marrow, and femur lengths were recorded to discover if there had been any effect on bone growth. The hematological data are shown in Table VIII, and the bone growth measurements are shown in Fig. 9. Eight months after administration of 4  $\mu\text{C}$  of  $\text{Ca}^{45}$  and 0.5  $\mu\text{C}$  of  $\text{Sr}^{90}$  there were no detectable differences between

<sup>10</sup> Norris, Tyler, and Brues, Retention of Radioactive Bone Seekers, *Science* 128, 456-462 (1958).

<sup>11</sup> Henry R. Frank, A Roentgenographic Study of Skull Development in Normal and Hypophysectomized Rats (Master's Thesis), University of California, 1953.

<sup>12</sup> M. P. Finkel, Relative Biological Effectiveness of Internal Emitters, *Radiology* 67, 665-672 (1956).

<sup>13</sup> Durbin, Asling, Jeung, et al., The Metabolism and Toxicity of Radium-223 in Rats, UCRL-8189, Feb. 1958.

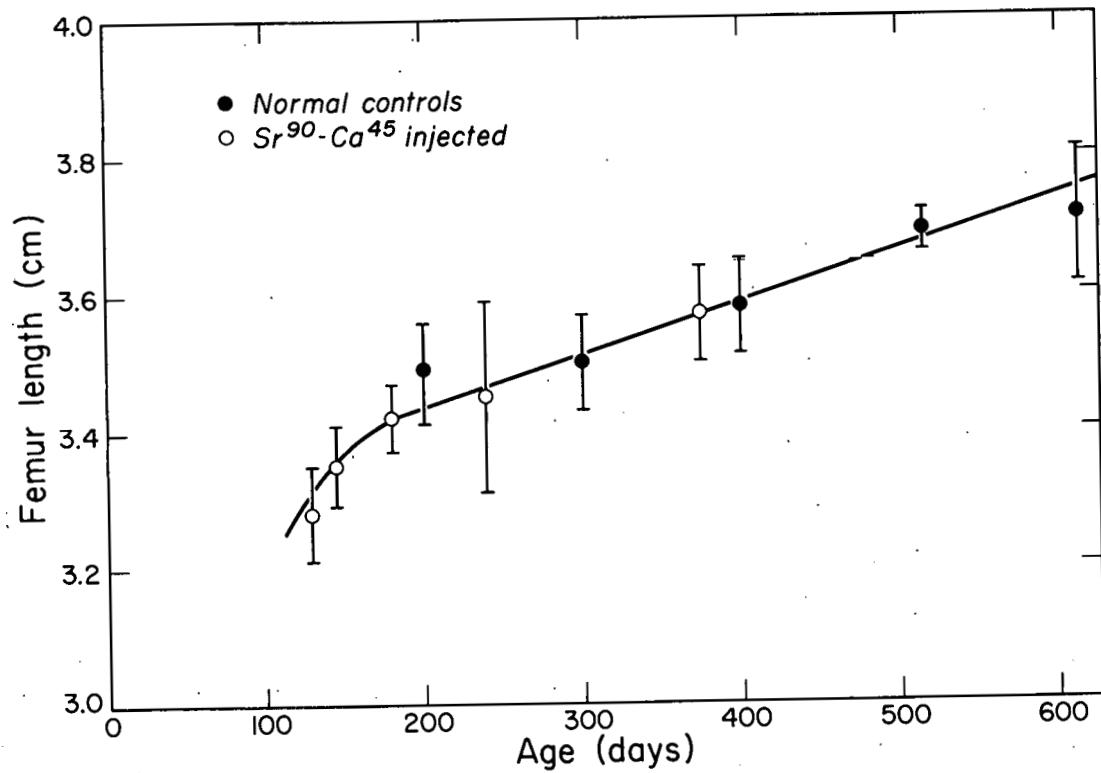
Table VIII

Comparison of some blood variables in normal control and  
 $\text{Sr}^{90}\text{-Ca}^{45}$ -injected rats

	200-day-old control	$\text{Sr}^{90}\text{-Ca}^{45}$ 180 days old (78-day group)	300-day-old control	$\text{Sr}^{90}\text{-Ca}^{45}$ 373 days old (263-day group)
Hematocrit ccRBC/cc blood	46.4	44.5	46.8	47.8
Hemoglobin g/100 cc	14.1	14.0	14.5	14.4
White cells/ $\text{mm}^3$ ( $\times 10^3$ )	10.96	9.57	8.03	11.28
% Lymphocytes	84.6	88.3	80.9	85.8

the hemograms and bone-growth patterns of injected and control rats. Radiation dosages to the skeleton were calculated from the  $\text{Ca}^{45}$  and  $\text{Sr}^{90}$  retention curves, on the assumption of a uniform isotope distribution and an average skeletal weight of 27 g. By the 68th day 2  $\mu\text{C}$  of  $\text{Ca}^{45}$  had delivered 11 rad, and 0.5  $\mu\text{C}$   $\text{Sr}^{90}$ , 33.4 rad. By the 263rd day the accumulated radiation dosage from 4  $\mu\text{C}$  of  $\text{Ca}^{45}$  was 48.7 rad, and from 0.5  $\mu\text{C}$  of  $\text{Sr}^{90}$ , 59.6 rad.

Three more rats were sacrificed 381 days postinjection, and nine injected animals remain. These will be followed individually with 1-week excreta samples each month for  $\text{Ca}^{45}$  as long as it can be detected, and to the end of the experiment for  $\text{Sr}^{90}$ . The remaining animals will be sacrificed in groups of three every 4 months.



MU-16250

Fig. 9. Growth of the femur of the female rat.

### THE INDUCTION OF MAMMARY TUMORS IN THE SPRAGUE-DAWLEY RAT

The initial observations on mammary tumor incidence in  $At^{211}$ -injected female Sprague-Dawley rats have been reported.<sup>14</sup> Several new groups of rats have been added to the experiment to explore this phenomenon further. Some of the unresolved questions are: (a) How do these results with  $At^{211}$  compare with those obtained by the Brookhaven Laboratory group using penetrating radiation? (b) What is the life-span incidence of mammary tumors in  $At^{211}$ -irradiated rats, and how does it compare with the life-span incidence in normal controls? (c) Is the  $At^{211}$  effect dose-dependent? (d) What are the relationships between the degree of hypothyroidism ( $At^{211}$  produces marked irreparable damage in the thyroid gland), the percentage of tumors induced, and the proportion of these that are malignant? and (e) What is the influence of ovarian hormones on tumor induction? Is the number of tumors reduced in both irradiated and normal rats following ovariectomy, and can it be increased in normal and irradiated intact rats or restored in ovariectomized rats by exogenous estrogen?

(a) In order to compare our results directly with those obtained by Shellabarger et al.<sup>15</sup> and Cronkite et al.<sup>16</sup> using penetrating radiation, and to conserve  $At^{211}$  which can be made only a few millicuries at a time, the 55-day-old age point has been abandoned in favor of younger, lighter animals. The Brookhaven group found mammary tumors in 79% of a group of female Sprague-Dawley rats during the 11 months following a single exposure to 250 kvp x-ray or  $Co^{60}$   $\gamma$ -ray when the rats were 43 days of age. For purposes of direct comparison thirty 43-day-old female rats of the same strain were given 0.5  $\mu$ C/g of  $At^{211}$ , and by the end of the 11th month 63% bore mammary tumors.

(b) Our earlier experiments with  $At^{211}$ -irradiated rats were terminated 1 year postinjection, and their control group was too small to provide reliable statistical information on the proportion of malignant tumors. The  $At^{211}$ -injected rats now on hand will be followed until 100% have developed mammary tumors or have died from other causes. A group of 103 control animals has been established. Fifty rats have been held 600 days or more; eight (16%) have developed mammary tumors--all of which were first seen after the 460th day of life-- and 14 have died of pulmonary disease or unknown causes.

(c) To test the dependence of tumor induction on dose,  $At^{211}$  was injected at the following dosages: 0.095  $\mu$ C/g, 0.27  $\mu$ C/g, 0.5  $\mu$ C/g, 0.6  $\mu$ C/g, and 0.76  $\mu$ C/g. The number of tumors developed in each group is shown in Table IX. Tumor induction in the various groups as a function of time after

<sup>14</sup> Durbin, Asling, Johnston, et al., The Induction of Tumors in the Rat by Astatine-211, *Radiation Research* 9, 378-397 (1958).

<sup>15</sup> Shellabarger, Cronkite, Bond, et al., The Occurrence of Mammary Tumors in the Rat after Sublethal Whole-Body Irradiation, *ibid* 6, 501-512 (1957).

<sup>16</sup> Cronkite, Shellabarger, Bond, et al., Studies of the Mechanism of Induction of Radiation-Induced Breast Tumors in the Rat, *ibid* 7, 311 (1957) Abstract.

Table IX

Mammary tumor incidence in female Sprague-Dawley rats as a function of At<sup>211</sup> dosage. Injections were intramuscular when rats were 43 days old.

At <sup>211</sup> dosage	Control	Percent of mammary tumor bearers <sup>a</sup>				
		0.095 $\mu$ C/g	0.027 $\mu$ C/g	0.50 $\mu$ C/g	0.60 $\mu$ C/g	0.76 $\mu$ C/g
No. of rats	18	22	21	30	20	20
Days postinjection when tumor appeared						
0-60	-	-	-	-	5	-
60-90	-	4.5	4.8	6.6	5	-
90-120	-	4.5	4.8	10	10	-
100-150	-	4.5	19.0	23.2	10	5
150-180	-	9.1	28.5	26.6	10	5
180-210	-	13.6	42.8	b32.1**	15	10
210-240	-	13.6	50*	44.5*	20	10
240-270	-	23.8	55	52.0	25	25
270-300	-	28.6	65	58.5	30	38.8**
300-330	-	28.6	70	63.0	40	47

<sup>a</sup> Percentage adjusted for deaths due to pneumonia.

<sup>b</sup> Asterisks denote number of pneumonia deaths (nontumor bearers) during interval.

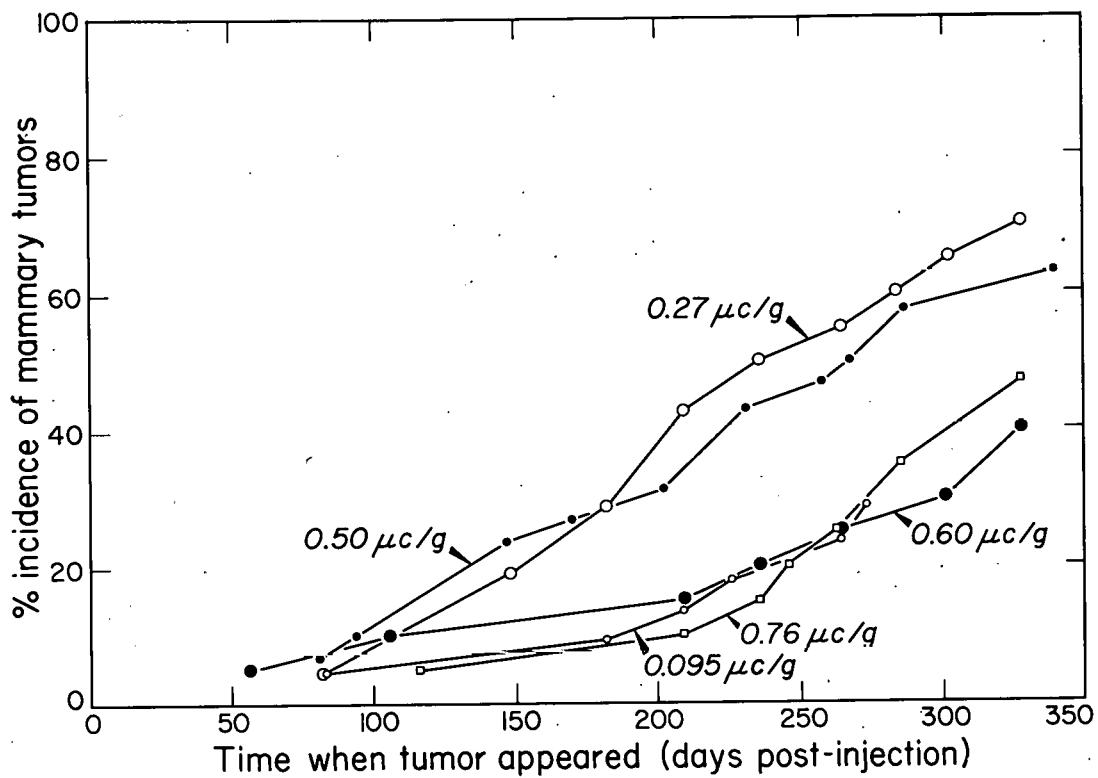
injection is shown in Fig. 10. From the time of appearance of the first tumor in this series of animals, 60 to 90 days after injection, until at least the 340th day, the  $0.27\text{-}\mu\text{C/g}$  and  $0.5\text{-}\mu\text{C/g}$  groups were developing tumors at the rate of 0.25% per day. The lowest-level group,  $0.095\text{ }\mu\text{C/g}$ , and the two highest-dosage groups,  $0.6\text{ }\mu\text{C/g}$  and  $0.76\text{ }\mu\text{C/g}$ , had a longer latent period of about 200 days before tumors began to appear at this same rate.

The incidence of tumors as a function of dose is shown in Fig. 11. Below  $0.27\text{ }\mu\text{C/g}$  tumor induction seemed to be dose-dependent, and a straight line through the first two points on the 340-day curve passes through a tumor incidence of 4% at zero dose. As the  $\text{At}^{211}$  dosage was increased the tumor incidence leveled off, and at the highest dosages it had fallen 30% below peak incidence. The relationships between irradiation of the developing mammary tissue, and the extent of  $\text{At}^{211}$  irradiation damage in the ovaries, are yet to be worked out. It is possible that the estrogen-producing interstitial tissue is impaired at the higher  $\text{At}^{211}$  dosages as the germinal tissue is at lower dosages, and the stimulus for mammary development and proliferation is reduced below that required for maximal tumor induction.

(d) Further attempts are being made to eliminate the complications of severe hypothyroidism by reducing the initial thyroid damage or by administration of exogenous thyroid hormone. Shellabarger et al.<sup>17</sup> found that the thyroidal uptake of  $\text{At}^{211}$  could be prevented to a large degree by prior administration of KSCN and, in addition, that the  $\text{At}^{211}$  accumulated by the thyroid gland was so loosely bound that much of it could be "washed out" by a subsequent single injection of KSCN. We are attempting to control thyroid damage due to  $\text{At}^{211}$  by giving KSCN before an injection of  $\text{At}^{211}$  and in a series of closely spaced injections for the first 24 hours thereafter. Some preliminary experiments with very young rats suggest that this mode of treatment will be reasonably successful in reducing the initial  $\text{At}^{211}$  thyroidal damage. The KSCN enhances urinary excretion of  $\text{At}^{211}$  somewhat, but not enough to change the whole-body radiation dosage by more than 10%. Three weeks after an  $\text{At}^{211}$  injection rats treated with KSCN had 24-hour thyroidal  $\text{I}^{131}$  uptakes of 50% of normal while  $\text{At}^{211}$ -injected rats that received KC1 had  $\text{I}^{131}$  uptakes that were less than 10% of normal. Other judgments of thyroid function are being made. These include endocrine gland weights, cytology, growth rate, and standard metabolic rates. A larger group of animals was recently treated with  $\text{At}^{211}$  and KSCN, but results are not available at the present time.

In the earliest experiments we attempted to control hypothyroidism by giving 1-thyroxine in the drinking water.<sup>12</sup> The concentration of thyroid hormone was apparently too low, and the results were equivocal. Fifteen additional rats have been given  $0.5\text{ }\mu\text{C/g}$  of  $\text{At}^{211}$  and are not receiving  $6\text{ }\mu\text{g}$  of 1-thyroxine by subcutaneous injection three times weekly. During the first 142 days after the  $\text{At}^{211}$  injection three rats have developed mammary tumors.

<sup>17</sup> Shellabarger, Durbin, Parrott, et al., Effects of Thyroxine and KSCN on Capacity of Rat Thyroid to Accumulate Astatine-211, Proc. Soc. Exptl. Biol. and Med. 87, 626-629 (1954).



MU-16251

Fig. 10. Incidence of mammary tumors in the female rat as a function of time after injection of various dosage of  $\text{At}^{211}$ .

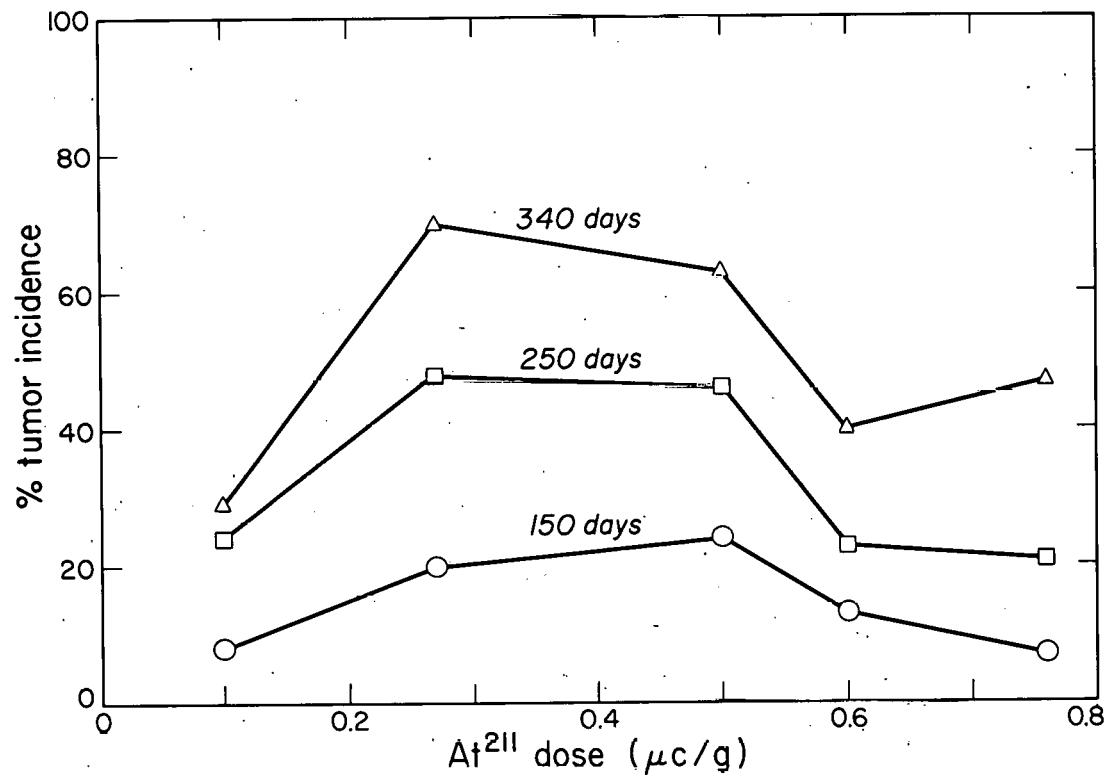


Fig. 11. Incidence of mammary tumors in the female rat as a function of  $\text{At}^{211}$  dose.

(e) Cronkite et al. observed that mature rats and ovariectomized rats develop fewer mammary tumors after a single exposure to x-ray than intact developing rats or ovariectomized rats subsequently given an ovarian implant.<sup>16</sup> A group of 110-day-old rats (sexually mature and nearly skeletally mature), and a group of 43-day-old ovariectomized rats were given 0.5  $\mu$ C/g of At<sup>211</sup>. No tumors have been seen in either group to date.

We are presently attempting to establish the dosage of exogenous estrogen and progesterone that will sustain normal uterine weight and normal mammary development in the Sprague-Dawley rat ovariectomized at 43 days of age.

#### PAPERS AND REPORTS

Work in this field has been reported during the period April-September 1958 as follows:

C. Willet Asling, Patricia W. Durbin, Muriel E. Johnston, and Marshall W. Parrott, Demonstration of the Concentration of Astatine-211 in the Mammary Tissue of the Rat (submitted to Endocrinology).

Patricia W. Durbin, C. Willet Asling, Muriel E. Johnston, Nyland Jeung, and Marilyn A. Williams, Early Effects of a Short-Lived Radium Isotope (Radium-223) on Bone Marrow, UCRL-8228 Abstract, April 1958. \*

Muriel E. Johnston and C. Willet Asling, Histophysiology of Calcium-45 Deposition in the Developing Skeletal Tissues of the Fetal Rat, UCRL-8229 Abstract, April 1958.

Muriel E. Johnston and Patricia W. Durbin, Autoradiography (submitted to Medical Physics).

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\* For International Congress of Radiation Research, Burlington, Vt., August 10-16, 1958.

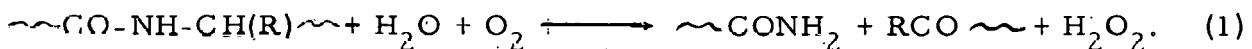
RADIATION CHEMISTRY

Warren M. Garrison in charge

Boyd M. Weeks, Michael E. Jayko, Winifred Bennett, Sibyl Cole,  
and Gordon Hughes

PRODUCTION OF AMIDE GROUPS (AND AMMONIA) IN  
THE RADIOLYSIS OF OXYGENATED PROTEIN SOLUTIONS

The indirect action of ionizing radiation on pepsin and gelatin in oxygenated solution has been shown to lead to the formation of high-molecular-weight products containing the carbonyl bond. It has also been found that conventional acid hydrolysis of the irradiated pepsin and gelatin solutions liberates a series of  $\alpha$ -keto acids. These observations are consistent with the proposal that a principal mechanism of indirect action involves cleavage of the peptide bond as represented by the over-all reaction<sup>1, 2</sup>



An inherent feature of this reaction is the prediction that carbonyl and amide groups should be produced in equal yield. Preliminary results of experiments now in progress substantiate this conclusion. A G value of  $\sim 1.1$  for formation of amide groups in the  $\text{Co}^{60}$   $\gamma$ -ray irradiation of oxygen-saturated gelatin solutions has been observed. This value is almost identical with the previously reported G for carbonyl production.<sup>2</sup> Ammonia is also formed with  $\text{G} \approx 0.3$ . The latter is assumed to be formed by oxidative deamination of terminal and (or) side-chain amino groups.<sup>3</sup>

Amide groups and free ammonia are determined in the following way: An aliquot of the target solution is made alkaline to phenolphthalein and distilled in a closed system (in vacuo) into a receiver containing a few ml of 0.1 N sulfuric acid. Nessler's reagent is used to determine ammonia recovered in the distillate. A second aliquot of the target solution is made 1 N in hydrochloric acid and hydrolyzed for 1 hr at  $95^\circ\text{C}$  to liberate ammonia from amide linkages.<sup>4</sup> Subsequent treatment is as described above. Gelatin having a low amide content was selected for these studies.<sup>5</sup> Solutions were dialyzed prior to irradiation to reduce the free-ammonia content to a minimum.

<sup>1</sup> M. E. Jayko and W. M. Garrison, Formation of  $>\text{C=O}$  Bonds in the Radiation-induced Oxidation of Protein in Aqueous Solution, Nature 181, 413 (1958).

<sup>2</sup> Garrison and group, in Biology and Medicine Semiannual Report, UCRL-8265, April 1958. p. 5.

<sup>3</sup> B. M. Weeks and W. M. Garrison, Radiolysis of Aqueous Solutions of Glycine, Radiation Research 9, 291 (1958).

<sup>4</sup> H. B. Vickery, Rate of Hydrolysis of Wheat Gliadin, J. Biol. Chem. 53, 495 (1922).

<sup>5</sup> Gelatin (purified calfskin), Batch 60-9680, Eastman Organic Chemicals.

Experimental conditions were adjusted so that amide and ammonia production by radiolysis was at least three times the background values. This involved a dose of  $\sim 10^{19}$  ev/ml for a 3.5% gelatin solution. Analyses on control and irradiated material are reproducible to within  $\pm 5\%$  for any particular stock solution.

#### PRODUCTION OF CARBONYL BONDS IN THE RADIOLYSIS OF OXYGENATED PROTEIN SOLUTIONS

The earlier studies on carbonyl production in the radiation-induced oxidation of protein were confined largely to the two materials pepsin and gelatin (see above). This work is being extended to include various other enzymes and proteins. Data on total carbonyl production in oxygenated solutions of chymotrypsin and yeast dehydrogenase have been obtained. The G for  $\text{C} = \text{O}$  production in  $\text{Co}^{60}$   $\gamma$ -ray-irradiated chymotrypsin solutions is approximately 1, as was found for pepsin under essentially identical conditions (concentration, 5 mg/ml; dose,  $10^{19}$  ev/ml). The corresponding value for the dehydrogenase system is, however, somewhat lower. This observation is consistent with the fact that the sulfhydryl content of dehydrogenase is much higher than that of any of the aforementioned enzymes and proteins. A protective action of the sulfhydryl derivative cysteine on carbonyl production in the radiation-induced oxidation of pepsin has been described.<sup>2</sup> Experiments are now under way to establish whether sulfhydryl oxidation and carbonyl production occur in parallel or in sequence in the radiolysis of such systems. This point is of particular significance from the radiation-biological standpoint.

In conjunction with these studies, efforts are being made to increase the accuracy of measurement of total carbonyl function associated with the high-molecular-weight oxidation products. A detailed description of the original method has been reported.<sup>1,2</sup> In brief, this procedure is as follows: Irradiated protein solutions are treated with 2,4-dinitrophenylhydrazine and then dialyzed to remove excess reagent and any products of low molecular weight. An appropriate aliquot of the dialyzed solution is then added to methanol-potassium hydroxide solution, and the hydrazone content is assayed spectrophotometrically with the assumption being made that the value of  $E_{\text{max}}$  is independent of the carbonyl structure.<sup>6</sup> Most of the protein data have been obtained by using the acetone hydrazone derivative as a reference standard. A series of studies has been made and is being made on several different modifications that do not depend on the aforementioned assumption regarding  $E_{\text{max}}$ . Three modifications show promise:

(a) displacement of reagent in protein hydrazone by treating with a reference carbonyl in excess,

(b) measurement of extinction coefficients in regions of the spectrum below 400 m $\mu$ . Values of  $E_{\text{max}}$  in the ultraviolet show less dependence on carbonyl structure.

(c) Determination of carbonyl bonds by measuring removal of hydrazine reagent rather than production of hydrazone derivative. Work is being continued.

<sup>6</sup> G. R. Lappin and L. C. Clark, Colorimetric Method for Determination of Traces of Carbonyl Compounds, Anal. Chem. 23, 541 (1951).

PRODUCTION OF C<sup>14</sup>-LABELED PRODUCTS IN THE RADIOLYSIS OF  
OXYGEN-FREE PROTEIN SOLUTIONS CONTAINING C<sup>14</sup>H<sub>3</sub>COOH AND HC<sup>14</sup>OOH

In the radiolysis of oxygen-free solutions containing protein molecules RH and a simple organic solute RH, there is a certain probability of radical combination reaction of the type R + R → R - R. If the nature of the radical R is known from independent studies, information on the configuration of the protein radical R can be obtained from the identification of products formed in conventional hydrolysis of the modified protein R - R. Studies in which RH is acetic acid (C<sup>14</sup>H<sub>3</sub>COOH) and RH is pepsin have shown that high-molecular-weight C<sup>14</sup>-labeled derivatives are produced and that hydrolysis yields a series of C<sup>14</sup>-labeled amino acids. A principal one of these shows an exact correspondence with authentic aspartic acid when chromatographed on an ion-exchange column after the method of Stein and Moore. Processes involved in the formation of the C<sup>14</sup> aspartic acid have been discussed.<sup>7</sup> The simplest explanation is that C<sup>14</sup>H<sub>2</sub>COOH radicals derived from acetic acid combine with protein-free radicals of configuration -NH-CH-CO-.

Because of the indicated importance of these concepts in the study of the radiation chemistry of proteins, a wholly independent confirmation of the aspartic acid identification seemed desirable. The procedure chosen involved application of the Van Slyke procedure in which primary amines are converted to the corresponding hydroxy derivatives by the action of nitrous acid. To 50 λ of freshly prepared nitrous acid solution (3.5 M in sodium nitrite, 3.5 M in acetic acid) was added the C<sup>14</sup> activity associated with the aspartic acid fraction (Peak A, Fig. 5, p. 15, Biology and Medicine Semi-annual Report, UCRL-8265). After the reaction had gone to completion (~15 min) about 20 mg of authentic malic acid was added, and the mixture was chromatographed on a silicic acid column with 35% butanol (v/v) in chloroform. Exact correspondence between C<sup>14</sup> activity and titer of the authentic malic acid was observed. Formation of C<sup>14</sup>-labeled aspartic acid is therefore confirmed. As noted previously, a series of C<sup>14</sup>-labeled acids, in addition to aspartic acid, is formed in the radiolysis of aqueous pepsin C<sup>14</sup>H<sub>3</sub>COOH solution. Identification studies of some of these are in progress. It is to be noted that on the basis of the proposed mechanism for incorporation of C<sup>14</sup> activity (i. e., -NH-C(R)-CO- + CH<sub>2</sub>COOH →), all the C<sup>14</sup>-labeled amino acids formed would be derivatives of succinic acid. It can be readily seen that only one of these, aspartic acid, corresponds to a natural amino acid.

Use of C<sup>14</sup>-labeled formic acid, instead of acetic acid, would appear to provide interesting possibilities for further experimentation along these lines. In this case the "indicator" would be the carboxyl radical-C<sup>14</sup>OOH. Combination reaction with the radical configuration -NH-C(R)-CO- would lead to a series of C<sup>14</sup>-labeled malonic acid derivatives. These on decarboxylation would be expected to give the original (naturally occurring) amino acid from which they were derived. Furthermore, since there is no great isotope effect in the decarboxylation reaction, the final products should contain roughly half the C<sup>14</sup> activity of the malonic acid derivatives. Measurement

<sup>7</sup> Garrison and group, in Biology and Medicine Semiannual Report, UCRL-8265, April 1958, p. 13.

of specific activity of the various amino acids in the protein hydrolyzate should then provide information on relative reaction probabilities at the variously substituted -CH(R)- groups of the peptide chain.

Preliminary data on the pepsin-  $\text{HC}^{14}\text{OOH}$  system have been obtained. Oxygen-free solutions ( $10^{-4}$  M pepsin,  $10^{-2}$  M  $\text{HC}^{14}\text{OOH}$ ) were irradiated with 40-Mev helium ions from the Crocker Laboratory 60-inch cyclotron for a total dose of  $\sim 2 \times 10^{18}$  ev/ml. The irradiated solutions were distilled in vacuo to recover  $\text{C}^{14}$ -labeled formic acid. The residue was dissolved in 1 N hydrochloric acid and dialyzed for 4 hours in cellophane against running water in the cold. The dialyzed fraction was made 4 N in hydrochloric acid, hydrolyzed (in vacuo) for 36 hours at  $95^\circ\text{C}$ , and chromatographed on a Dowex-50 column according to the amino acid separation procedure used in the pepsin- $\text{C}^{14}\text{H}_3\text{COOH}$  studies. A series of distinct  $\text{C}^{14}$ -labeled peaks was observed. Identification studies are now in progress.

#### DOSE-RATE EFFECTS IN THE RADIOLYSIS OF AQUEOUS ACETIC ACID-OXYGEN MIXTURES

Earlier reports have described the development of analytical techniques for the quantitative study of radiation-induced oxidation in aqueous acetic acid-oxygen solutions.<sup>8</sup> A detailed study of mechanism in the radiolysis of this system has been completed. The work has been reported separately.<sup>9</sup> The following gives a brief summary of the results.

The major oxidation products are glyoxylic, glycolic, and oxalic acids, formaldehyde, and carbon dioxide. Formation of these products apparently occurs through parallel processes initiated by formation of a common precursor, viz., the peroxy radical  $\text{O}_2\text{CH}_2\text{COOH}$  ( $\text{RO}_2$ ). There is no evidence for a chain reaction involving the  $\text{RO}_2$  intermediate. The problem of elucidating the mechanism of  $\text{RO}_2$  removal in this study has been found to be closely related to the classical problem of formulating the mechanism of chain termination in hydrocarbon oxidation. The experimental results in the acetic acid-oxygen studies have been found to be consistent with the concept that the removal steps are of the type  $2\text{RO}_2 \longrightarrow$  products,  $\text{RO}_2 + \text{H}_2\text{O} \longrightarrow$  products. The evidence for the occurrence of these competing reactions (which are second-order and first-order in  $\text{RO}_2$  respectively) was obtained from observed effects of radiation intensity on product yields. A complete discussion of detailed processes is included in the article.<sup>8</sup>

<sup>8</sup> Garrison and group in Biology and Medicine Quarterly Report, UCRL-3880, June 1957, p. 19.

<sup>9</sup> Bennett, Cole, Garrison and Haymond, Radiation-Induced Oxidation of Aqueous Acetic Acid-Oxygen Solutions, Radiation Research (in press).

## SYNTHESIS OF HIGHER-MOLECULAR-WEIGHT PRODUCTS IN THE RADIOLYSIS OF OXYGEN-FREE FORMIC ACID SOLUTIONS

It has been shown that irradiation of dilute, oxygen-free formic acid solutions with cyclotron-produced protons or helium ions leads to the synthesis of a number of products of higher molecular weight. The compounds identified include oxalic, glyoxylic, glycolic, mesoxalic, tartronic, and tartaric acids and glyoxal.<sup>8</sup> These products are not formed in appreciable yield by neutron and  $\gamma$ -ray irradiation. This system is of considerable interest in that it is the simplest aquo-organic system that can be used to obtain information on the radiation chemistry of the carboxyl linkage. More recent studies have contributed information on the effect of pH on the chemical properties of the COOH free radical. A summary of this work has been issued as a separate report.<sup>10</sup> A detailed discussion of mechanism is included.

## RADIOLYSIS OF GLYCINE-WATER SYSTEMS AT ELEVATED TEMPERATURES

In the radiolysis of aqueous glycine solutions above 1 M, the value  $G(\text{NH}_3)$  at room temperature increases almost linearly with concentration up to the solubility limit, which is  $\sim 3$  M. The simplest explanation is that a direct action of radiation on glycine contributes to ammonia production. It has been pointed out that a radiation-induced reaction of the type  $\text{NH}_2\text{CH}_2\text{COOH} \rightsquigarrow \text{NH}_2 + \text{CH}_2\text{COOH}$  followed by  $\text{NH}_2 + \text{NH}_2\text{CH}_2\text{COOH} \longrightarrow \text{NH}_3 + \text{NH}_2\text{CHCOOH}$  would satisfy the known experimental requirements.<sup>3</sup> There is also evidence for a competing direct-action process leading to the formation of methylamine:  $\text{NH}_2\text{CH}_2\text{COOH} \rightsquigarrow \text{CH}_3\text{NH}_2 + \text{CO}_2$ . It was felt that radiolysis studies of aqueous glycine at elevated temperatures would be of interest because information on the direct-action processes could be obtained over a much greater range in glycine concentration. The experiments would at the same time provide information on the temperature dependence of the elementary processes.

Preliminary thermal studies were required to establish the maximum temperature and the corresponding glycine concentration that could be used without introducing problems of thermal decomposition. A series of glycine-water mixtures with increasing glycine/water ratio were heated in heavy-walled pyrex tubes (evacuated) to the minimum temperature required for complete dissolution of the glycine. The amounts of material taken were such that  $\sim 5$  ml of solution was obtained in each case. Glycine solutions at temperatures above  $160^\circ\text{C}$  begin to show thermal decomposition. The saturated solution at this temperature corresponds to a concentration of  $\sim 10$  M. A series of 12 tubes covering the concentration range 0.25 M to 10 M has been irradiated with fast neutrons produced by bombardment of beryllium with 24-Mev deuterons. The tubes were mounted uniformly on a rotating wheel in a glycerine bath which was kept at a temperature of  $155 \pm 5^\circ\text{C}$ . On the basis of analytical results completed to date, it appears that the ammonia yield increases regularly with glycine concentration up to the

<sup>10</sup> Bennett, Cole, and Garrison, Synthesis of Higher-Molecular-Weight Products in the Radiolysis of Aqueous Solutions of Formic Acid, Radiation Research (in press).

10 M value. The methyl amine yield, on the other hand, tends to level off at concentrations above about 3 to 4 M.

### RADIOLYSIS OF ORGANIC LIQUIDS

Use of ICN as a new type of "scavenger" for the study of reactive intermediates formed in radiolysis of organic liquids has been described.<sup>11</sup> Information obtained on the  $\gamma$ -ray radiolysis of solution of ICN in methanol, n-hexane, cyclohexane, and benzene has been abstracted for the Spring 1958 meeting of the American Chemical Society.<sup>12</sup> Subsequent work has been devoted to a detailed study of the  $\gamma$ -ray radiolysis of benzene containing IC<sup>14</sup>N. The results confirm an earlier suggestion that phenylcyanide is a major product. A summary report of the work with ICN including a detailed discussion of mechanism is now being prepared for publication.

### PAPERS AND REPORTS

Work in this field has been reported during the period April-September 1958 as follows:

Winifred Bennett, Sibyl A. Cole, Warren M. Garrison, and Herman R. Haymond  
Radiation-Induced Oxidation of Aqueous Acetic Acid Acid-Oxygen Solutions  
(submitted to Radiation Research).

Warren M. Garrison, Radiation Chemistry of Organic Compounds Containing  
the N-C Bond, UCRL-8436 Abstract, September 1958 (for AAAS Symposium  
on Radiation Chemistry, Washington, December 1958).

Gordon Hughes and Warren M. Garrison, Radiolysis of Solutions of ICN in  
Organic Liquids, in Abstracts of Papers, 133rd Meeting, American  
Chemical Society, April 1958.

Michael E. Jayko and Warren M. Garrison, Formation of  $>C \approx O$  Bonds in  
the X-Ray-Induced Oxidation of Protein in Aqueous Solution, in  
Abstracts of Papers, 133rd Meeting, American Chemical Society,  
April 1958.

Michael E. Jayko, Boyd M. Weeks, and Warren M. Garrison, Mechanism in  
the Radiolysis of Aqueous Protein Solutions, in Proceedings of the  
Second International Conference on Peaceful Uses of Atomic Energy  
(United Nations, New York, 1958).

Boyd M. Weeks and Warren M. Garrison, Formation of C<sup>14</sup>-Labeled  
Protein Derivatives in the Radiolysis of Aqueous Pepsin (Abstract),  
Radiation Research 9, 202 (1958). (Presented at the International Congress  
of Radiation Research, Burlington, Vt., August 1958).

<sup>11</sup> Garrison and group, in Biology and Medicine Semiannual Report, UCRL-8265, April 1958. p. 14.

<sup>12</sup> Gordon Hughes and Warren M. Garrison, Radiolysis of Solutions of ICN in Inorganic Liquids, UCRL-8287 Abstract, June 1958.

Boyd M. Weeks and Warren M. Garrison, Radiolysis of Aqueous Solutions of Glycine, Radiation Research 9, 291 (1958).

Garrison

BIOLOGICAL STUDIES OF RADIATION EFFECTS

John H. Lawrence in charge

Donner Laboratory of Biophysics and Medical Physics  
University of California, Berkeley, California

RADIATION THERAPY AND BIOLOGY

IRRADIATION OF THE PITUITARY IN HUMANS

James L. Born, Richard A. Carlson, and Franco F. Sangalli

The current investigation on the effects of irradiation on the human pituitary gland, using the beam from the 184-inch cyclotron, has been in progress since the Fall of 1954. The purpose of this study is to determine the possible beneficial effects on various neoplastic and metabolic diseases that are under endocrine control and can be mediated through the pituitary.

During the first year of the study, 30 patients were irradiated with the 340-Mev proton beam from the cyclotron. During the next two years studies of the patients continued, but no further patients were irradiated because the cyclotron was torn down and rebuilt. One year ago the cyclotron was again placed in operation, and irradiation on human patients with various diseases was resumed. Because of energy changes a 900-Mev alpha-particle beam having tissue-destructive properties comparable to the proton beam was used in place of the proton beam.

Seventy-six patients have been irradiated, 64 of these because of metastatic breast cancer. Of the remaining twelve patients, three have diabetes mellitus with complications, and five have acromegaly; the other four have had respectively, chronic lymphatic leukemia, acute lymphatic leukemia, embryonal dysgerminoma, and malignant exophthalmos. Since our purpose is to determine the effects of this irradiation on the course of the particular disease and on certain physiological processes, a comprehensive clinical and laboratory study is carried out before irradiation and every 4 to 8 weeks thereafter. The comprehensive evaluation includes:

1. Complete physical examination
2. Appropriate x-ray examinations
3. Blood chemical tests of: electrolytes, renal function, liver function, thyroid function (PBI)
4. Twenty-four-hour urinary gonadotropins, ketosteroids, hydroxysteroids, estrogens, and calcium
5. Urinalysis, BSP, EKG
6. Biopsy and other diagnostic procedures, as needed
7. Blood volume, body volume, and in appropriate cases red cell life studies, erythropoietic studies, carbon-14 carbohydrate metabolic studies, and growth-hormone studies.

Of the 76 patients irradiated, 29 are living at present: the longest-surviving patients are living at 39 and 36 months respectively. Of the deceased patients, six of the seven who were terminal at the time of irradiation died during treatment, four died of other causes, and the remainder eventually died of their disease.

Of the 29 living patients 21 have metastatic breast cancer. In seven of these the period of follow-up has been too brief to determine the effects of therapy. We have observed subjective and objective improvement in seven of the remaining fourteen patients. In one there has been subjective improvement alone. In six we have so far noted either no improvement, or progression of their disease.

Laboratory and clinical data indicating the degree of completeness of hypophysectomy are being collected on all patients. The changes indicating successful hypophysectomy appear gradually and are by no means confined to those showing improvement. In 18 of the patients with breast carcinoma who are now deceased, there was objective evidence of temporary arrest or regression of their disease.

Early encouraging results are being observed in a group of eight patients with diabetes mellitus and acromegaly. All three diabetics treated had advanced eye complications. One also had advanced diabetic glomerulosclerosis with the nephrotic syndrome, uremia, and severe anemia. Following irradiation this patient's insulin needs dropped to zero and he lost a large quantity of edema fluid. The other two diabetics have experienced arrest or improvement of their diabetic retinitis and their insulin needs have decreased.

Five patients with acromegaly have been irradiated. One treated 6 months ago has shown a gradual reduction in insulin requirements and diphenyl biguanide, and his carbohydrate metabolism now appears to be normal. Another patient who is 1 month postirradiation has been able to eliminate insulin entirely although he was taking 80 units per day prior to irradiation. Two other patients show less striking evidence of improvement, while one shows no evidence of improvement.

In both groups of patients irradiated--those with the 340-Mev proton beam and those with the 900-Mev alpha beam--it is to be noted that the earlier cases in both groups received smaller radiation doses over longer periods of time than those irradiated later in the series. Also, more of the earlier breast cases had far advanced disease, some even near terminal. One can reasonably expect better results as patients are treated earlier in the course of their disease with the 900-Mev alpha-particle beam in the higher dose ranges. The early results of pituitary irradiation in the acromegalics and diabetics is encouraging. Pituitary irradiation will be continued in patients with breast carcinoma, acromegaly, diabetes mellitus, and other conditions that are under endocrine control through mediation of the pituitary. The results observed in the treated patients have been sufficiently encouraging to warrant continuation of the study so that a statistically significant number can be evaluated. The interrelated metabolic investigations yield data of importance in gaining an underlying knowledge of the disease processes involved.

## ALIGNMENT FOR PITUITARY IRRADIATION

Graeme P. Welch

Alignment of patients in the irradiation apparatus is now routinely made with the aid of electronic image amplifiers. The x-ray fluoroscopic image of the patient's pituitary is observed directly while alignment adjustments are being made. This results in more efficient operation and less strain on the patient, since x-ray pictures are now taken only as a final check.

## HISTOLOGICAL EFFECTS OF ACCELERATED PARTICLES DIRECTED AT THE HYPOPHYSIS OF RATS

Gilles LaRoche, Paul Blanquet, and Cornelius A. Tobias

In this group of experiments the hypophyses of rats were irradiated by alpha particles or deuterons accelerated by the 184-inch cyclotron. Animals that had received a single dose of 10,000 rads did not show any detectable histological changes at the level of the hypophysis itself, the thyroid, the adrenals, or the testes 8 weeks after irradiation. On the other hand, in those administered 20,000 rads the hypophysis showed obvious signs of degeneration. The gland was considerably reduced in size and demonstrated large concentrations of pyknotic nuclei, separated by strands of remaining cytoplasm where cell boundaries were invisible. Among these animals there was impressive reduction in the height of the epithelial cells of the thyroid glands, accompanied by a significant increase in the size of the colloid vesicles.

## ELECTRON MICROSCOPY OF THYROID TISSUE

Thomas Hayes

Techniques for fixation, staining, and sectioning of tissue specimens for electron microscopy have been established in this laboratory. A Porter-Blum ultramicrotome and an RCA EMU-2E electron microscope are used. During the last six months, particular attention has been focused on the thyroid tissue of rats, both normal and hypophysectomized. Hypophysectomy was carried out by use of the cyclotron beam or by surgery, and an attempt was made to see the effect of such a removal of the pituitary on the sub-microscopic structure of the thyroid.

A prominent feature of the thyroid cell as seen in the electron microscope is a system of cytoplasmic membranes. These membranes are about 50 Å thick, have small granules attached to one side, and have been postulated to be intimately concerned with thyroid secretion.

The most striking effect of hypophysectomy on the thyroid ultrastructure was the loss of these cytomembranes. It is possible that these results can be used to develop a morphological test of thyroid function that would be of value in assessing the efficiency of hypophysectomy following cyclotron irradiation.

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## THYROID PHYSIOLOGY

Paul Blanquet, Ann Rhodes, David Yudilevich, and Gilles La Roche  
Iodinated Products in the Thyroid

A method has been developed by Blanquet, Meyniel, and co-workers for separation of the iodinated amino acids of the thyroid gland on an anion-exchange resin. A pancreatin hydrolyzate of the gland is placed on a column of Dowex-1×2 in the OH<sup>-</sup> form, and elution is carried out with 0.2N HCl containing ethyl and isoamyl alcohols.

The iodothyronines, monoiodotyrosine, and diiodotyrosine are successively eluted, while inorganic iodide remains absorbed on the column. The thyroid glands used are taken from rats given doses of the order of 100  $\mu$ C of I<sup>131</sup>, one or two days before sacrifice. Thus the iodinated constituents are labeled with I<sup>131</sup>, and the process of the elution is followed by Geiger counting. The radioactivity serves as a measure of the amount of each constituent present, namely, thyroxine, mono-, and diiodotyrosine.

By use of this method, studies were made of the thyroid function of animals that had been subjected to intense alpha-particle or deuteron irradiation of the pituitary area. The effect of such irradiation, when given in sufficiently high dose (20,000 rads), is qualitatively similar to that of surgical hypophysectomy.

Similar irradiation of the eminentia medialis area of the hypothalamus was also carried out. Intense irradiation of the median eminence appears to result in thyroid abnormalities that are different from previously known destructions of this gland. The thyroxine content of the thyroid is decreased to about 1/10 normal, while I<sup>131</sup> uptake and mono- and diiodotyrosine content are near normal. It is possible that in this condition the thyroid metabolism is still normal but the thyroxine is released immediately as it is formed. Another explanation would be the existence of a new hormone acting on formation of thyroxine, which would act under the control of nerve centers located in or near the median eminence.

Electron microscopy of the thyroid of the animals used in these studies resulted in demonstration of striking subcellular morphological changes, correlated with hormonal functions. These are discussed in another section of the report.

### Iodinated Products in the Plasma

An attempt is being made to separate various plasma components through the use of ion-exchange resins. In a first step the proteins would be separated from the free amino acids. In a second step the proteins would be submitted to further fractionation (ion exchange, salt, or ethanol) and purification, while the free amino acids could be partitioned according to the procedure described for thyroid hydrolyzates.

It is hoped that through this fractionation a better knowledge of various iodinated products in the plasma will be gained, thus furnishing a dependable measure of thyroidal activity.

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STUDIES ON  
THE HYPOTHALAMO-HYPOPHYSEAL FUNCTION IN THE DOG

Orland K. Anderson, Julian P. Henry, and Gilles La Roche

Under this program alpha particles accelerated by the 184-inch cyclotron are used to produce well-defined lesions at the level of various hypothalamic nuclei in male beagles. Preliminary histological and x-ray studies of the regions surrounding the 3rd ventricle have permitted the location of the paraventricular nuclei and their position with respect to cranial bony landmarks.

A new and greatly simplified holder was designed to stabilize the head of the animal during irradiation. The holder is fixed to a rotary mechanism and the high-energy beam, 1/16-inch in diameter, is focalized on the desired region. In order to prevent extensive vascular damage at the site of irradiation, fractionated doses of 2000 rads per nuclei, up to a maximum of 24,000 rads, are administered. At this time the animal is kept under observation to ascertain the possible endocrine or peripheral effects connected with the destruction of these nucleated centers.

The principal advantages in the use of the cyclotron rather than the usual surgical stereotaxic techniques resides in the fact that, following irradiation, slowly developing lesions in limited regions restricted by the diameter of the beam, permit a gradual disabling of the "target" centers.

ELECTROENCEPHALOGRAPHIC INVESTIGATIONS  
ON BRAIN-IRRADIATED RATS

Cornelius A. Tobias and George M. Tsiljar-Lentulis \*

It has been known for some years that whole-body-irradiated animals or animals that have been subjected to intense brain irradiation of many thousand roentgens exhibit central nervous system symptoms in the post-irradiation period. Some of these resemble rage, others are connected with unusual hyperactivity of the animals, and still other symptoms resemble epileptic shocks in the human. The use of the cyclotron beam enables us to irradiate a small localized portion of the brain; therefore a systematic study was started to determine the immediate effect of proton and alpha particle irradiation on neural function, including electrical properties of brain, and also to see whether physiological changes are associated with delayed effects described above. As part of this study a simple electronic arrangement was built which enables us to follow the electroencephalogram of a rat either during irradiation or any time in a postirradiation period. Preliminary experiments indicate that there are important changes associated with the electroencephalogram during and after irradiation of a rat. For successful results the rat should not be anesthetized during the experiment. During whole-head irradiation the amplitude of the brain waves was found to increase by a significant factor, but immediately after irradiation the amplitude returns to normal. There is an effect on the frequency spectrum of the brain waves. If the rat receives a dose of 30,000 rad or more to his brain, then in

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the postirradiation period there follow occasional shocks, resembling epilepsy, which may lead to the death of the animal. If irradiation is turned on continuously until the animal dies then the over-all amplitude of the brain waves are found to continuously decrease until its vanishing at or near the time of death. Somewhat similar electroencephalographic changes already have been previously reported by Russian workers and by some workers in the United States. Our efforts are directed to better understanding of the mechanism of development of these effects and also toward finding regions in the brain that may be responsible for the frequency and amplitude distributions of brain waves.

### BIOLOGICAL EFFECTS OF HEAVY-ION BOMBARDMENT

Donald Fluke

The completion in 1957 of a new linear accelerator for ions of carbon, nitrogen, oxygen, and neon has made possible a study of radiobiological effects at higher values of ion density than are encountered with alpha rays. The work is of interest in considering effects of fission fragments and of heavy cosmic-ray primaries outside the earth's atmosphere, as well as in more theoretical aspects of radiobiology. Following assembly of apparatus suitable for use with the new machine and for coping with the unusual problems of dosimetry involved, a study has been made of direct-action effects in bacterial spores, in a bacteriophage, and in several enzymes. The greater efficiency of slow alpha rays in rendering bacterial spores incapable of producing microcolonies has been followed by decreasing efficiency at still higher ion density values, much as in earlier work with vegetative yeast cells. The heavy-ion studies of effects on infectivity and host-killing properties of bacteriophage have emphasized the remarkably constant response of this organism to all "ordinary" values of ion density, but at highest ion densities a saturation effect is seen. The effect of delta rays on this system is clearly involved, and both spores and phage show behavior at high ion density which can reasonably be represented as saturation of a cross section for the incident particles. That the enzyme work has not shown such saturation is probably associated with the fact that efficiency begins to decline at lower ion density. The decline continues smoothly throughout the region studied, and a reasonable delta-ray correction gives no assurance that a target concept alone can represent the radiobiological processes involved in the inactivation of the catalytic property. The apparatus has now been modified so that vegetative cell preparations can be studied, and it is expected that the work will next concentrate on high-ion-density effects in metabolically active cells.

### HEMATOLOGICAL EFFECTS OF LOW DOSES OF RADIATION IN HUMANS

Howard Parker

The double-nucleated lymphocyte response to repeated low-level doses of ionizing radiation has previously been confirmed in this laboratory. During the past year studies have been carried out in an attempt to establish what size of single whole-body dose of ionizing radiation would give rise to this response. There is an indication that the response is detectable in two

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humans who received the highest doses--of the order of 2.5 r gamma radiation.

Repetition of such studies and further animal experiments will be necessary to establish the response at this dose level and to elucidate the dose-response relationship.

These studies are still in progress, and require the counting of literally tens of millions of white blood cells individually by eye, with the use of motor-driven-stage microscopes. Investigation of the feasibility of performing these cell counts automatically was recently carried out with the help of the Data-Reduction Electronics Group, UCRL. Automation in such a problem still presents very great difficulties (and hence is still prohibitively expensive). However, an automatic counter has a would be tremendously valuable in cell counting of many sorts if it could be made to discriminate elements of form and color as well as size.

Information on the binucleated lymphocyte response should be of value in furthering our understanding of the radiosensitivity of the lymphoid system, and may incidentally be of help in developing a biological method for observing small doses of radiation in the human.

#### WHOLE-BODY HUMAN COUNTER AND LOW-BACKGROUND COUNTING FACILITY

Howard Parker

Still in the early development stage is the human whole-body counter at Donner Laboratory.

In recent months the problem has been studied, similar facilities visited and experts consulted, the counting equipment ordered, and architectural plans for the facility begun.

The facility will consist of a well-shielded underground steel room containing a large NaI crystal 100-channel scintillation spectrometer, as well as associated low-background rooms for other biological studies.

The human counter will be prepared for diagnosis in radioactivity accident cases, either occupational or resulting from a disaster in any near-by community. It will also be used in research in natural and fallout activities in humans and animals, in assessment of body K<sup>40</sup> activity (and hence "lean body weight"), and in the measurement of blood loss by total-body Fe<sup>59</sup> activity in some Donner Clinic patients. Many new uses for this facility are expected to be found. It will identify and quantitate gamma-emitting isotopes in amounts as small as 10<sup>-9</sup> curie.

The counter will produce a great volume of potentially useful data, and hence plans are being made to use card-punch data storage and automatic computer methods wherever possible.

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## LIPOPROTEIN AND SURVIVAL STUDIES AFTER X-IRRADIATION

John Hewitt

Investigations into the nature of the postirradiation hyperliproteinemia are continuing. The effects of combining x-irradiation and other treatments capable of producing lipemias, such as  $CCl_4$  poisoning and excessive bleeding, are being examined.

Also in progress is a study of the possibility of using  $Fe^{59}$  to determine the relative radiosensitivity of the animals of a given group. Irradiation depresses the incorporation of  $Fe^{59}$  into the red blood cells. This effect is a function of x-ray dose, and is reported to be detectable at doses as low as 5 r. To test the relationship between this depression of erythropoietic activity and survival of the individual animal, rats are being irradiated with low doses of x-rays and postirradiation incorporation of  $Fe^{59}$  into their red blood cells is being measured. The animals will later be irradiated with an  $LD_{50}$  dose and their survival following this dose will be compared with their  $Fe^{59}$ -incorporation response after the previous test dose.

## RADIATION EFFECTS OF THE PERMEABILITY OF YEAST CELLS TO SODIUM AND POTASSIUM IONS

Kwan Hsu

Yeast cells grown in potato dextrose broth for 36 hours are harvested, washed, and resuspended in sterile distilled water. They are irradiated in the distilled water suspension with 250-kvp x-ray (Philips 250-kv x-ray machine, with 0.5 mm Al filter). After irradiation, the same suspension is divided into three aliquots. At different time intervals the cells are centrifuged and the supernatants are assayed for Na and K. Potassium is found to leak out from the cells into the suspension within 10 minutes after irradiation. The potassium content in the water increases exponentially within the first 3 hours and then reaches a constant value from 10 to 18 hours after irradiation. Preliminary studies have given the following results:

Strain	Time after irradiation (hr)	Potassium in water ( $\mu$ g/10 <sup>8</sup> cells)	
		Irradiated	Control
X-320 (diploid)	1	3.6	2.4
	2	5.2	3.7
	3	6.4	4.6
	4	7.0	5.3
	10	8.1	6.4
X-362 (hexaploid)	1/2	10.0	6.6
	1	12.6	9.2
	2	15.4	11.8
	3	17.0	13.0

The experiment is being continued to study the leakage of potassium from haploid, diploid, tetraploid, and hexaploid cells at different dose levels.

### NEUTRON-ACTIVATION ANALYSIS

Kwan Hsu

Neutron-activation analysis is a method of determining the constituents of a sample by exposing it to thermal neutrons, which by nuclear reactions will make some elements radioactive. From the characteristics of the radioactive isotopes thus induced the unknown elements may be quantitatively determined. For many elements this method gives higher accuracy and greater precision than other microchemical methods. It provides a powerful method for determining the trace elements in biological tissues.

Yeast cells of different strains and blood serum are irradiated in the center of the Livermore water-boiler reactor. The thermal-neutron flux varies from  $2.42 \times 10^{10}$  n/cm<sup>2</sup>-sec in the center to  $1.83 \times 10^{10}$  at 4 inches from the center. The samples are irradiated for 2 hours and are then assayed for Na and K, primarily to investigate the reproducibility of the method. NaCl and KCl, either in crystal form or in water solution, are used as standards. The activities are counted in G-M and scintillation counters at different time intervals. Activities after the 2-hour irradiation can be determined from extrapolation of the decay curves. For example, in one particular experiment,  $5 \times 10^{-4}$  g of NaCl in 2 ml solution gave  $1.74 \times 10^4$  cpm of  $^{24}\text{Na}$  activity (half life 15 hours), and  $10^{-3}$  g of KCl in 2 ml solution gave  $1.33 \times 10^4$  cpm of  $^{42}\text{K}$  activity (half life 12.4 hours). The counting was done in a well-type scintillation counter (Harshaw, type 7F8). The sodium and potassium contents in the biological samples are determined in many ways. The ratio of the Na and K contents in the tissues investigated is such that it is very difficult to determine accurately by pulse-height analyzer. At present, separation of the two isotopes by ion-exchange resin seems to work best. For blood serum, the Na and K contents determined by neutron-activation analysis fall within the range as determined by flame photometer. Yeast cells of different strains grown in minimal culture medium gave Na content from 2 to  $10 \mu\text{g}/10^8$  cells, and potassium content from 100 to  $242 \mu\text{g}/10^8$  cells.

The Livermore LTPR reactor, now in operation, has a maximum thermal flux of  $2.5 \times 10^{13}$  n/cm<sup>2</sup>-sec. The detection limit for Na and K should be of the order of magnitude of  $0.00035 \mu\text{g}/\text{ml}$  and  $0.004 \mu\text{g}/\text{ml}$ .<sup>1</sup> Progress is now being made in investigation of the procedure for irradiation of biological samples by the thermal flux of the Livermore LTPR reactor.

<sup>1</sup> W. W. Meinke, Trace-Element Sensitivity: Comparison of Activation Analysis with Other Methods, Science 121, 177-184 (1955).

BLOOD BIOCHEMISTRY AND CIRCULATION

**PHYSIOLOGICAL EFFECTS OF HUMAN URINARY ERYTHROPOIETIN**

Donald Van Dyke

It is apparent from the work in this and other laboratories that one of the primary factors in the control of division and growth of the hemocytoblasts of the marrow is a hormonal factor called erythropoietin. The screening of a large number of patients in the past two years has demonstrated that certain individuals with severe anemia not only have high levels of erythropoietin in their plasma but constantly excrete the erythropoietic hormone in the urine. Methods have been developed for concentrating this material in 1/1000 of the solids of the urine. In the last year an intensive effort has been made to determine the physiological and chemical properties of this urinary erythropoietin. Ultrafiltration provides an active fraction which is sufficiently free of toxic side effects to be usable in most physiologic studies. The administration of this material for a period of 14 days to mice, rabbits, and monkeys has resulted in the production of a polycythemia. An adult male monkey given 100 mg intravenously daily for 15 days showed a 63% increase in the total circulating red cell volume. In studies of the spleen in rats, it has been shown that an increase in DNA precedes an increase in incorporation of radioiron, indicating that the primary effect of this material is to stimulate division of the hemocytoblasts of erythropoietic tissue. Experiments are in progress, using marrow suspensions and perfusions of isolated limbs, in order to further our understanding of the mechanism of action. The knowledge gained from studies on monkeys about the route of administration and the dose required in primates warrants a limited program investigating the possible therapeutic usefulness of this material in certain types of anemia. This will be undertaken in the near future.

**STUDIES OF  
THE EFFECT OF ERYTHROPOIETIN ON BONE MARROW IN VITRO**

James Beck

It is yet to be shown that erythropoietin acts directly on bone marrow. An attempt to demonstrate this is being made by means of measuring  $Fe^{59}$  uptake of rat bone marrow cells in suspension in a synthetic medium. Some increase in iron uptake by marrow cells in the presence of anemic rat serum over that by marrow cells in the presence of normal rat serum has been found thus far, though results have been inconsistent. If this can be dependably repeated, the next step is conditioning the suspension of marrow cells with human erythropoietin. Thus, in addition to the fundamental significance of demonstrating a direct action, the technique may be useful for assay. The method would also be helpful in determining the nature of the process of iron uptake by these cells, in finding an organ source, etc.

CHEMICAL SEPARATION AND CHARACTERIZATION  
OR URINARY ERYTHROPOIETIN

Howard C. Mel

Starting from the proteinlike material collected by high-pressure ultrafiltration of urine of patients containing erythropoietin activity, various physicochemical procedures have been carried out. Solubility measurements at pH 8 indicate that at total concentrations greater than 6% the solubility of the biological activity is not exceeded, whereas the limits of solubility of some of the nonactive material have already been reached. By continuous electrochromatographic procedures in conjunction with detailed spectrophotometric and pH analysis, specific activity has been increased by a factor of 5.5 or greater. Composition of the most active portions is significantly different from that in the more acidic or basic fractions of the urine ultrafiltrate.

PROGRESS IN METHODOLOGY, IN PHYSICAL CHEMISTRY, AND  
IN STUDY OF THE METABOLISM OF HUMAN SERUM LIPOPROTEINS

Frank T. Lindgren and Alexander V. Nichols

A simplified procedure has been developed for the study of the serum lipoproteins which requires only 2 ml of serum (or plasma). Lipoprotein isolation and analysis are achieved in a NaBr medium of density 1.20 g/ml. All classes of the serum lipoproteins may be studied with this procedure, including the complete low-density spectrum for which equivalence with the established low-density classification ( $S_f^0$  0-400 values) has been determined.

Another analytical development for the study of lipoproteins has been a method to isolate lipoproteins on a sedimentation equilibrium salt gradient in the preparative ultracentrifuge, using such high-molecular-weight salts as NaBr and CsCl. Such a method allows the physical and chemical study of very narrow lipoprotein bands of greater physical homogeneity than was heretofore possible. A preliminary study of the  $S_f^0$  6-8 low-density lipoprotein band isolated from five normal adult individuals revealed some chemical unhomogeneity of this class (from person to person) with respect to glyceride, cholesteryl ester, and unesterified cholesterol content.

Other physical-chemical studies in lipoproteins include partial ether extraction. Partial degradation of both  $S_f^0$  20-400 and high-density lipoproteins by this process gives rise to lipoproteins exhibiting a wide range in such physical properties as hydrated density and particle size. Many classes of these product lipoproteins are chemically different from the physically corresponding normal (undegraded) lipoproteins. The high-density lipoproteins exhibited considerably more stability to ether extraction than the  $S_f^0$  20-400 lipoprotein. Nonetheless, it was possible to obtain from high-density lipoprotein by ether extraction an essentially lipid-free protein fragment of 43,000 molecular weight units.

The effects of storage on lipoproteins and their stability to freeze-thawing cycles has also been studied. Prolonged storage at  $-28^{\circ}\text{C}$  was found to be more injurious to lipoproteins than storage at either from 0 to  $-5^{\circ}$  or from 0 to  $+5^{\circ}\text{C}$ . However, degradation of the lipoproteins occurred at all

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temperatures; there was extensive damage after a few months of storage. No ultracentrifugal effects were observed to occur when lipoproteins were frozen and thawed five times or less. However, repeated freezing and thawing resulted in extensive and progressively greater damage. These effects were not influenced by the rate of the freeze-thawing process.

In studies on the metabolism of lipoproteins the effects of a 30-g/day soy lecithin supplement was investigated in a group of Stockton State Hospital patients. The absorption and the chemical form present in the blood stream of tritium-labeled stearic acid fed before and during the period of lecithin supplementation were studied. However, no effect of the lecithin could be demonstrated. Further, studies of the total serum lipids and the total serum lipoproteins made before, during, and after the lecithin period revealed no such beneficial lowering effects as had been previously reported (by Morrison).

In connection with both metabolic and physical-chemical studies of lipids and lipoproteins, a radiological ( $S_r 90$ ) gas chromatographic unit designed specifically for fatty acid analysis has nearly been completed. This unit soon will allow full chemical characterization and quantitative analysis of the fatty acids present in extremely small lipid samples (less than 1 mg).

#### BLOOD HEPARIN AND LIPOPROTEIN LEVELS

Tadao Yasugi

In further studies of the role of heparin in lipid metabolism, an investigation has been carried out with the object of correlating the natural level of heparin in circulating blood with concentrations of cholesterol and the various classes of lipoproteins. The population sampled was a group of 105 schizophrenic males, age 40 to 50, in the State Hospital at Stockton, California. Subjects were in a fasting state when blood was drawn. The method of heparin analysis (modified from Bassiouni, J. Clin. Path. 6, 39 (1953)) measures total "heparinlike" substances, and gave a mean value of 3.07 mg/100 ml of blood. Lipoprotein measurements were made by the standardized ultracentrifugal techniques of this laboratory. Significant inverse correlations were demonstrable for the following pairs of variables:

$$S_f^0 0-12 \text{ vs "heparin"} \quad r = -0.49 \text{ (p < .001)}$$

$$S_f^0 12-20 \text{ vs "heparin"} \quad r = -0.34 \text{ (p < .001)}$$

$$\text{Cholesterol vs "heparin"} \quad r = -0.47 \text{ (p < .001)}$$

Correlation coefficients with all classes of high-density lipoproteins and with the very low-density lipoproteins were small and not statistically significant.

## ATHEROSCLEROSIS AND BLOOD PRESSURE

Wei Young

In analysis of the data obtained by studying arterial sections it has been found that the relationship between cerebral atherosclerosis and blood pressure (both systolic and diastolic) is highly significant. This relationship drops off as age increases to 80-89. In coronary atherosclerosis, on the other hand, there is no correlation with systolic blood pressure throughout the age groups studied (60-89), but there is a statistically significant relationship with diastolic pressure in the age groups 60-69 and 70-79. This relationship also disappears in the 80-89-year age group.

## PHYSIOLOGICAL ROLE OF LIPOPROTEINS

Wei Young

In order to learn more about the physiological role and possibly the structural configuration of lipoproteins, we have carried out perfusion studies, using vagal heart preparations and observing the effects of vagal stimulation. It has been found that the serum protein protects the integrity of vagal effect. Lipemic serum potentiates the vagal inhibitory effect, while albumin, or a serum protein fraction from which substantially all lipids and lipoproteins have been removed ultracentrifugally, tends to reduce it.

Preliminary experiments using lipid components in the medium show that a certain fraction of phospholipids abolishes the vagal inhibitory action. This needs to be verified by repetition of the experiment, using purified and better characterized material.

## INFRARED SPECTROMETRY AND LIPID CHEMISTRY

Keith Freeman

The simplified infrared spectrophotometric method of serum lipid analysis, recently developed in this laboratory, has been applied to three groups of subjects, classified clinically as follows: (a) normal; (b) multiple sclerosis; (3) Hodgkin's disease. This work has had a twofold purpose: first to study the distributions of serum concentrations for total lipid and for the various individual lipids for each of these groups, searching for possible characteristic aberrations in the disease groups; and secondly to evaluate the method more thoroughly by comparison with independent chemical analysis of many of the sera. With respect to the second objective, the order of disagreement with chemical analysis for the various components is indicated in the following table:

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<u>Number of comparisons</u>	<u>Measured component</u>	<u>Compared with</u>	<u>Average discrepancy</u>
35	Total lipid	Weight	$\pm 2.6\%$
41	Total cholesterol	Chemical	$\pm 6.0\%$
53	Total phospholipid	Lipid phosphorus anal.	-11.6%
19	Esterified fatty acids	Saponification	$\pm 7.3\%$

The differences appear to be random in direction except in the case of phospholipids, for which the infrared determination is nearly always higher than the chemical value. Furthermore, the magnitude of the error in this particular component shifts from one set of analyses to the next, i. e., for one group of determinations the discrepancy may range from 0 to 10%, and for another group done at a different time it may be 10 to 20%. Possible causes of the systematic difference and the fluctuation are being investigated.

Comparison of the three groups on a statistical basis has been only partially carried out. Hodgkin's disease cases, in particular, have accumulated slowly, and for many of them clarification of their clinical status is necessary. From the available data it appears that in Hodgkin's disease both total lipids and total cholesterol tend to be lower than in normals (71% of total lipid values and 82% of total cholesterol values are below the medians of the corresponding normal distributions). In multiple sclerosis, on the other hand, the lipid distributions are substantially the same as in normals. The medians are slightly, but not significantly, higher.

#### PROLIFERATION IN THE LIVER RETICULOENDOTHELIAL SYSTEM

Ernest L. Dobson, Lola S. Kelly, Caroline R. Finney, and J. Dorothy Hirsch

Studies of liver reticuloendothelial (RE) system proliferation have continued. We have reported previously that the injection of Zymosan produces a dramatic increase in liver DNA synthesis which is due to the proliferation of littoral cells.<sup>1</sup>

In a recent paper Heller et al. demonstrated that massive doses of a variety of estrogenic substances stimulated phagocytic activity, but concluded from indirect evidence that hyperplasia of RE elements was not involved.<sup>2</sup> However, we had observed previously (in unpublished experiments on the radiation-protective properties of estrogens) that liver DNA synthesis was increased. These observations suggested that phagocytic activity and DNA synthesis should be compared directly. DNA synthesis was measured by the 2-hour incorporation of P<sup>32</sup> into liver DNA and the phagocytic activity by the rate of removal from the blood of intravenously injected colloidal carbon. Figure 12 presents the results obtained after a single subcutaneous injection of 1 mg of estradiol in sesame oil. They confirm the increased phagocytic activity and make it clear that this is associated with very active cell proliferation in the liver. Other tissues are currently under investigation. This finding is of interest in the interpretation of the radiation-protective properties of estrogen.

<sup>1</sup> UCRL-8265, April 25, 1958, p. 41.

<sup>2</sup> Heller, J. H., Meier, R. M., Zucker, R., Mast, G. W. Endocrinology 61, 235, 1957.

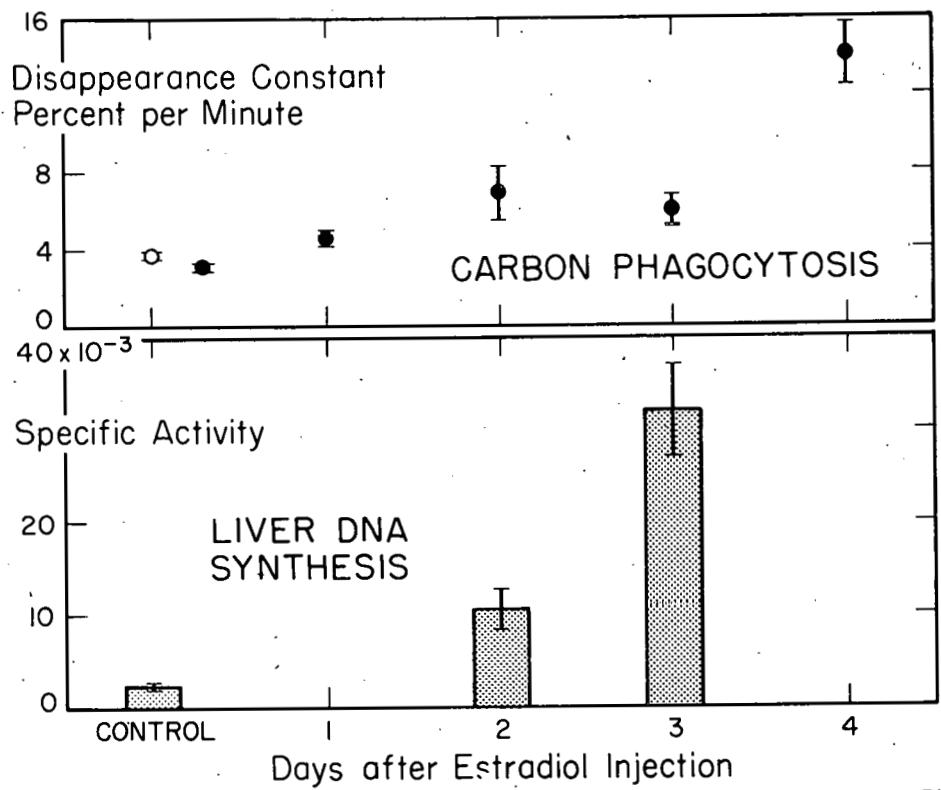


Fig. 12. Phagocytic function and DNA synthesis at various times after subcutaneous estradiol injection.  
(Specific activity =  $(\text{cpm}/\text{mg P})/\text{cpm injected/g}$ ).

## THE ROLE OF THE THYMUS IN LYMPHOCYTE PRODUCTION

John C. Schooley and Lola S. Kelly

A controversy exists in the literature on whether the small cells of the thymus (thymocytes) and the lymphocytes of the lymph nodes and blood are identical cell types and, in fact, whether the cells produced in the thymus ever leave this organ. Experiments are in progress to clarify this problem. Long-Evans rats were thymectomized at 6 days of age. At 60 days the thoracic duct was cannulated at its entrance into the subclavian vein and the output of lymphocytes determined. At autopsy the remaining lymphoid tissues were weighed. In another group of operated animals the DNA synthesis of the remaining lymphoid tissue was determined 3 hours after the injection of  $P^{32}$  by a modified Schmidt-Thannhauser technique. The results are shown in Tables X and XI.

It is clear that there was no significant difference in DNA specific activities in the lymph nodes of the thymectomized and sham-operated animals, indicating normal rates of cell renewal. The DNA content per unit weight of lymphoid tissue was unchanged. There was a decrease in the weight of the lymphoid tissue of the thymectomized rat, therefore, the total cell production must have been reduced in proportion to the decrease in weight.

The thoracic duct lymphocyte output of the thymectomized animals showed a dramatic decrease to 27% of the control values. The blood lymphocyte levels were also reduced in the operated animals, but this decrease was not so marked as the thoracic duct differences. This rather marked decrease in thoracic duct output in the thymectomized animals might be interpreted as indicating that the lymphocytes produced in the thymus do leave this organ via the afferent lymphatics and empty into the thoracic duct; however, part of this decrease may simply result from the decreased mass of the remaining lymphoid tissues.

Further experiments are in progress using an autoradiographic technique with tritiated thymidine as a cell label.

Table X

Specific activity of DNA in thymectomized and sham-operated rats  
3 hr after  $P^{32}$  injection

Tissue	Thymectomized rats (11 animals)		Sham-operated controls (7 animals)	
	DNA specific activity <sup>a</sup>	DNA specific activity, % acid-soluble	DNA specific activity <sup>a</sup>	DNA specific activity, % acid-soluble
Spleen	20.9 $\pm$ 5.1 <sup>b</sup>	14.6 $\pm$ 4.3	12.4 $\pm$ 4.0	8.49 $\pm$ 2.3
Lymph nodes (mesenteric and cervical)	12.7 $\pm$ 0.39	6.89 $\pm$ 0.44	10.8 $\pm$ 0.54	6.53 $\pm$ 0.32

<sup>a</sup> Given as  $\frac{\text{cpm/mg DNA phosphorus}}{\text{cpm/g body weight}} \times 100$

<sup>b</sup> Errors shown are standard errors of the mean.

Table XI

Thoracic duct outputs and lymph-node weights in thymectomized and sham-operated rats.

Treatment	Animal weight	WBC per mm <sup>3</sup> blood	Lymphocytes per mm <sup>3</sup> blood	Thoracic duct lymph flow (mm <sup>3</sup> /hr)	Lymphocytes per mm <sup>3</sup> thoracic duct lymph	Lymphocyte output in thoracic duct per hr per Kg body weight	Mesenteric lymph nodes (mg/100 gm body weight)	Cervical lymph nodes (mg/100 gm body weight)
Sham-operated controls (12 animals)	207 ± 16 <sup>a</sup>	20,130 ± 1,700	17,500 ± 1,500	640 ± 62	38,800 ± 4,500	(1.17 ± .14) × 10 <sup>8</sup>	118 ± 10.3	96 ± 8.1
Thymectomized (11 animals)	196 ± 15	15,100 ± 1,400	10,500 ± 1,000	623 ± 52	10,190 ± 1,200	(0.31 ± .10) × 10 <sup>8</sup>	75.6 ± 7.8	68 ± 5.5

<sup>a</sup> Errors shown are standard errors of the mean.

CELLULAR BIOLOGY  
GROWTH OF YEAST  
UNDER CYCLIC APPLICATION OF VARIOUS STIMULI

June Barr

A study of the effects of cycling various stimuli--specifically oxygen and nitrogen, complete and depleted media--on a population developing from a starved inoculum of diploid yeast has been undertaken with the expectation that such studies may disclose patterns involved in growth, in budding, and in storage of food reserves. Such patterns may elucidate those variables which are responsible for obtaining a high degree of synchrony. A synchrostat has been constructed which automatically, by way of solenoid valves actuated by cam switches, alternates oxygen and nitrogen, discharges depleted medium, and dispenses fresh medium according to a predesignated cycle which can be varied from a total of 15 minutes to 8 hours. Modifications are in progress on the synchrostat which will allow for a more accurate timing mechanism and which will permit, among other things, subperiods corresponding to the generation time under different sets of conditions, (varying oxygen, nitrogen, and natural and synthetic complete media). Preliminary experiments have been performed to ascertain the degree of synchrony obtainable following various durations of starvation in the depleted growth medium or in succinate buffer. The nature of the cellular changes occurring during succinate starvation have been sought in spectral studies suggested by earlier cytological findings.

THE EFFECTS OF ELEVATED TEMPERATURES ON  
THE GROWTH AND INHERITANCE OF SACCHAROMYCES CEREVISIAE

Freddie Sherman

A comparative study was made of the growth of yeast in various media at the optimum temperature ( $30^{\circ}$ ) and at supraoptimum temperatures. It was found that at elevated temperatures there is a decrease in the ability of yeast to grow, which may be alleviated by (a) increasing the percentage of yeast extract in the medium, (b) adding oleic acid to the medium, or (c) using an inoculum of cells which have been previously grown at the elevated temperature. Because of these findings, it is believed that growth at elevated temperatures results in an increased nutrient requirement which may be eliminated by induced adaptation.

When yeast were grown at elevated temperatures or exposed for a short time to lethal temperatures it was found that there was a great increase in the fraction of respiratory-deficient mutants (petites). It was shown that the increase of mutants did not arise because of selection, and that the elevated temperatures actually induced the mutation. From the results of various genetic analyses it was shown that these respiratory-deficient mutants are very similar, if not identical, to vegetative petites occurring spontaneously or induced by acriflavine.

Lawrence

## ELECTRON MICROSCOPY OF YEAST

Walter Birnbaum

Efforts have been made to visualize yeast cells in the electron microscope. It had been shown that only yeast cells grown in a very particular way (Lindgren's Medium) showed a normal internal structure when prepared with standard techniques.

By investigating various fixatives and stains, it was hoped to extend the electron microscopy of yeast to include cells grown on any medium. Phosphotungstic acid proved to be of value in increasing the contrast in the cell, particularly the cell membranes.

In order to eliminate the possibility that the cell wall is interfering with staining, the yeast cells were stained after sectioning. Some success has been achieved with this technique using asmic acid and phosphotungstic acid as the electron stains.

## PHYSIOCHEMICAL STUDIES OF YEAST MICROSOMES.

James K. Ashikawa

Microsomes of different sizes and stabilities have been observed in starving, nonproliferating, and proliferating yeast cells.

When starving cells are given utilizable nitrogen, the growth curve shows a characteristic lag phase corresponding to the degree of starvation. During this phase the principal microsomal component (80S) appears to be degraded and reconstituted.

Ultracentrifugal sedimentation studies have shown that this phase is followed by the appearance of four new microsomes corresponding to the logarithmic growth phase of the cells. The concentration of microsomes reaches a maximum during log growth. As the cells pass through stationary phase a gradual quantitative and qualitative change occurs in these components.

There are studies indicating that microsomes may actively participate in protein and lipid synthesis. It is therefore, very possible that this change in the microsomes regulates biosynthesis by regulating enzymic activities.

## EFFECTS OF pH AND ANOXIA ON GROWTH AND X-RAY SENSITIVITY OF ESCHERICHIA COLI

Thornton Sargent III

The effects of hydrogen ion concentration during growth, irradiation, and postirradiation incubation on the survival of x-irradiated Escherichia coli have been studied.

Weatherwax reported that the hydrogen ion concentration during post-irradiation incubation had a large effect on this organism after ultraviolet irradiation. A much smaller but similar effect was found here after

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x-irradiation; survival was lower when the incubation medium was alkaline. The effect was largest when the organism had been grown anaerobically in acidic medium before irradiation.

A culture of Escherichia coli grown aerobically in nutrient broth becomes alkaline, but an anaerobic glucose broth culture becomes acidic. Cultures were grown aerobically and anaerobically with the hydrogen ion concentration maintained at a constant value by addition of acid or alkaline phosphates during growth. Cells from these cultures were irradiated under the same conditions of acidity or alkalinity as those under which they were grown and also under opposite conditions.

Aerobically grown cells showed the same survival regardless of hydrogen ion concentration during growth and irradiation. Anaerobically grown cells are known to show a sigmoidal or multiple-hit type of survival curve when grown under normal acidic conditions. Greatly increased radio-sensitivity was found in these cells, however, when they were grown under alkaline anaerobic conditions.

Cells irradiated under conditions of acidity or alkalinity opposite to those under which they were grown showed no modification in survival that could be attributed to an effect of hydrogen ion concentration during irradiation. Since radiochemical reactions are known to be pH-dependent this is considered evidence against intercellular indirect action by x-rays.

The well-known effect of enhancement of radiation damage by oxygen was invariably observed when cells under the various conditions above were irradiated in air and in nitrogen. The enhancement ratio was always the same, demonstrating an independence of the oxygen effect from other effects observed.

Cells from acid anaerobic cultures were found to contain many nuclei, while alkaline anaerobic cells were found to have fewer. This difference was shown by microscopic observation and by analysis of the deoxyribose nucleic acid content per cell. If killing of a cell by x-rays is due to nuclear inactivation, the difference in numbers of nuclei explains the difference in sensitivity of cells from alkaline and acid cultures. These findings, and the complete independence from the oxygen effect, provide a different explanation from that of Hollaender and his co-workers; they proposed that the protection afforded by anaerobic growth in this organism is due to anaerobiosis during irradiation caused by endogenous removal of oxygen.

It is proposed that the nuclear multiplicity of acid anaerobic cells is due to inhibition of an enzyme system responsible for separation of daughter cells after nuclear reproduction.

## THE CELL-CULTURE PROGRAM

Jim Koehler

A program of cell culture has been undertaken for the purposes of broadening and extending work done on microorganisms in this group as well as the investigation of problems pertaining specifically to cell culture. The HeLa-cell has been chosen as the initial cell line because of its ease of cultivation and applicability to the cancer problem. The strain designated by Theodore Puck as the S<sub>3</sub> clone was acquired through the courtesy of Dr. Mary McClain of the Naval Biological Research Station and is being cultured by the methods employed in that laboratory. Routine culture is being carried out in stoppered 8-oz milk dilution bottles incubated at 37°C in balance nutrient media containing 10% lamb serum. Because the cells attach to the glass within 24 hours, they can be removed for transfer or reinoculation only by a special trypsinization technique. The usual inoculum consists of about 10<sup>6</sup> cells, which increases to about 10<sup>7</sup> in the course of 3 to 4 days, at which time the cells must be transferred. Initial attempts at cultivation have been very successful, sterility requirements being no more stringent than those employed in general bacteriology.

Several problems are currently under investigation, including the morphological effects of estrogen treatment and possible uses of such changes in a rapid bio-assay of these hormones, and some basic radiation-effect work.

## MEASUREMENTS OF CELL MEMBRANE POTENTIAL

Cornelius T. Gaffey

Research efforts are to be directed at the understanding of interface phenomena at the cell membrane, such as the propagation of impulses along nerve fibers. Since nerve membrane is only 100 Å thick, the possibility of direct chemical study is largely ruled out. Analysis by electrical techniques coupled with isotopic tracer methods offers the special advantage that minute changes in membrane properties (such as resistance, capacitance, and potential difference) of the living cell are readily measurable. Studies of this sort focused on the problem of radiosensitivity of nerves to high-energy protons and deuterons permit one to obtain information about the organization of the macromolecules which are the structural elements of the cell membrane.

## AGING

### BIOLOGICAL MEASUREMENTS AND AGING

Arthur R. Tamplin

Two major disease states account for 80% of the deaths in the United States today. The number one killer is disease of the cardiovascular system, which accounts for 60% of the deaths, and the number two killer is cancer, which accounts for 20% of the deaths. The population death rates for these diseases show an exponential increase with age. Thus either individuals are more susceptible to the diseases as they grow older or the disease itself runs a course in time. In other words, something is happening in an individual with time which increases his tendency to die. Biologists have long distinguished between chronological age and physiological age; that is, they have recognized that at a given chronological age individuals will exhibit different tendencies toward death. Thus biological experience indicates that a population is a group of individuals who are undergoing physiological aging at some assortment of rates and that this physiological aging leads to death.

Obviously it would be very useful to be able to measure the physiological age or death tendency of individuals, because then those with a very high death tendency could be compared with those who have a very low death tendency in an effort to gain some insight into these aging processes. In a study of the evolution of coronary artery disease Gofman introduced a highly effective approach to this problem. Gofman found that individuals who developed clinically manifest symptoms of coronary artery disease or who died from the disease had, on the average, higher concentrations of certain serum lipoproteins. However, there was considerable overlapping of the lipoprotein distributions in the two groups. That is: many individuals of the group who didn't develop clinically manifest disease or die of the disease had levels equal to or greater than those who did develop clinically manifest disease or die of it. At this point one could say qualitatively that higher lipoprotein concentrations were somehow related to coronary artery disease. In an effort to quantitate this relationship, Gofman divided the ordinate on the disease distribution by the ordinate on the nondisease distribution at the same lipoprotein concentration, and thereby constructed a curve of probability of dying versus lipoprotein concentration. Thus here was a relatively simple method of quantitating the physiological age or death tendency in a population. Obviously by the inclusion of more biological measurements a more critical estimate could be made.

Furthermore, in this study of lipoproteins and coronary artery disease, it became apparent that the mean of those who died of the disease approached the mean of the remainder of the population as one went to the more advanced ages. In fact, if the study had been confined to individuals above 60 years of age, one might have concluded that no relationship existed. This is what one would anticipate if the lipoproteins were a measure of the rate at which individuals were developing death tendency. In other words, only individuals who were developing death tendency at a very high rate would die at younger ages, and as time went on individuals with lower and lower rates would be

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included. Thus it evolved from this study that ordinary statistical methods were not efficient in studies of aging, and a mathematical approach suited to the particular needs of the problem was necessary. This is a continuing project which is now in progress.

Broadly the concept is that an individual's physiological age or death tendency can be estimated by an appropriate combination of biological measurements. These measurements can be separated into three groups:

1. Measurements which are related to the amount of accumulated aging.
2. Measurements which are related to the rate of aging.
3. Measurements which are related to death tendency but which are constant with time or bear no relationship to time.

If we let "T" represent physiological age and let  $(n_1, n_2, \dots, n_x)$  represent some set of biological measurements, then we can express the concept above as follows:

$$T = dT = \left( \frac{\partial T}{\partial n_1} \right) dn_1 + \left( \frac{\partial T}{\partial n_2} \right) dn_2 + \dots + \left( \frac{\partial T}{\partial n_x} \right) dn_x. \quad (1)$$

Furthermore, if "D" represents the death rate, then we have

$$D = f(T); \quad (2)$$

and if the measurement is adequate, the following equation should hold:

$$\left( \frac{\partial D}{\partial t} \right)_T = 0, \quad (3)$$

where  $t$  represents time.

In practice one would calculate the relationship between "D" and the biological measurements directly without the introduction of "T", as was done for coronary disease. Then these various probabilities would be combined according to the laws of probability theory. This is a simple and rather straightforward process with independent measurement, but when the measurements are intercorrelated the complexity is enormous. However, since this does represent a bounded mathematical problem, for each population and set of parameters the various solutions are unique. Thus one can predict the maximum relationship of any one variable as the number of equally related variables is increased; or one can predict the maximum relationship of all remaining variables as the relationship of one of them is increased. Mathematically this can be expressed as follows:

$$P(D/n_0 L)_{n_0=\infty} = \pi \sum_{i=1}^n \frac{N_L^{n_0 n_i}}{N_L^{n_0}} P_A(D/n_i L),$$

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where

$P(D/n_0 L)_{n_0 = \infty}$  = extrapolated probability of dying as a function of the value of the biological measurement ( $n_0$ )

$\frac{N_L^{n_0 n_i}}{N_L^{n_0}}$  = ratio of the number of people living with a particular value of  $n_0$  and  $n_i$  to the total number of people alive with that value of  $n_0$ .

$P_A(D/n_i L)$  = absolute probability of dying provided that the individual has the particular value of  $n_i$  and is alive. This probability is measured when the probability of dying on all other parameters is unity.

The effort at present is directed toward mathematical models which will give quantitative examples of these equations. Such data should be of great value in experimental design and in evaluating experimental results. They should represent an additional set of statistical tables, which will allow workers in the field of aging to set additional quantitative limits on their results.

## GENETICS

### FRUIT FLY GENETICS

Curt Stern, Gerald Braver, Philip Hildreth, and Gweneth Carson

#### Studies of the Spouse Effect on Spontaneous and X-Ray-Induced Lethal Mutations in *Drosophila melanogaster*

Hildreth and Carson have shown that the frequency of spontaneous lethals in X chromosomes derived from wild-type males was influenced by the type of female that had been inseminated.<sup>1</sup> This phenomenon, called the "spouse effect," has been studied along the following lines:

(a) Tests were made to determine whether differences in the genetic backgrounds of the males examined for lethality were involved in the spouse effect. When the genetic background of the high-frequency line was replaced with that of the low-frequency line the X-chromosome lethals remained lethal.

(b) No clear-cut spouse effect was demonstrated for x-ray-induced lethals.

(c) Further tests of spontaneous lethals are being carried out with a new experimental design that uses one line of inbred males and three different lines of females.<sup>2</sup>

<sup>1</sup> Philip Hildreth and Gweneth Carson, Proc. Natl. Acad. Sci. U.S. 43, 175-183 (1957).

<sup>2</sup> G. L. Carson and G. Braver, Proc. Tenth. Intern. Congr. Genet. 2, 44 (1958).

### Studies on Mating Preferences and Behavior in Drosophila

As part of the design of experiments in connection with the above-described spouse effect on mutations, single males were placed in vials with one each of two genetically different females, A and B. Sequence of matings was recorded. First matings of males were equally frequently with A and with B. A male which had mated with A mated with B in only 40% of the cases and did not mate again in 60% (during a fixed period of 2 hours and 15 minutes). In contrast, a male which had first mated with B mated subsequently with A in 60% of the cases and did not mate again in 40%. Various aspects of these mating properties are under study. They have a bearing on problems of population genetics and the interpretation of some experimental determinations of fitness. A manuscript dealing with mating properties is nearly completed.

### FURTHER GENETIC STUDIES

Curt Stern, Gerald Braver, Philip Hildrath, and Gweneth Carson

### Human Twin Data: The Ratio of Monozygotic to Dizygotic Affected Twins and the Frequencies of Affected Twins in Unselected Data

A theoretical analysis of ascertainment of human twin pairs has led to the following conclusions: the ratio of monozygotic to dizygotic twin pairs ascertained on the basis of a given trait may be from one half to one times the ratio in the general population, and the ratio of twin pairs to all individuals from one to two times as high as in the general population.<sup>3</sup>

### Radiation and Population Genetics

A review of the above topic has been prepared for the Atomic Energy Commission: Curt Stern, in Radiation Biology and Medicine (Addison-Wesley, Reading, Mass., 1958), Chap. 9, pp. 206-227.

### RADIATION-INDUCED REVERSIONS

Ralph L. Gunther

Studies of radiation-induced reversion at a number of genetic loci have been carried out, using diploid cells homozygous for the particular loci under study. X-ray-induced reversions were reduced in frequency by approximately twofold when irradiation was performed in nitrogen instead of air. Some indication of differential nitrogen protection for different loci is afforded by the results, although all strains showed a "dose-reduction" factor greater than 1.7. A fiftyfold variation in mutation sensitivity occurred in different strains at doses which cause little inactivation. Of particular note is the

<sup>3</sup> Curt Stern, Acta Genet. Med. et Gemellol. 7, 313-318 (1958); Proc. Tenth Intern. Congr. Genet. 2, 275 (1958).

tenfold difference in back-mutation sensitivity between two different strains, each homozygous for one of two heteralleles at the arg-4 locus. Radiation-induced reversion of one haploid was half that of the diploid homozygous for the same marker, and DRF due to nitrogen was the same in both ploidies. Other haploid strains showed a much higher induced reversion frequency than corresponding diploids, or showed equivocal results because spontaneous mutations masked the induced ones. Thus homozygous diploid auxotrophes are favored to avoid phenotypic expression of suppressor mutations.

For the same survival, reversion due to ultraviolet light was generally higher than that due to x-rays; uv-induced reversion was highest in those strains showing highest reversion from x-rays, and conversely.

Preliminary studies with alpha particles at one locus indicated a lower efficiency for reversions for this radiation than for x-rays, as contrasted with a greater efficiency for inducing lethality.

#### PAPERS AND REPORTS

Work in this field has been reported during the period April-September 1958 as follows:

James A. Ashikawa, Ultracentrifugal Studies of Microsomes from Starving Nonproliferating and Proliferating Yeast, to be published in Proceedings of the Biophysical Society.

Ann C. Birge and Joseph A. Sayeg, The Effects of Accelerated Carbon Nuclei and Other Radiations on the Survival of Haploid Yeast. Part I: Dosimetry of the Cyclotron Beams. Part II (with Carl A. Beam and Cornelius A. Tobias): Biological Experiments; submitted to Radiation Research.

James L. Born, Orland K. Anderson, Hal O. Anger, Ann C. Birge, Paul Blanquet, Tor Brustad, Richard A. Carlson, Donald C. Van Dyke, Donald J. Fluke, Joseph F. Garcia, Julian P. Henry, Ralph M. Knisely, John H. Lawrence, Charles W. Riggs, Bo G. Thorell, Cornelius A. Tobias, Paul Toch, and Graeme P. Welch, Biological and Medical Studies with High-Energy Particle Accelerators.\*

Tor Brustad and Donald J. Fluke, Effect of Stripped Carbon and Oxygen Ions in Lysozome (Abstract), Radiation Research 9 (July 1958). (Reprint 1958-133)

R. Lowry Dobson and Mary M. Chupp, Hematologic Effects in Man of Low-Level Radiation Exposure.\*

Donald J. Fluke and Tor Brustad, Effect of Stripped Carbon and Oxygen Ions on Bacterial Spores and Bacteriophage (Abstract), Radiation Research 9, (July 1958). (Reprint 1958-134)

\* Presented at the Second International Conference on the Peaceful Uses of Atomic Energy, Geneva, September 1-13, 1958.

Joseph F. Garcia and Donald C. Van Dyke, Dose-Response Relationships of Human Urinary Erythropoietin,\*

Morgan Harris and Roman J. Kutsky, Growth Rates of Fibroblasts from Chick Skeletal Muscle in Cultures Supplemented with Homologous Nucleoproteins, Cancer Research 18, 585-91 (1958). (Reprint 1958-113)

Paul Howard-Flanders and Pierre Jockey, The Effect of Nitric Oxide on Radiosensitivity and the Mechanism of Radiation Injury (Abstract), Radiation Research 9 (July 1958). (Reprint 1958-12)

Baruch S. Jacobson, Factors Affecting the Response of Chlamydomonas to Fractionation of X-Ray Doses (Abstract), Radiation Research 9 (July 1958). (Reprint 1958-135)

John H. Lawrence and William G. Donald, Jr., Giant Follicular Lymphoblastoma: Its Treatment with Radioisotopes, Ann. Internal Med. 49, 1-16 (1958). (Reprint 1958-154)

Frank T. Lindgren, N. Keith Freeman, Alexander V. Nichols, and John W. Gofman, "The Physical Chemistry of Lipoprotein Transformation", in The Blood Lipids and the Clearing Factor, a record of the Third International Conference on Biochemical Problems of Lipids, July 26-28, 1956.

Robert K. Mortimer, Radiobiological and Genetic Studies on a Polyploid Series (Haploid to Hexaploid) of Saccharomyces cerevisiae, Radiation Research 9, 312-326 (1958). (Reprint 1958-33)

Myron Pollycove, Isotopic Measurements of the Life Span of Human Erythrocytes, Leukocytes, and Platelets.\*

M. J. H. Smith, Action of Salicylate on Metabolism of Acetate-2-C<sup>14</sup> in the Rat, Science 128, 423 (1958). (Reprint 1958-35)

M. J. H. Smith, Effects of Salicylate on the Metabolic Activity of the Small Intestine of the Rat, Am. J. Physiol. 193, 29-33 (1958). (Reprint 1958-34)

Donald J. Rosenthal and John H. Lawrence, Radioisotopes in Medicine, in Radiation Biology and Medicin: Selected Reviews in the Life Sciences, Walter D. Claus, Ed. (Addison-Wesley, Reading, Mass., 1958), Chap. 20.

Charles A. Sondhaus, The Hemoglobin Content of Single Erythrocytes in Cell Aging and Hemopoietic Disturbance (Thesis), UCRL-8203, March 1958.

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\*Presented at the Second International Conference on the Peaceful Uses of Atomic Energy, Geneva, September 1-13, 1958.

Curt Stern, Radiation and Population Genetics, in Radiation Biology and Medicine: Selected Reviews in the Life Sciences, Walter D. Claus, Ed. (Addison-Wesley, Reading, Mass., 1958), Chap. 9.

E. H. Strisower, J. W. Gofman, B. Strisower, and O. deLalla, Physiologic Effects of 1-Triiodothyronine, J. Clin. Endocrinol. and Metab. 18, 721-735 (1958). (Reprint 1958-136)

Cornelius A. Tobias and Tor Brustad, Radiobiological Studies with Accelerated Heavy Ions, to be presented at the Symposium on the Physics and Medicine of the Atmosphere and Space, San Antonio, Texas, November 1958.

Cornelius A. Tobias, Kwan Hsu, and James L. Born, Biological and Medical Studies with Accelerators, in Radiation Biology and Medicine: Selected Reviews in the Life Sciences, Walter D. Claus, Ed. (Addison-Wesley, Reading, Mass., 1958) Chap. 22.

C. A. Tobias, H. C. Mel, and D. G. Simons, Cosmic Radiation and Space Travel, Science 127, 1508-1510 (1958). (Reprint 1958-107)

Donald C. Van Dyke, The Pituitary Erythropoietic Factor, Ann. N. Y. Acad. Sci. (in press).

Donald C. Van Dyke, Joseph F. Garcia, and John H. Lawrence, \* Biological and Chemical Characteristics of Urinary Erythropoietin.

Donald C. Van Dyke, Miriam E. Simpson, Alexei A. Koneff, and Cornelius A. Tobias, Long-Term Effects of Deuteron Irradiation of the Rat Pituitary, Endocrinology (in press).

Graeme P. Welch, A Positive-Feed Fluid Pump with Variable Flow Rate, UCRL-8342, June 1958.

T. Yasugi, J. W. Gofman, O. deLalla, A. R. Tamplin, and K. Oshima, Relationship of Blood Heparin Levels to Serum Lipoproteins and Cholesterol Levels in Fasting Subjects, Proc. Soc. Exptl. Biol. 98, 46-49 (1958). (Reprint 1958-109)

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\* Presented at the Second International Conference on the Peaceful Uses of Atomic Energy, Geneva, September 1-13, 1958.

RADIATION DETECTION AND PROTECTION

Lawrence Radiation Laboratory  
University of California  
Berkeley, California

HEALTH CHEMISTRY

Nelson B. Garden in charge

Reported by Rosemary J. Barrett

GENERAL

The University of California made available meeting space and other accommodations for the 1958 Annual Meeting in Berkeley of the Health Physics Society, in June. Those groups of the Radiation Laboratory most directly concerned with this meeting--primarily Health Chemistry, Health Physics, and the Medical group--joined with representatives from the U. S. Naval Radiological Defense Laboratory, Tracerlab, General Electric (San Jose), the Atomic Energy Commission San Francisco Operations Office, and others in organizing the three-day meeting. Health Chemistry presented exhibits of their standard 2-inch portable lead shield and manipulator box assembly, the 6-inch lead cave's low-leak ventilation system, and a train for total capture of off-gas in slug-dissolving operations, used with the 6-inch cave work.

EQUIPMENT DEVELOPMENT

Maintaining the policy of keeping all radioactive work in enclosures required the fabrication of 66 units during this period. Many were standard units involving gloves or 2-inch lead shielding, and some were special items to provide atmosphere control or to furnish enclosures for unusual mechanical processes.

The routine of developing new or improving old equipment and techniques has produced progress in engineering and gadgetry as represented by the following.

Accessory equipment for use in the Donner Clinic: improvements were made on syringe shields, cone holders, shielded "cocktail" cups, and carrying cases, all of which further reduce chronic-exposure possibilities.

Foil punch for use in 2-inch lead caves: an improved actuating mechanism was made for the punching of thicker and harder foils within the caves.

Hand-tool rack in atomic-beam oven-loading box: the placement of hand tools in a convenient rack in this enclosure rather than on its floor has reduced the contamination of the tools.

Connection for liquid-waste carboy: the "Y" connection from the hoods to the liquid-waste-receiving units has been redesigned for elimination of breakage and for ease of handling of the operation.

Garden

Target cart for 60-inch-cyclotron targets: a cart has been designed to accommodate "window assembly target blocks" (consisting of target and collimator unit), for this type of target assembly is too large to be used with the existing microtarget equipment; this assembly is also prone to becoming more radioactive under the cyclotron beam bombardment; some give readings which indicate that the radiation at the surface is as high as 10,000 r/hr.

Polyethylene-sealing welding head: broader acceptance of the sealable polyethylene enclosures has required improvements in the welding head, and has necessitated quantity production to meet the diversified application of this technique.

Centrifuge head: the head of the centrifuge used in the gloved boxes has been redesigned for achievement of greater strength and rigidity.

Miscellaneous shielding: unique problems have been presented in achieving shielding for the atomic beam equipment; special size, shape, and mobility have been incorporated in lead shields in three of these work boxes.

#### INSTRUMENT GROUP

The health aspects of the separation of californium from pile-irradiated americium necessitates the following of the count of spontaneous-fission neutrons. This information is also most valuable for the chemical operations, and has heretofore been done by transferring the samples from the separation box to the counting area. This procedure is time-consuming and conducive to spills. A neutron-counting probe has been adapted for installation within the box itself, eliminating these difficulties.

#### LIQUID WASTE DISPOSAL

During this period 550 gallons of aqueous liquid waste and 55 gallons of organic liquid waste (oils) were solidified.

Sampling of all aqueous wastes discharged through the acid-waste system in Building 70 is being continued on a routine basis. No radioactivity above permissible levels has been recorded.

In line with the goal of having zero activity reach the sewer system, the installation of a monitoring system for the sink wastes from the chemistry laboratory in Building 71 (Hilac) has been completed, and the system is now in operation.

The cement-vermiculite method of waste solidification has been modified for use with high-level alpha-emitting wastes. Trials are now being made.

A report by John A. Kaufmann and Nelson B. Garden, "The Disposal of Radioactive Waste Materials at the University of California Radiation Laboratory" was issued as UCRL-8380, June 1958.

Garden

### AIRBORNE ACTIVITY CONTROL

Fallout-rainout data showed continuance of reportable fission-product activity and reports have been made to the Division of Biology and Medicine of the Atomic Energy Commission; data have also been supplied to the State Department of Public Health and to various Donner Laboratory researchers.

All "historical" atmospheric fission-product monitoring data pertinent to this site, covering the period 1951 to date, were compiled and graphed. A low concentration during July and August of each year, of unknown cause, was characteristic. A second obvious feature was the upward trend in the activity over the years.

The Airborne Activity Control Group was invited to submit its design and specifications for an active gas sampler (employing stacked activated-carbon layers) to the American Committee of Governmental Industrial Hygienists' Air Sampling Committee for inclusion in the forthcoming revision of the Encyclopedia of Industrial Hygiene Instrumentation (University of Michigan), and a draft has been submitted.

A modified and improved "general purpose scrubber" unit has been completed and laboratory-tested and is ready for use for high-level-activity applications as needed.

A special rig was devised for accomplishing three purposes in an enclosure for holding radioactive-animals; the rig achieves (a) air cleaning and ventilation; (b) cage cleaning and refuse collection (preparatory to waste disposal) by vacuum within the unit; (c) minimizing of operation exposure to penetrating radiation by enhancing speedy work.

Modifications to Hilac-target close-capture air-cleaning gear (to prevent spread of contaminants) continue as new target gadgetry is developed.

Owing not only to a shortage of personnel but also to an increased number of areas needing coverage, a cutback in frequency of operating-area air sampling became necessary (30 rooms are now on weekly instead of daily sampling). Currently, 97 areas are variously covered by the air sampling-evaluation program.

### SPECIAL PROBLEMS GROUP

During this period 66 special radioactive sources were fabricated according to the specifications (quantity and energy) of the researchers. Fifty-six sources already in existence were inspected, adjusted, and (or) repaired. Nineteen new commercial materials were tested for their suitability for laboratory purposes. In the course of detecting and identifying radioactive contamination, 280 assays were made.

Garden

HEALTH PHYSICS

Burton J. Moyer in charge

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

Beta-gamma meters	22
I D L meters	21
Juno ion chamber	20
Abacus logarithmic ion chamber	30
Recording-intensity meters	8
Victoreen proteximeter	3
Slow-neutron proportional counters	15
Fast-neutron proportional counters (portable)	11
Slow-neutron portable unit	4
Balanced chamber--fast neutron--portable	3
Special tissue wall survey instrument	1

Survey Instruments in Storage

Beta-gamma meters	7
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Personnel Meters in Use (Berkeley only)

Total personnel covered with film badges	2000
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Summary of instrument use:

Instruments	Buildings	Number per day (approx.) for 6 months	Man days of coverage	
Electrosopes	51	25	4500	
	53 and 80	15	2700	
	71	15	2700	
	70	5	900	
	2	10	<u>1800</u>	12,600 total
Dosimeters	53 and 80	15	2700	
	2	10	<u>1800</u>	4,500 total
Slow-neutron chambers	53 and 80	15	2700	
	2	10	<u>1800</u>	4,500 total

Cases of Monthly Exposure above 0.5r (excluding Livermore) (gamma readings only)

Monthly film expos. above	184-inch area	60-inch area	Bldg. 51	Chem.	Other	Total
0.5	0	12	5	8	21	46
1.0	0	0	0	1	11	12
1.5	0	0	0	0	1	1
2.0	0	0	0	1	0	1
3.0	0	0	0	2	0	2
6.0	0	0	0	0	0	0

Film-Badge Program

During Fiscal Year 1958 film badges were changed every month instead of every week, and it has been found that this permits the determination of smaller integrated exposures. In addition, study leading to design of a new film-badge holder was begun; at present it is contemplated that the new film-badge holder will be a wallet-size card and will hold two monitoring films.

**RADIATION SURVEY WORK AND RESEARCH PROJECTS**

1. Initial surveys at points remote from Building 51 were begun, to measure the radiation produced by the Bevatron. Locations include both UCRL proper and portions of residential Berkeley.

2. A high-efficiency neutron-detection system was set up and maintained for Project Sherwood. A similar system was checked prior to shipment to the Geneva Conference.

3. The design was completed and construction initiated of three very sensitive integrating ionization chambers for use under any environmental conditions.

4. A 50-channel pulse-height analyzer was put into operation to study, with a sodium iodide crystal:

- a. The gamma spectra from fallout.
- b. The gamma spectra from very sensitive neutron threshold detectors.
- c. Requirements for a low-background counting facility.

5. A facility for the activation by thermal neutron capture of various elements to provide beta and gamma calibration sources has been put into operation.

REPORTS AND PAPERS

Project reports prepared during the period April through September 1958:

H. Wade Patterson, Wilmot N. Hess, Burton J. Moyer, and Roger W. Wallace,  
The Flux and Spectrum of Cosmic-Ray-Produced Neutrons as a Function  
of Altitude, UCRL-8208, May 1958.

Alan R. Smith,  
The Stray Radiation Field of the Bevatron, UCRL-8377, July 1958.

H. Wade Patterson, Alan R. Smith, and Lloyd Stephens,  
Fallout and Natural Background in the San Francisco Bay Area, UCRL-8401,  
August 1958.

Papers for presentation at the Health Physics Society meeting in Berkeley,  
June 9-11, 1958, were indicated by these abstracts:

Lloyd D. Stephens,  
Fast-Neutron Surveys Using Indium Foil Activation, UCRL-8205 Abstract,  
March 1958.

Joseph B. McCaslin,  
High-Energy Neutron Dosimeter, UCRL-8206 Abstract, March 1958.

Theodore M. Jenkins,  
Noble Gas Scintillation for Neutron Spectroscopy, UCRL-8209 Abstract,  
March 1958.

A paper was prepared for the International Conference on the Peaceful  
Uses of Atomic Energy, Geneva, 1958:

R. Wallace, J. S. Handloser, B. J. Moyer, H. W. Patterson, L. Phillips, and  
A. R. Smith,  
Safety Problems Associated with High-energy Machines, UCRL-8069,  
March 1958.

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Information Division  
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