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UNIVERSITY OF CALIFORNIA

Radiation Laboratory

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MEDICAL AND HEALTH DIVISIONS

QUARTERLY REPORT

July, August, September, 1948

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I THE METABOLIC PROPERTIES OF PLUTONIUM

AND ALLIED MATERIALS

J. G. Hamilton

Project 48A-I

Radioautographic Studies

Dr. C. D. Van Cleave from the University of North Carolina spent two months at this laboratory learning various radioautographic methods at the request of the Atomic Energy Commission. The results he obtained with europium liver autographs were preliminary and further experiments will have to be undertaken with this element before any conclusions may be drawn.

Bone radioautographs with europium have been set up and will be completed in the next month.

Cadmium radioautographs of liver are being continued. Fixation in Bouin's was found to be unsatisfactory, so the study of alcohol fixed material is underway.

A preliminary study of the deposition of radio-strontium at short time intervals in bone has been completed. The results were inconclusive and indicated the advisability of repeating the experiment using only 2 of these time intervals. These experiments have just been started.

Ansco has made available for our work a small amount of uncoated emulsion. Using undecalcified bone, we have found it difficult to obtain satisfactory autographs. The density, fragility, and staining properties of the bone have slowed up this work considerably.

Radioautographs of praseodymium in adult rats sacrificed at 4 days resembled those obtained with cerium, actinium, and yttrium, i.e. surface deposition on the shaft and on the trabecular bone, with a somewhat spotty distribution in the shaft.

Experiments with adult animals using protoactinium have to be repeated due to the low activity in the tissue.

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Tracer Studies

Actinium. Intramuscular studies in rats with Ac^{227} have been completed up to 256 days after administration. At this time period, 5 percent remained at the site of injection. The major part of the activity absorbed was eliminated in the urine and feces. The balance of the retained activity was primarily found in the skeletal system, the skin, and in the liver. The average values of the actinium deposition in the other tissues are to be found in Table I.

Arsenic. The deposition of carrier-free As^{74} has been completed up to 32 days. These values are similar to those reported earlier with the exception of more uniformity among the observations. As was reported earlier, the major part of the arsenic is included within the red blood cells. The relative deposition of arsenic in blood as well as the other tissues is summarized in Table II. In addition to the above studies, carrier-arsenic as inactive arsenic was added to the carrier-free material in concentrations of .01, .1, and 1.0 mg, being administered to rats by intramuscular injection. As increasing amounts of carrier were added, a slight drop in the ability of the red blood cells to combine with the arsenic administered was observed, although the effect was not large. In addition to this, the fecal excretion rose at the expense of the urinary excretion. These data are summarized in Table III. The site of deposition of arsenic in the red blood cells has been investigated. The major part of the arsenic is combined with the globin fraction of the hemoglobin of the red cell and this increases with the addition of arsenic carrier. These studies are being continued in order to determine if the same situation exists in the human as in the rat, since this method of labeling a red cell when combined with the radioautographic technique should enable one to determine the life of the red cell in the body in normal and abnormal conditions.

Columbium. A series of rats have been studied after the intramuscular injection of carrier-free Cb^{95} which has been complexed with sodium citrate. One, four and sixteen day animals received 1.5 mg. sodium citrate with the activity. The 32 and 64 day animals received 3. mg. sodium citrate. When columbium is complexed in this manner, it is relatively easily absorbed from the injection site, since at 1 day following administration, 33 percent remained. This value dropped to 18.9 percent at 4 days, 8 percent at 16 days, 6 percent at 32 days, and 4.2 percent at 64 days. After absorption of Cb^{95} , the major amount of the activity remaining in the animal is deposited in the skeleton, with measurable amounts present in the liver and blood at 1 and 4 days after administration. Later time periods show rather high skeleton, liver, and kidney values, but relatively lower blood values. Columbium is excreted almost equally by both the urine and feces. This data can be seen in Table IV. In addition to the above studies, columbium was also complexed with oxalate. As with the citrate, this material also enhances absorption from the injection site. Data showing these results are to be found in Table V.

Europium. Europium with carrier has been completed to 32 days following intramuscular administration. This member of the lanthanide series of rare earths is absorbed from the injection site with difficulty. Sixty-four, sixty-three, fifty-eight, and fifty-three percent of the administered material remained at the injection site 1, 4, 16, and 32 days respectively. Of the material absorbed, the major regions of deposition were the skeleton, liver, kidney, and skin. The material found in the soft tissues is apparently gradually eliminated in the urine

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TABLE I

DEPOSITION OF Ac^{227} IN THE RAT 256 DAYS FOLLOWING INTRAMUSCULAR
ADMINISTRATION. VALUES CORRECTED FOR RECOVERY AND EXPRESSED AS
PERCENT OF DOSE.

Tissue	% per organ	% per gram
Heart	<.01	<.01
Lungs	.03	<.01
Spleen	.03	.04
Blood	<.01	<.01
Liver	.38	.05
Kidney	.06	.04
Adrenals	<.01	-
Thyroid	<.01	-
Lymph Gland	-	.03
Pancreas	<.01	<.01
Brain	<.01	<.01
Fat	-	<.01
Stomach	<.02	<.01
Sm Int	<.04	<.01
Lg Int	<.03	<.01
Skeleton	18.4	1.15
Muscle	.64	<.01
Skin	1.62	.07
Eyes	.02	.05
Pituitary	<.01	-
Gonads	<.01	<.01
Urine	2.54	-
Feces	76.2	-
	100.0	

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TABLE II

DEPOSITION OF CARRIER-FREE As^{74} IN THE RAT FOLLOWING INTRAMUSCULAR
ADMINISTRATION. VALUES CORRECTED FOR RECOVERY AND EXPRESSED AS
PERCENT OF DOSE

Tissue	4 D		8 D		15 D		32 D	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.66	.54	.26	.28	.42	.44	.57	.43
Lung	1.26	.56	.57	.31	.75	.39	1.43	.44
Spleen	.66	.70	.58	.67	.66	.75	.61	.56
Blood	44.4	2.53	39.3	2.54	37.8	2.18	39.9	2.49
Liver	2.41	.19	3.19	.57	2.47	.19	3.12	.34
Kidney	.85	.33	.56	.29	.66	.27	.67	.28
Adrenal	.01	-	-	-	.01	-	.01	-
Thyroid	.01	-	-	-	.01	-	.01	-
Lymph Gland	-	.04	-	.01	-	.01	-	.04
Pancreas	-	.03	-	.02	-	.05	-	.03
Brain	.06	.04	.03	.02	.04	.03	.04	.02
Fat	-	.01	-	-	-	.01	-	.01
Stomach	.07	.02	.03	.02	.07	.01	.05	.03
Sm Int	.15	.02	.20	.03	.15	.01	.19	.02
Lg Int	.14	.01	.10	.02	.18	.03	.10	.01
Muscle	1.47	.01	1.39	.02	1.62	.01	1.71	.01
Skeleton	2.33	.06	2.73	.08	2.12	.05	1.99	.06
Skin	.46	.01	1.36	.03	.75	.02	.70	.05
Pituitary	.01	-	-	-	.01	-	.01	-
Gonads	.06	.02	.03	.02	.04	.01	.05	.01
Fur	-	.03	-	.06	-	.10	-	.05
Urine	42.3	-	46.6	-	47.7	-	42.5	-
Feces	2.89	-	3.00	-	4.47	-	6.36	-

TABLE III

COMPARITIVE DEPOSITION OF CARRIER-FREE AND CARRIER-ARSENIC IN THE RAT
FOUR DAYS AFTER INTRAMUSCULAR ADMINISTRATION. VALUES GIVEN AS PERCENT
OF DOSE CORRECTED FOR RECOVERY.

Tissue	Carrier-free		.01 mg		.1 mg		1.0 mg	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.66	.54	.72	.61	1.02	.79	.43	.32
Lungs	1.26	.56	.79	.47	1.37	.58	.72	.43
Spleen	.66	.70	.74	.92	.69	.96	.65	.74
Blood	44.4	2.53	48.4	2.89	42.2	2.02	36.9	1.73
Liver	2.41	.19	4.28	.34	6.88	.45	3.97	.29
Kidney	.85	.33	.95	.38	1.50	.50	.94	.31
Adrenal	.01	-	.01	-	.01	-	.01	-
Thyroid	.01	-	.01	-	.01	-	.01	-
Lymph Gland	-	.04	-	.07	-	.07	-	.05
Pancreas	.02	.03	.05	.04	.04	.04	.01	.02
Brain	.06	.04	.07	.04	.07	.04	.06	.03
Fat	-	.01	-	.01	-	.02	-	.01
Stomach	.07	.02	.08	.03	.07	.03	.03	.02
Sm Int	.15	.02	.18	.02	.26	.03	.10	.01
Ig Int	.14	.01	.10	.01	.11	.02	.09	.01
Muscle	1.47	.01	2.35	.02	3.81	.03	1.63	.01
Skeleton	2.33	.06	2.63	.07	4.84	.10	2.67	.06
Skin	.46	.01	.60	.02	1.57	.03	.89	.02
Pituitary	.01	-	.01	-	.01	-	.01	-
Gonads	.06	.02	.05	.01	.06	.02	.06	.02
Fur	-	.03	-	.02	-	.02	-	.03
Urine	42.3	-	33.3	-	21.7	-	38.6	-
Feces	2.89	-	4.66	-	13.8	-	12.2	-

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TABLE IV

DEPOSITION OF CARRIER-FREE Cb^{95} COMPLEXED WITH CITRATE* IN
THE RAT FOLLOWING INTRAMUSCULAR ADMINISTRATION. VALUES
GIVEN IN PERCENT OF DOSE CORRECTED FOR RECOVERY.

Tissue	1 D		4 D		16 D		32 D		64 D	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.42	.56	.16	.16	.17	.17	.16	.17	.13	.22
Lungs	.81	.63	.47	.23	.59	.25	.30	.18	.46	.20
Spleen	.24	.54	.19	.23	.39	.36	.32	.34	.25	.47
Blood	22.2	1.71	7.72	.48	.68	.04	.21	.01	.17	.01
Liver	3.97	.58	8.42	.61	4.54	.35	2.56	.24	2.08	.22
Kidney	1.45	.77	2.92	1.20	1.85	.64	2.14	.80	.89	.41
Adrenals	.04	-	.03	-	.03	-	.01	-	.01	-
Thyroid	.02	-	<.01	-	<.01	-	<.01	-	<.01	-
Lymph Gland	-	.26	-	.09	-	.47	-	.27	-	.37
Pancreas	.14	.38	.17	.13	.13	.13	.17	.16	.10	.17
Brain	.04	.02	.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01
Fat	-	.32	-	.08	-	.08	-	.15	-	.07
Stomach	.89	.08	.28	.05	.13	.03	.12	.06	.13	.04
Sm Int	1.96	.24	1.94	.16	1.56	.12	.94	.10	.73	.07
Lg Int	4.80	1.01	4.13	.40	1.32	.16	1.13	.11	.52	.05
Skeleton	23.6	1.43	16.2	.88	9.05	.42	8.38	.43	6.89	.37
Muscle	14.1	.14	7.96	.07	8.86	.07	6.83	.06	3.89	.04
Skin	12.0	.40	8.48	.20	10.4	.20	6.90	.16	4.94	.10
Gonads	1.29	.52	1.41	.41	1.46	.63	1.27	.38	1.14	.69
Eyes	.06	.16	.04	.09	.03	.08	.03	.11	.02	.08
Pituitary	<.01	-	<.01	-	<.01	-	<.01	-	<.01	-
Urine	7.18	-	18.0	-	31.5	-	44.3	-	35.6	-
Feces	4.84	-	21.4	-	27.2	-	24.2	-	42.0	-
	100.0		100.0		100.0		100.0		100.0	

* 1, 4, and 16 day animals received 1.5 mg citrate; 32 and 64 day animals 3.0 mg citrate.

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TABLE V

DEPOSITION OF CARRIER-FREE Cb^{95} COMPLEXED WITH OXALATE IN
THE RAT 4 AND 64 DAYS AFTER INTRAMUSCULAR ADMINISTRATION.
VALUES GIVEN AS PERCENT OF DOSE AND CORRECTED FOR RECOVERY.

Tissues	4 Days		64 Days	
	% per organ	% per gram	% per organ	% per gram
Heart	.21	.33	.07	.09
Lungs	.71	.59	.34	.15
Spleen	.44	.87	.21	.33
Blood	4.22	.38	.10	.01
Liver	4.12	.62	1.91	.26
Kidney	3.00	2.09	1.39	.87
Adrenals	.05	-	.02	-
Thyroid	.06	-	.01	-
Lymph Gland	-	.89	-	.29
Pancreas	.10	.32	.07	.07
Brain	.02	.01	.01	.01
Fat	-	.44	-	.07
Stomach	.37	.07	.08	.03
Sm Int	1.73	.29	.66	.07
Lg Int	2.20	.32	.52	.06
Skeleton	25.9	2.20	10.7	.72
Muscle	7.49	.09	1.99	.02
Skin	10.3	.33	2.82	.10
Eyes	.06	.16	.01	.07
Pituitary	.01	-	.01	-
Gonads	.20	.82	.03	.15
Urine	29.8	-	41.5	-
Feces	9.08	-	37.6	-
	100.0		100.0	

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and feces so that 32 days after administration the primary organ of deposition is skeleton. These data are summarized in Table VI.

Protoactinium. Pa^{230} has been completed up to 64 days following intramuscular administration. This member of the actinide series of elements is absorbed with difficulty. There remained at the site of injection 71 percent, 71 percent, 67 percent, and 59.2 percent at 4, 16, 32, and 64 days respectively. Of the material absorbed, the major portion was in the skeleton and in addition in soft tissues such as liver, kidney, and muscle. Protoactinium is excreted in both the urine and the feces and 64 days after intramuscular administration, approximately 40 percent of the material was excreted. These data are summarized in Table VII.

Praseodymium. Intramuscular studies on praseodymium have been completed. This material, which was reported previously is now being prepared for declassification, and will be submitted as a complete report.

Uranium. Intramuscular studies using U^{233} have been completed up to the 254 day period. Less than 1 percent of this material remained at the injection site at this time period; the primary avenue of excretion being the urine. Of the total material absorbed, 3.2 percent remained in the skeleton, .36 percent in the kidney, and traces in muscles and skin. (See Table VIII)

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TABLE VI

DEPOSITION OF EUROPIUM* IN THE RAT FOLLOWING INTRAMUSCULAR
ADMINISTRATION, USING Eu^{154} AS A TRACER. VALUES CORRECTED
FOR RECOVERY AND EXPRESSED AS PERCENT OF DOSE.

Tissue	1 D		4 D		16 D		32 D	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.12	.06	.07	.07	.06	.06	.04	.02
Lungs	.18	.06	.18	.11	.11	.06	.11	.06
Spleen	.18	.18	.15	.22	.17	.19	.08	.11
Blood	.53	.04	.15	.01	<.01	<.01	<.01	<.01
Liver	33.1	2.70	26.3	2.50	11.9	1.16	6.40	.59
Kidney	2.76	1.06	2.54	1.14	1.74	.86	1.95	.70
Adrenals	.02	-	.01	-	<.01	-	<.01	-
Thyroid	<.01	-	.01	-	.01	-	.01	-
Lymph Gland	-	.06	-	.11	-	.08	-	.08
Pancreas	.02	.03	.02	.02	.01	.02	.01	.01
Brain	.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01
Fat	-	.01	-	.26	-	.01	-	.01
Stomach	.35	.12	.18	.04	.14	.06	.11	.04
Sm Int	.88	.12	.59	.07	.80	.08	.97	.11
Lg Int	6.40	.71	.59	.07	.83	.14	1.00	.08
Skeleton	24.3	.76	33.3	.99	37.8	1.05	38.6	.95
Muscle	1.35	.01	6.58	.04	8.17	.06	1.12	<.01
Skin	3.88	.12	4.30	.11	1.74	.03	2.84	.06
Gonads	.06	.04	.07	.02	.17	.08	.06	.02
Eyes	.01	.03	.01	.03	<.01	.03	<.01	.02
Pituitary	<.01	-	<.01	-	<.01	-	<.01	-
Urine	12.9	-	11.0	-	12.6	-	16.2	-
Feces	13.0	-	14.0	-	23.7	-	30.5	-
	100.0		100.0		100.0		100.0	

*Approximately .6 micrograms of Europium was administered with the Eu^{154} .

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TABLE VII

DEPOSITION OF PROTOACTINIUM IN THE RAT FOLLOWING INTRAMUSCULAR
ADMINISTRATION. VALUES CORRECTED FOR RECOVERY AND EXPRESSED
AS PERCENT OF DOSE

Tissue	4 D		16 D		32 D		64 D	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.25	.31	.20	.23	.22	.32	.20	.26
Lungs	.76	.52	.54	.37	1.10	.65	.65	.42
Spleen	.66	.95	.53	.68	.95	1.28	1.04	1.67
Blood	2.83	.25	.78	.06	.78	.06	.44	.04
Liver	7.79	1.24	4.33	.51	5.04	.48	4.84	.66
Kidney	4.05	2.63	2.57	1.41	4.10	2.16	2.97	1.87
Adrenals	.06	-	.03	-	.07	-	.06	-
Thyroid	.02	-	.01	-	<.01	-	.01	-
Lymph Gland	-	1.92	-	2.44	-	3.02	-	.87
Pancreas	.21	.45	.13	.31	.40	.59	.15	.30
Brain	.04	.02	.01	.01	.01	.01	.02	.01
Fat	-	.15	-	.10	-	.18	-	.10
Stomach	.23	.04	.14	.04	.25	.06	.20	.10
Sm Int	1.28	.17	.81	.09	1.39	.16	1.34	.17
Lg Int	1.61	.21	.88	.10	1.56	.16	.88	.14
Skeleton	45.1	2.85	45.9	1.99	35.2	2.16	36.4	2.29
Muscle	7.09	.08	5.78	.06	7.83	.07	5.84	.06
Skin	6.12	.23	6.30	.18	6.06	.21	4.73	.17
Pituitary	<.01	-	<.01	-	<.01	-	<.01	-
Eyes	.06	.21	.04	.17	.09	.23	.05	.20
Gonads	.10	.79	.03	.67	.10	1.22	.10	.72
Urine	9.03	-	11.2	-	15.6	-	19.6	-
Feces	12.7	-	19.8	-	19.2	-	20.4	-
	100.0		100.0		100.0		100.0	

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TABLE VIII

DEPOSITION OF U^{233} IN THE RAT 254 DAYS AFTER
INTRAMUSCULAR ADMINISTRATION. VALUES CORRECTED FOR RECOVERY
AND EXPRESSED AS PERCENT OF DOSE.

Tissue	% per organ	% per gram
Heart and Lungs	<.01	<.01
Spleen	<.01	<.01
Blood	<.01	<.01
Liver	<.01	<.01
Kidney	.36	.15
G. I.	<.01	<.01
Skeleton	3.20	.08
Muscle	<.05	<.01
Skin	<.02	<.01
Urine	81.5	-
Feces	14.9	-
	100.0	

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Decontamination and Bone Metabolism Studies

Effect of Zirconium Citrate on the Elimination of Radiocerium. Prompt treatment with massive doses of zirconium citrate has been shown to cause a marked increase in the urinary excretion of radioyttrium and plutonium, with a corresponding reduction in skeletal deposition. Such treatment has been found ineffective in the case of radiostrontium. The present experiment was undertaken to determine the effect of zirconium citrate on the excretion and bone retention of radiocerium. Cerium was chosen as a representative of the group of rare earths and actinided earths which have a high initial liver activity, and are excreted largely by way of the feces.

Procedure: Adult female rats, with weights ranging from 275 - 375 grams, were injected intravenously with 0.25 ml. of an isotonic salt solution (pH 5-6) containing approximately 5.5 microcuries of carrier-free Ce^{144} . Five rats were then injected intraperitoneally with 1.6 ml. of a zirconium citrate solution (containing 40 mg. Zr). A second group of five rats received an equivalent amount of sodium citrate (1.6 ml. of a 10 percent solution), while a third, untreated group served as controls.

The animals were placed in individual metabolism cages, fed stock diet, and urine and feces were collected separately. At the end of three days, the rats were sacrificed and tissues and excreta were analyzed for Ce^{144} .

Results: The results expressed as percent of the administered dose, are shown in Table IX. The average recovery was 94.3 percent. Both sodium citrate and zirconium citrate caused a significant increase in the urinary excretion of Ce^{144} , although the effect was not as striking as was the case with radioyttrium and plutonium. Less Ce^{144} was found in the livers of the zirconium citrate treated animals, but the difference was not statistically significant. There was also no significant reduction in the skeletal deposition of Ce^{144} or in its retention in the carcass.

Conclusion: Zirconium citrate injections (ineffective with radiostrontium) do cause an increase in the urinary excretion of Ce^{144} , although the effect is much less marked than for radioyttrium and plutonium. However, the effect appears to be too small to be of any value in decontamination.

Effect of Massive Doses of Parathormone and Zirconium Citrate on the Elimination of Radioyttrium. Although massive injections of zirconium citrate will increase the urinary excretion of radioyttrium and plutonium, the effect is greatly reduced if sufficient time has elapsed for these elements to become fixed in bone. Since large doses of parathormone produce active bone resorption, it was hoped that such treatment might free the previously bound elements so that they might be eliminated with zirconium citrate.

Although the rat is rather resistant to the action of parathormone, previous experiments (Quarterly Report - April, June and July 1948) indicate that intraperitoneal injection of 500 units of the hormone will produce histological evidence of very active bone resorption with 24 hours. (This dose increased the urinary excretion of radiostrontium, but did not significantly reduce the bone retention of the element.) The effect of this dose of parathormone, in conjunction with .

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TABLE IX

THE EFFECT OF ZIRCONIUM CITRATE AND SODIUM CITRATE ON Ce^{144}

Tissues	Controls	Sodium Citrate 1.6 ml. 10%	Zirconium Citrate 40 mg. Zr.
Urine	3.6 ± 1.2	9.7 ± 1.1	12.8 ± 2.4
Feces	1.6 ± 0.3	2.0 ± 0.7	2.6 ± 0.9
Femur	0.6 ± 0.1	0.7 ± 0.04	0.5 ± 0.04
Liver	57.1 ± 9.8	60.6 ± 2.9	42.2 ± 6.3
Kidney	4.6 ± 5.4	2.8 ± 1.6	1.5 ± 0.6
Spleen	3.2 ± 2.0	2.6 ± 0.8	3.6 ± 0.9
Carcass	25.5 ± 3.4	25.4 ± 2.7	22.5 ± 6.4

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zirconium citrate, was studied in the following experiment.

Procedure: Young female rats (weight range 120 - 150 grams, age 6 - 7 weeks) were used because of their greater susceptibility to the action of parathormone. Each was injected intravenously (foot vein) with 0.25 ml. of an isotonic salt solution containing 45 microcuries of carrier-free Y^{90} and 10 micrograms of Pu^{239} . The pH was around 5. The rats were divided into 6 groups according to the treatment given.

- (1) Control group - 6 rats, no treatment.
- (2) Pretreated - 4 rats, injected IP with 40 mg. Zr as citrate in 1.6 ml. 48 hours prior to the administration of Y^{90} and Pu^{239} .
- (3) Immediate treatment - injected IP with 40 mg. Zr at the same time as the administration of the Y^{90} and Pu^{239} .
- (4) Post-treated - injected IP with 40 mg. Zr, 48 hours following the administration of the radioyttrium and plutonium.
- (5) Parathormone treated - injected IP with 500 units of parathormone (E.Lilly) in 5 ml., 24 hours following administration of Y^{90} , Pu^{239} .
- (6) Combined parathormone and zirconium citrate treatment - injected IP with 500 units (5 ml.) parathormone 24 hours and 40 mg. Zr as citrate 48 hours after the administration of the radioyttrium and plutonium. The Zr treatment was timed to coincide with the maximum effect of parathormone.

The rats were placed in individual metabolism cages, and urine and feces were collected daily. They were fed the regular stock diet. At the end of six days, the animals were sacrificed and tissues and excreta were analyzed for Y^{90} and Pu^{239} . At present, data is only available for the Y^{90} .

Results: The results of this experiment are shown in Table X. The values are expressed as percent of the administered dose and the figures given are the averages for each group, - the standard deviation.

The urinary excretion of Y^{90} was significantly increased in all the groups treated with Zr, the most marked effect being obtained when treatment was given immediately following the administration of Y^{90} . The massive dose of parathormone had no effect on urinary radioyttrium, whether given alone or with zirconium.

Skeletal uptake of radioyttrium was significantly reduced in the rats pretreated with Zr, and in those given injections of Zr immediately after the dose of Y^{90} . When the treatment was given 48 hours after the dose of radioyttrium, the reduction in bone uptake was slight, and unaffected by parathormone. The massive dose or parathormone by itself had no effect on the concentration of radioyttrium in bone, despite the very active bone resorption produced by this dose.

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TABLE X

EFFECT OF PARATHORMONE AND ZIRCONIUM
CITRATE ON THE METABOLISM OF Y^{90}

Tissues	Control	Pretreated 40 mg. Zr 48 hrs. before Y^{90}	Immediate Treatment with Zr Citrate	Parathormone 500 units 24 hours	Zr Citrate at 48 hours	Parathormone 24 hours, Zr Citrate 48 hr.
Urine	9.7 $\pm$ 0.8	16.0 $\pm$ 1.3	28.0 $\pm$ 6.2	8.6 $\pm$ 0.6	16.1 $\pm$ 1.5	13.6 $\pm$ 0.9
Feces	9.5 $\pm$ 0.9	11.6 $\pm$ 0.2	7.4 $\pm$ 3.0	8.5 $\pm$ 1.0	12.7 $\pm$ 1.2	13.1 $\pm$ 2.8
Femur	2.9 $\pm$ 0.1	1.8 $\pm$ 0.1	1.6 $\pm$ 0.4	3.4 $\pm$ 0.5	2.4 $\pm$ 0.4	2.6 $\pm$ 0.3
Liver	10.1 $\pm$ 1.3	13.7 $\pm$ 2.7	17.2 $\pm$ 3.9	7.8 $\pm$ 2.6	8.2 $\pm$ 1.6	10.7 $\pm$ 1.8
Kidney	2.3 $\pm$ 0.2	1.2 $\pm$ 0.1	1.0 $\pm$ 1.3	1.5 $\pm$ 0.3	1.9 $\pm$ 0.2	1.8 $\pm$ 0.2
Spleen	0.2 $\pm$ 0.03	0.5 $\pm$ 0.2	0.7 $\pm$ 0.3	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.02
Carcass	62.8 $\pm$ 4.4	55.5 $\pm$ 6.4	43.5 $\pm$ 7.8	71.7 $\pm$ 4.3	56.4 $\pm$ 3.3	56.7 $\pm$ 2.7
Recovery	97.7 $\pm$ 3.7	99.9 $\pm$ 5.1	99.4 $\pm$ 3.4	101.9 $\pm$ 3.2	97.9 $\pm$ 1.7	99.0 $\pm$ 3.3

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The animals treated with Zr had lower carcass values for radioyttrium, but the difference was only significant in the group given immediate Zr treatment.

Conclusions:

(a) Despite the active bone resorption produced by massive doses of parathormone, such treatment appears to have no effect on the skeletal uptake or excretion of radioyttrium.

(b) Intraperitoneal injection of large amounts of zirconium citrate (40 mg. Zr) 2 days before, or immediately after the administration of Y^{90} produces an increased urinary excretion and a reduced uptake by bone.

(c) While zirconium citrate injections hold some promise as a de-contamination procedure, there appears to be little value from parathormone treatment.

Work in Progress

(a) Effect of parathormone and zirconium citrate on Pu^{239} . Plutonium assays are being made on the tissues and excreta of the animals reported above.

(b) Kinetic studies of radiostrontium uptake and excretion.

(c) Effects of severe phosphorus deficiency on bone depletion.

(d) Endocrine factors in bone formation: The effect of thyroid and growth hormone on the deposition of radiostrontium.

Radiochemical Isolation

An improved distillation method was found for separating carrier-free tin from alpha-bombarded cadmium. The low yields previously attributed to the absorption of tin to the walls of the containing vessel have been eliminated. In the modified technique, concentrated hydrochloric was used to remove tin bromide from the carbon dioxide carrier-gas and citric acid was added to this solution prior to evaporation. Tartaric and oxalic acids were also found effective in preventing the absorption loss.

Carrier-free arsenic was separated from two deuteron-bombarded germanium targets. A distillation method which has been previously reported was used. Approximately 75 microcuries of arsenic was obtained from each target.

A 10 millicurie sample of Oak Ridge carrier-free Yttrium (Y^{91}) was purified. A contamination of Ce^{144} was found and removed using a thorium iodate precipitation method.

An improved technique for separating columbium from zirconium was worked out. The method involves a preliminary manganese dioxide precipitation to separate columbium from zirconium followed by a separation of columbium and manganese carrier using a ferric hydroxide scavenger.

Several other Oak Ridge shipments were worked on: carrier-free Sm^{151} was submitted to Mr. Stewart for radio-purity determination and it was found to contain only one activity; solutions of Eu^{154} and Eu^{155} were characterized and physiological solutions were prepared.

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Method of Preparing Anthracene Crystals Suitable
for Scintillation Counters

Mr. John Alley has spent considerable time in developing the following method for the preparation of anthracene crystals.

Anthracene has been demonstrated to be one of the better phosphors for use with the scintillation counter (1) (2) (3). The following method utilizes equipment available in most laboratories and has produced water clear crystals of anthracene up to ten grams. The majority of crystals over four to five grams have contained small internal cracks. It is hoped however, that proper annealing techniques may eliminate this type of imperfection.

The process in use at this laboratory consists of two steps: purification of the anthracene, and formation of the crystals. If Eastman Kodak 480-X anthracene is used, purification may be accomplished by two recrystallizations from absolute ethyl alcohol*. Because of the low solubility of anthracene in alcohol, 25% by volume of benzene may be added. A recrystallizing apparatus similar to Fig. 4 is suggested for the purification phase.

Anthracene is placed in the chamber (A) above the sintered glass filter with the alcohol-benzene mixture below (B). When heated, the vapors will bypass the filter, condense, and dissolve the anthracene, bringing it through the filter and into the lower chamber. After the anthracene has been dissolved from the upper chamber, and recrystallized in the lower, it is cooled to room temperature, filtered, and washed with absolute alcohol. The filtrate and washings are discarded. Residual alcohol remaining on the crystals is removed with a vacuum pump. However, for storing, the anthracene may be left damp, as the vapor will facilitate the removal of air in the next step.

After purification, the anthracene is placed in a flask made from pyrex tubing and shaped as shown in Fig. 3. Anthracene will decompose if heated in the presence of oxygen and therefore the flask is evacuated and sealed off.

The neck of the flask may be constricted for sealing under vacuum, after being filled with anthracene if the air in contact with the anthracene is first displaced with an inert gas such as helium. To insure a good seal all anthracene should be removed from the surface to be constricted by gentle heating before applying the full heat of the torch.

To form the crystal, the anthracene in the flask is melted and then slowly cooled from the bottom toward the top. To effect directional cooling, a cylindrical electric combustion furnace is placed on end and insulated so that cooling will

*If anthracene other than Eastman Kodak 480-X is used, thermal recrystallization in the furnace may effect a sufficient separation of impurities so that a more efficient and convenient process may be developed (recrystallization from an appropriate solvent found by solubility tests on the impurity).

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tend to start from the bottom, (Fig. 1). The sealed flask, from which all air has been evacuated, is put inside the furnace and covered with a heat shield (Figs. 1 and 2). This is done to further facilitate directional cooling, and to minimize rapid temperature changes. Two variable voltage transformers are connected in series with the furnace, one of which is used to bring the furnace up to the melting point of anthracene (217°C). The full range of the other transformer is then used to slowly reduce the power supply.

Originally the transformer was turned down manually at an initial rate of approximately one two-hundredth of the total power per hour. After determining a rate which gave satisfactory results, manual operation was replaced with a constant speed motor and reduction gears. A thermometer was placed in the system (Fig. 1) and crystals were formed by cooling the melt at an initial rate of 1 to 2°C per hour. After 24 to 30 hours the cooling rate was increased to 4 to 5°C per hour.

Because there is a temperature gradient through the anthracene, the temperature measured at one point in the furnace during crystal formation will go through a range which will depend upon the gradient in the system and depth of crystal being formed.

The crystal is taken out of the furnace when it reaches room temperature and is removed from the flask by carefully breaking away the glass. It can then be cut or sawed, and polished with a soft rag and benzene.

It is suggested that room temperature variation, if rapid or large, be avoided and that a constant voltage transformer be used if the line voltage is not regulated.

There is no reason, at present, to believe that the dimensions of the equipment are critical. However, the combustion furnace used has an internal diameter of 7.8 cm. The heat shield is of 64 mm. tubing and 24 cm. in length. It is covered with aluminum foil and asbestos sheeting which was softened with water. The edges were glued together with sodium silicate solution. The furnace was also insulated with asbestos sheeting. Cracks and protruding metal parts were covered with asbestos and water mixed to the consistency of putty. Insulation on sides and top was three to four times as thick as on the bottom of the furnace.

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FIG. 1

FIG. 2

FIG. 3

RECRYSTALLIZING APPARATUS

FIG. 4

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II BIOLOGICAL STUDIES OF RADIATION EFFECTS

J. H. Lawrence - in charge

Project 48A-II

Biological Use of High Energy DeuteronsC. A. Tobias, Paul Rosahn*, Margaret Lewis**, Hal Anger,
and J. H. Lawrence***

In the Quarterly Progress Reports for March-June, 1948, methods of observation and measurement of light nuclei for the purpose of biological experimentation were outlined. It was pointed out that the use of high speed protons, deuterons, or alpha particles offers two distinct advantages:

- (1) One should be able to irradiate deep-lying tissues or organs of animals selectively and without inflicting severe damage to the skin and underlying layers. Thus there is a possibility for experimental tumor therapy and for the observation of the nature of biological effects due to localized treatment.
- (2) These particles are suitable for carrying out certain crucial experiments concerning the nature of the biological effects on chromosomes and on certain micro-organisms. Significant advances may be expected in the comparative study of radiations with different specific ionization.

Like all new experimental tools the methods for biological use of the cyclotron also have to undergo a considerable period of development, during which instrumentation and the method of measurement is continually improved. Since accurate measurement of dosage is of importance, considerable time has been spent on the problem. In addition, several biological experiments were begun. In reading the data presented below, one should bear in mind that, as in previous reports, the work is in a preliminary stage, and the conclusions reached should not be regarded as final.

Measurement of the Physical Characteristics of Deuteron and Alpha Beams. When the high energy alpha or deuteron beam is brought out to the air from the cyclotron; it has to pass through three different focusing devices - an electric deflector, a magnetic deflector, and an external focusing magnet. The variable characteristics of these deflecting devices allow shifting of the position of the external beam and considerable variation of its intensity distribution at the exit port. For biological experimentation it was necessary to align the treatment bench exactly with the beam. In order to accomplish this a track system was devised to carry all measuring instruments and biological specimens. Since one is not allowed to be present in the bombardment room while the beam is turned on, the table is equipped with remote control. The position of the beam with respect to the treatment table

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*** With the assistance of Mrs. Constance Tregillus and Mrs. Jean Luce.

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is indicated by Quadrant ionization chambers (BP 109). Photograph 1 shows the complete table with some of the monitoring ionization chambers and other equipment used.

The most convenient monitors are parallel wall ionization chambers which measure the rate of ionization in the air between two parallel electrodes. In order to test the accuracy of this method and to obtain absolute calibration, it appeared worthwhile to begin an investigation of the dependence of ion current on the size of the chamber, the material of the electrodes, the applied voltage, etc. For this purpose an extrapolation chamber was built (shown in Photograph 2). Briefly, the following conclusions were reached by the use of this chamber:

(a) Ionization chambers of this type have a wide plateau in dry air, ranging from 600 volts/cm to at least 800 volts/cm field. The slope of the plateau is better than 1 percent per 100 volt/cm (see Diagram 1).

(b) Using a constant deuteron or alpha particle beam and staying on the flat part of the plateau, the ion current varies proportionally with the distance of the electrodes (see Diagram 2).

(c) When one of the electrodes is replaced by another electrode made of different material, there seems to be no significant change in the ion collection as long as the thicknesses of the electrodes correspond to equivalent stopping power. Four different electrode materials were tried: Graphite on polystyrene, Al, Cu, and Pt. Within the error of measurement (S. E. 2 percent) of these electrodes did not seem to influence the ion current at all. However, when a fine beryllium copper wire screen was used as an electrode, the ionization was 4 percent higher than with the other electrodes. This phase of the study is still being continued, and it is possible that with refined measuring methods there will be a small electrode effect detected in measuring the ion current. From a biological viewpoint it is important to estimate the fraction of ionization which is due to recoil nuclei from elastic collisions since recoil nuclei with high specific ionization may exert relatively larger biological effects (possibly by a factor of 10 higher) than those with low specific ionization.

(d) Distribution of beam ionization in a plane perpendicular to the direction of the beam was studied in the extrapolation chamber with a set of circular electrodes of different electrode areas. Diagram 3 shows typical curves obtained for the current density when the beam is limited with an aperture and after it has gone through different thicknesses of Al absorber. The ionization in air behind these layers of absorber is higher than without the absorbers since slower particles ionize more corresponding to the Bragg curve of ionization. Also scattering of the particles in the absorber will make the apparent width of the beam greater, as may be seen clearly from the diagram. These sets of electrodes enable us to study the mean angle of scattering as well as the mean displacement of the particles due to scattering. Such data will be of value to depth therapy as well as to Physics. Detailed results of the measurements will follow in a later report.

(e) The ion current was collected on a capacitor and measured by means of a slide back vacuum tube volt meter which operates on the principle of manual compensation of the current. Using this instrument, absolute evaluation of the charge collected in the ionization chamber was made, and the measurements were compared to the dosage measured in other types of instruments. First, the standard Victoreen X-ray thimble ionization chambers were tried. For reasons unknown

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several of these chambers proved to be entirely unreliable in their response when placed in the alpha or deuteron beam. They showed more than 200 percent fluctuation in response and sometimes they were completely discharged after a small dose. None of these effects appeared when the instruments were retested in a gamma ray field. Later members of the Health Physics group, Drs. Bert Moyer, and Roger Hildebrand, built some gelatin walled ionization chambers filled with air and saturated water vapor. The ionization in these chambers agreed quite well with the ionization measured by the method described above, and these results are shown in Table I. At the present time thus there are two independent methods of dosage measurements available, both of which usually give the same result within 6 percent standard deviation. Another set of measurements was carried out to compare with the calibration of Failla and Rossi (Columbia University). The results of this comparison are not fully evaluated as yet.

The above described measurements are part of a program to describe completely the range, ionization, and scattering of high energy particle beams with sufficient accuracy for all biological experiments.

Preliminary Studies of Radiation Effects on Mouse Tumors. Having made a preliminary study of the lethal effect of the beam (Quarterly Progress Report for March-June, 1948), it seemed worthwhile to carry out a few experiments to localize the ionization of the deuterons in some tumors. These experiments have already proved indicative of the possible future usefulness of high energy nuclear radiation.

The choice of the experimental animals was somewhat difficult. It is of advantage to use as large an animal as possible for two reasons: (1) The size of the tumor at the time of irradiation should be small compared to the size of the animal. If this is the case the tumor may be reached by a pencil of radiation without having to irradiate too large a part of the body of the animal. (2) The size of the tumor should be larger than 1 cm because the beam will scatter out to at least this diameter as it passes through the body of the animal, even if very small apertures are used. Both of these conditions favor the use of rabbits over rats or mice. The Brown-Pierce carcinoma available in rabbits, however, spontaneously regresses and fails to cause lethal effects in a large percentage of the animals. Since clear-cut evidence of the radiation effect was needed, mice were used in the first experiment. The data reported here refer throughout to Type A (Strong) mice carrying a transplantable mammary carcinoma (1). This strain has been carried on for many years in the Donner Laboratory. With care there are 100 percent takes among the transplants and 100 percent of the mice having such tumors die from the carcinomas. At the onset of these experiments it was quite clear that one could not hope for a 100 percent regression of tumors after the treatment since several conditions had to be rigorously fulfilled. The tumor had to be irradiated over its entire volume; at the same time the body should not receive more than a moderate dose. These conditions are only partially satisfied in the mice where the skin lying over the tumor will unavoidably receive much radiation. Metastasis of the tumor also might influence the outcome. Using small numbers of mice (there was only limited cyclotron time available), the tumor lethal dose was first established. This was done by pulling the entire tumor, enclosed in a skin fold, away from the body of each animal and endeavoring to direct the beam in such a way that only the tumor and the skin fold would be hit; the rest of the body was avoided. Table II

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shows the results, and it was found that 2,000, 3,000, or 4,000 d_{190}^* can cause complete regression in about one-half of the animals, in harmony with the observations of Lawrence, et al (2). 1000 d_{190} appeared to have retarded the tumor growth only for a limited time period. Diagram 4 indicates the weight loss of these animals and their survival time as compared to controls.

The principle which we intended to prove in a practical way was that by the use of deuterons, protons, or alpha particles it is possible to deposit a larger amount of energy in a tumor than in the tissues of the animal lying between the tumor and the radiating device. Since the mouse tumors were transplanted under the skin, the mice were placed in the next experiments in such a way that the deuteron beam had to pass through their bodies first before reaching the tumors. The regression dose of the tumor was several times the lethal dose for whole body irradiation of the animals and therefore it was decided to further enhance the effectiveness of the treatment by a technique equivalent to multiple port irradiation.** The tumors of the animals were fixed in space and the animals themselves were rotated slowly around the tumor, describing an arc of 160 degrees. This allowed distribution of the beam in the animal body outside of the tumor and also increased the probability of the most ionizing part of the beam to hit the tumors uniformly. The device actually used is shown in photograph 3. The mice were placed in a lucite tube in a vertical position, anesthetized, and partially immersed in water of body temperature. The tumor of the animals was aligned in the center line of the cylindrical water bath. Absorbers were placed in the path of the deuterons to bring the peak of ionization to the same spot where the tumor was located. The dose rate was about 800 d_{190} per minute and each mouse received treatment of only three or four minutes duration. Two separate experiments were performed. In each of these there were twenty mice irradiated and twenty tumor-bearing mice passing through the same treatment, but receiving no radiation. The dose selected was 2800 d in the first experiment, and 3500 d in the second experiment. The beam aperture was adjusted to fit the size of the tumors of the individual mice.

The effect on the irradiated mice (see Table II) in each instance was quite uniform. Tumor tissues exposed to the most ionizing portions of the beam exhibited rapid regression, in some instances quite complete ten days after irradiation, leaving only scar tissue. At no time thereafter was there any tumor growth observed in any of the mice at or near the central spot of irradiation. A good number of the mice, however, in the treated group still showed continuing tumor growth, resulting apparently from two different causes: (1) The beam was not aligned perfectly in a number of instances and parts of the tumors not affected by the beam continued to grow. (2) There were metastases of the original tumors far from the original location which appeared to show uninhibited growth after irradiation was administered. In some cases these tumors were along the path of the trocar used for transplanting the tumor, and possibly they represented the deposition of cell fragments at the time of transplantation of these tumors. In spite of these disturbing effects and of the additional irradiation to other parts of the body of the animals where the beam passed through, five of the first twenty treated animals and four of the second group of twenty mice survived the treatment with no ill effects.

*"1 d_{190} " corresponds to a beam of deuterons which produces 1 e.s.u. of ions of either sign in 1 cm^3 air at N.T.P., when the deuteron energy is 190 Mev. Since such a beam penetrates tissue, the dosage will vary with depth in a manner described by the Bragg curve, and influenced by scattering.

**The ratio tumor dose to skin dose at point of entry was about 3:1 to the multiple port treatment. It increased to about 10:1 with the rotation method.

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Altogether nine animals out of forty treated were found which survived up to the present time (3 1/2 months after irradiation) with their tumors completely regressed and disappeared. These animals today are apparently normal.

Photographs 4 and 5 show two of the unirradiated control mice about 30 days after transplantation of the tumor, and a few days before death occurred. One of the tumors is necrotic. The irradiated animals are shown in photographs 6 - 13. Photographs 6 - 10 were taken 30 days after transplantation and 20 days after irradiation treatment. Photographs 6 and 7 show animals where the treatment was not entirely successful. Note the area of bombardment indicated by arrow and the necrotic tumors growing at a different site. Photographs 8, 9, and 10 show tumor mice successfully irradiated. Complete absence of the tumors may be noted; they were replaced by scar tissue. The skin of these animals was epilated and reddened about ten days after irradiation treatment. Some of the areas treated became ulcerated, others showed dark red blisters, and hemorrhagic blebs. If no metastases develop, these animals usually recover from the skin effects in two or three months; otherwise most of them die by this time. At the end of three months the skin appeared normal in color, the epidermis somewhat thickened at the site of irradiation, and in most of the animals the hair began to grow back. Such animals are shown in Photographs 11, 12, and 13. These animals are being kept for further observation. By the end of three months all of the control animals had died due to uncontrolled growths of their mammary carcinomas. Diagrams 5 and 6 show survival time, weight loss, and change in tumor size of the various groups.

The irradiated tumor animals lost weight early but gained it back in a short time. Their white and red cells and hemoglobin became somewhat low, as evidenced by the fact that in two of the animals the white blood counts were approximately 5,000 on the third day after irradiation, and the red blood count had an average level of four million. The normal mice in this group have white and red counts of 10,000 and 10,000,000 respectively.

The statistics of the experiments carried out so far are not as yet too convincing. Nine animals out of a treated forty had complete regression of tumors and have now survived for three months after bombardment. Of forty controls only two animals are still alive and in these two the transplanted tumors did not take. All of the irradiated animals had well developed tumors at the time of irradiation. The results furnish an indication, however, as to what could be accomplished if better techniques of localization of tumors and more knowledge of the dosimetry were available. They show that selective deposition of large doses of radiation is quite possible deep in the animal body, by judicious use of accelerated light nuclei. These preliminary results would seem to establish for the first time the fact that unlike X-rays, it is possible to pass deuterons through the animal body and exert a lethal effect on a tissue without irreversibly damaging the normal tissues through which the beam passes in order to reach the tissue to be destroyed. This ratio of greater biological effect in the depths compared to the surface dose is present because of the nature of the ionizing mechanism. The relationship between surface and depth dose for these high speed deuterons is much more favorable toward a relatively greater depth dose for deuterons than high speed beta rays from a betatron and we felt these known physical facts should be demonstrated and established on a living animal.

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Chromosomal Derangements Caused by Deuteron and Alpha Particle Beams. Dr. and Mrs. Norman H. Giles from the Biology Laboratory at Oak Ridge spent several weeks in Berkeley carrying out experiments on the chromosomes of tradescantia pollen in association with the Donner group. The aim of these experiments was to evaluate the relative biological effectiveness of portions of the beam with high specific ionization vs. portions with low specific ionization. The high specific ionization proved to be much more effective in producing chromatid and chromosome effects than low specific ionization in apparent agreement with previous work done with neutrons, gamma rays and X-rays. A detailed report will follow at a later time.

Acknowledgements: The authors wish to thank Professor E. O. Lawrence, Dr. Robert Thornton of the Radiation Laboratory, for their interest in this problem, and Mr. James Vale and the Cyclotron crew for their kind cooperation.

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TABLE I

COMPARISON OF TWO METHODS OF DOSE MEASUREMENT
(DEUTERON BEAM)

I		II	II/I
Tissue Wall Chamber Calibrated Using γ rays of Ra.		Extrapolation Chamber Based on Measurement of Charge Collected.	Ratio of Numerical Values of Dose
No.	Dose in r	Dose in d*	
A	51.2	50.8	0.995
A	45.4	44.5	0.940
A	27.4	28.8	1.05
A	27.6	31.2	1.13
B	47.6	46.4	0.975
B	42.4	43.5	1.03
B	55	52	0.94
C	78.7	77.0	0.950
C	27.6	31.2	1.13
Average ratio			1.02
Standard deviation			0.06

* For definition of the d see footnote, p. 6.

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TABLE II

RESULTS OF DEUTERON IRRADIATION OF MAMMARY
CARCINOMA IN "A" MICE

THE BODY OF THE MICE SHIELDED AND THE TUMOR EXPOSED

Dose	No. of Animals	Percent Recovered Permanently with Complete Regres- sion of Tumors	Death Apparently Due to Growth of Tumors	Percent Died Due to Radia- tion Effects
Controls- no dose	20	6.7%	100%	0%
1,000 d	5	0 %	100%	0%
2,000 d	19	42 %	52.6%	5%
3,000 d	18	33 %	50%	17%
4,000 d	19	50 %	43.8%	6%

TUMOR EXPOSED THROUGH THE BODY OF THE
MICE IN THE MANNER DESCRIBED IN
TEXT

2,800 d	20	25%	60%	15%
3,500 d	20	20%	18%	62%

*For definition of the d see footnote p. 6.

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Rate of the Retardation in the Appearance of Iron⁵⁹
In the Circulating Hemoglobin of Rats Following
Irradiation with 180 Kv X-rays at Varying Dose Levels

Rex L. Huff and Thomas G. Hennessy

Twenty five female rats weighing from 180 to 210 grams were divided into five groups and given 5, 25, 125, 250 and 0 roentgens total body radiation. Measurement of the radiation was with a Victoreen Ionization Chamber and a lucite phantom approximating the size of a rat. One day following radiation each of the rats was given intravenously .002 milligrams of iron 59 as ferric chloride buffered with sodium citrate having a specific activity of 1×10^{-10} . At regular intervals thereafter one half ml. of blood was drawn from each of the rats by cardiac puncture, and analyzed for its iron 59 content. Following is the averaged values per milliliter of packed red cells from the various groups of five animals at the specified time periods.

TABLE I

Counts per Minute per Milliliter Packed Red Blood Cells

Roentgens	Days Following Administration of Fe ⁵⁹					
	1	2	3	5	8	12
0	-	-	To be Reported		-	-
5	936	1346	1652	1527	1421	1424
25	568	965	1265	1387	1554	1577
125	227	395	672	668	975	975
250	266	160	206	385	561	635

It is readily seen from Table I that there is an inverse relationship between the amount of total body radiation given a rat and the rate at which radio iron appears in the peripheral red cells. This relationship also appears to hold for the percent of the total dose which is incorporated in red cells for the twelve day period of observation.

To calculate the total amount of radio iron which has been incorporated in the red cells the total red cell mass must be known. In this laboratory red cell mass has been determined in this species of rat with both the iron and phosphorous tagged cell method. The data from these determinations indicate that the blood volume constitutes approximately 4.34% of the body weight. Following is a graph showing the percent of administered dose in red cells calculated on the basis that 4.34% of body weight is blood volume against increasing periods of time.

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PLATEAU OF PARALLEL WALL ION CHAMBER
MEASURING 190 MEV PULSED DEUTERONS

DISTRIBUTION OF INTENSITY IN THE CROSS SECTION OF 190 MEV DEUTERON BEAM DIFFERENTIAL CURVE

DIAGRAM 3

SURVIVAL AND WEIGHT LOSS OF IRRADIATED TUMOR BEARING MICE
DIAGRAM 4

SURVIVAL AND WEIGHT LOSS OF TUMOR MICE IRRADIATED THROUGH THEIR BODY

DIAGRAM 5

CHANGE IN TUMOR SIZE FOLLOWING DEUTERON IRRADIATION
DIAGRAM 6

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PHOTOGRAPH-1

Bench for exposure of biological specimens with high energy light nuclei from the 184" Cyclotron. The particles travel as indicated by the arrows. Legend: (A) Brass diaphragm. (B) Monitoring ionization chamber. (C) Absorber wheel. (D) Container to expose film. (E) Ionization chamber for centering the bench. (F) Ionization chamber for tissue dosage measurements. (G) Extrapolation chamber. (H) Telescope. (I) Servo mechanism.

PHOTOGRAPH 2

Extrapolation chamber (shown on the left). The distance of the electrodes and the size of electrode areas may be changed.

PHOTOGRAPH 3

Device for exposing mice to the particle beam. Beam enters from the right as indicated by the arrow. "A" is a lucite container. An anesthetized mouse is placed in it with his head on top. The whole tube is then placed in container "B" in such a way that the part of the mouse to be irradiated is at the center of tube "B" marked by "X". Irradiation may be carried out by the device with the tube "B" at rest, or "B" may be rotated into a different angular position. Continuous rotation of "B" is also possible. "C" indicates absorbers which are usually placed in the beam in order to bring the maximum specific ionization to the right depth "X".

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PHOTOGRAPH 4

PHOTOGRAPH 5

The above photographs show a mice thirty days after transplantation of mammary carcinoma not treated by radiation.

PHOTOGRAPH 6

PHOTOGRAPH 7

Mice treated on the tenth day after tumor transplantation with deuterons. The beam was directed through the body. In these animals the radiation did not hit a large enough area and metastasized tumors finally caused death. The photographs were taken 30 days after irradiation.

PHOTOGRAPHS 8 and 9

PHOTOGRAPH 10

Mice with mammary carcinoma successfully irradiated with deuterons. The pictures were taken 30 days after transplantation and 20 days after irradiation. The tumor tissue has completely disappeared with only scar tissue remaining. Note the lack of hair and the ulceration where the beam was directed.

EXPERIMENT #79
BOMBARDED WITH DEUTERONS
PICTURE TAKEN OCT. 8, 1948

EXPERIMENT #79
BOMBARDED WITH DEUTERONS
PICTURE TAKEN OCT. 8, 1948

PHOTOGRAPHS 11 AND 12

EXPERIMENT #79
BOMBARDED WITH DEUTERONS
PICTURE TAKEN OCT. 8, 1948

PHOTOGRAPH 13

Photographs 11, 12, and 13 show mice which were successfully treated with deuterons. The photographs were taken 100 days after transplantation and 90 days after irradiation. Note that the skin has healed, is normal in color, and the hair is beginning to grow back. There are no signs of tumors. The mouse in photograph 8 is the same as the one in photograph 11, and the one in photograph 9 is the same as the one in photograph 13.

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III BIOLOGICAL EFFECTS OF RADIATIONS FROM EXTERNAL AND INTERNAL SOURCES

Robert S. Stone, - in charge

Project 48C

University of California Medical School

Statement of the Program

R. S. Stone

As has been stated before, the Arthritic Clinic of the University of California Hospital, composed of physicians from the Medical, Orthopedic and Radiological Divisions, became interested some years ago in the effects of total body irradiation on chronic arthritics, particularly those with ankylosing spondylitis. Such patients are therefore selected for this type of treatment by physicians having no connection whatever with the Atomic Energy Commission, and their treatment and follow-up is given by physicians in their capacities as members of the staff of the University of California. Project 48-C, a hospital portion of the Radiation Laboratory, has taken advantage of the fact that these patients were being treated in this manner to study the effects of total body irradiation on the blood.

From the inception of the program in 1942 until October 1946, the patients were treated by exposure of their whole bodies to x-rays of various energies from 100 to 1000 kv. Starting in December 1946 radiophosphorus was used as the source of radiation, being given intravenously, and it has been used exclusively since that time. The last patient to be treated in this group received her final treatment on May 24, 1948.

The treatment of arthritic patients with x-ray has been going on for many years. The treatment of a large portion of the body for arthritis was started about 1920 by S. Gilbert Scott in England. The first treatments of this type at the University of California Hospital were given in 1942. In 1944 Doctor Hans Wayne, an internist with a particular interest in arthritics, began referring patients to Doctor Low-Beer to study the effects of P^{32} on this incapacitating disease. The first patients treated were not studied as to the effects on the blood for the Atomic Energy Commission. These patients were not clinic patients of the University of California, but private patients of Doctor Wayne. It was only after this type of therapy had been utilized on these patients that it occurred to us that they would be an excellent group on whom to study the hematological changes. Consequently patients treated since that time, either private or clinic have had blood studies made under the auspices of the Atomic Energy project. The Arthritic Clinic and the private physicians concerned with arthritis are still continuing to send us patients for treatment with total body irradiation, local x-ray irradiation and P^{32} . We intend to continue treating these patients in our capacities as staff members of the University Hospital and private physicians, and hope that the Atomic Energy project will not object to this excellent material being utilized.

Patients with diseases of the thyroid report to the Thyroid Clinic of the

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University of California Out-Patient Department or to the various private physicians on the hospital staff who are interested in thyroid disease. From these sources they are referred to the Radiological Division for studies with radioactive iodine. The various studies carried out are reported from time to time in these reports. We had hoped to do more blood studies as well as metabolic studies on the patients being so treated, but to date various factors including lack of enough funds to employ extra technicians and consultant hematologists has kept us from doing this work. We consider that studies of the blood on these patients would be very valuable to the Atomic Energy Commission program.

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Hematological Effects of Total Body Irradiation

B.V. Low-Beer

X-Ray. Nine of the 32 patients who were treated prior to October 1946 with x-rays applied to the entire body for the disease from which they were suffering are still continuing to be followed by means of blood counts. Little of significance has been found in the present period, but we would like to call attention to two items.

In the first place, it is significant that the three patients who were treated with 100 kv x-rays have not shown any changes which we could detect either during or since the completion of their treatment. Two of them received 10 r per day to a total of 300 r and the third received 20 r per day to a total of 300 r.

Second, it had seemed that the lobe index of patients who had had treatments a considerable time ago, was continuing to fall and this was a cause of some worry. It now appears that by current techniques and technicians the lobe indices of new patients before any treatment is given are at roughly the same level as the lobe indices of those patients who were treated some years ago. It would thus seem as if there is either a technical variation in the way the counts were done or an actual change in the lobe indices of the people coming to our clinic. This matter is being given further technical and statistical analysis, but in view of the current findings it would seem to be connected with some other factor than the irradiation of the patient.

Insofar as possible, the patients who were irradiated with x-rays are being kept under continuing observation.

Radiophosphorus. Blood studies were started on 22 patients who were being treated with P^{32} . Eighteen of these are still being followed. The longest period of observation being 605 days and the shortest 106.

In the previous report (UCRL-98) we stated that the P^{32} used was carrier-free. We were and still are using the P^{32} supplied from Oak Ridge which we now believe to contain approximately 5 mg of inert phosphate per millilitre.

Table I shows the amounts of P^{32} given to the patients and the intervals between doses. The patients can be divided into two groups, since 9 of them received totals of between 6000 and 7000 microcuries of P^{32} , and 12 patients received 8000 microcuries. Practically all of these patients have shown some effect on the total white cell count, the neutrophilic granulocyte count and lymphocyte count. Some of the patients had rather definite changes in the platelet and erythrocyte counts and the hemoglobin concentration.

The lobation indices of these patients has changed, but as was mentioned above in connection with the studies on patients who were treated with x-rays, the change of technicians and possibly some other factors have apparently had a greater effect on the lobation index than the radiation. This point is being investigated further.

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Calculations are being made of the dosage rate in rep per day and of the total dose from the beta rays of the P^{32} . The present indications are that a patient who has had a total of 6000 microcuries of P^{32} injected in a period of 14 days, receives a total of 57 rep at daily rates not exceeding 2.5 rep. The changes observed are so much greater than one would expect from a dose of 57 reontgens of 200 kv x-rays given at the same rate or even as one single dose that it is difficult to conceive of a satisfactory correlation between dosage from internal emitters and external sources. This matter will be discussed more fully in our next report.

The experimental treatment of arthritic patients with P^{32} was started in 1944 by Hans Waine and B.V.A. Low-Beer. Such treatment was based on the fact that the arthritic lesions and particularly those associated with rheumatoid arthritis, are characterized by inflammatory hyperplasia and increased vascularity of connective tissue. Experimental studies by Doctor Low-Beer have shown that inflammatory tissue takes up a much larger proportion of P^{32} than normal tissues. X-ray treatment to large parts of the body was advocated by S. G. Scott as a means of treating this generalized disease process. On theoretical grounds, it appeared justifiable therefore to treat with P^{32} . Waine and Low-Beer so treated 5 patients with peripheral rheumatoid arthritis. One showed marked improvement, subsidence of fever and of local active arthritis, gain in weight and return of the sedimentation rate to normal. One showed a temporary improvement with lessened pain and muscular stiffness, coinciding with a decrease of sedimentation rate. The remission lasted for only 3 months. Two showed no change, and one had an exacerbation of her disease following treatment. Hematological examinations which were made frequently during and after treatment showed no lasting untoward effects from the treatment. This experience encouraged us to continue to treat arthritic patients experimentally with P^{32} , since the marked relief of even one patient in five is a great accomplishment in this disease.

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TABLE I

Pt. No.	P ³² dose in μ c	Interval Days	P ³² dose in μ c	Interval Days	P ³² dose in μ c	Interval Days	P ³² dose in μ c	Interval Days	P ³² dose in μ c	Total dose in μ c	Total Time in Days
33	2000	7	2000	7	2000	7	1000	7	1000	8000	28
34	2000	7	2000	7	760	7	1620			6380	21
35	2000	7	2000	7	1000	7	3000			8000	21
36	2000	6	2000	7	2000	7	700			6700	20
37	2000	7	1548	7	2452	7	482			6482	21
38	2000	7	2000	7	2000	7	659			6659	21
39	2000	7	2000	7	2000	27	2000			8000	41
40	2000	7	2000	7	2000					6000	14
41	2000	32	2000	7	2000					6000	39
42	2000	26	2000	7	2000	7	819			6819	40
43	2000	7	1650	patient did not return						3650	7
44	2013	7	1998	7	2000	7	2012			8023	21
45	2000	7	2000	7	2000	6	2000			8000	20
46	2020	7	2000	6	2000	7	2000			8020	20
47	2000	8	2000	7	2000	7	2000			8000	22
48	2000	7	2000	7	2025	9	2000			8025	23
49	2000	7	2000	5	2000	9	2000			8000	21
50	2000	7	2000	7	2000	7	2000			8000	21
51	2000	7	2000	7	2000	7	2000			8000	21
52	2000	7	2000	9	2000	7	2000			8000	21
53	2000	7	2000	7	2000					6000	14
54	2000	7	2000	7	2000					6000	14

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Metabolism and Effects of Radio-Iodine (I^{131})

Earl R. Miller

The results of the investigation on metabolism and effects of radio-iodine I^{131} have not been sufficiently different from those in the last report to warrant detailed discussion at the present time.

Studies are being made on the use of an ionization chamber designed and built by Doctor B. J. Moyer at the Radiation Laboratory. This ionization chamber is about two inches in diameter and ten inches long. It has attached to it an electrometer circuit with an amplifier and the ionization current is read on a microammeter. Preliminary calibration studies on this instrument show that it is a stable measuring instrument and one whose calibration apparently has remained fairly constant over this short period of observation. It has four settings of sensitivity. Full scale deflection on the microammeter is attained with the exposure of the ionization chamber to the radiations from 181, 835, 4850 and 21,600 microcuries of Iodine I^{131} , when these amounts are put in a bottle and the bottle placed in contact with the ionization chamber. Preliminary studies show that under these conditions, as would be expected, the readings obtained from a given amount of iodine would be very definitely dependent upon the size of the bottle in which the iodine is placed. If the largest of a series of bottles is put in contact with the ionization chamber, and smaller bottles are placed so that their centers occupy the position previously occupied by the center of the large bottle when it was in contact with the chamber, then the readings from the same amounts of iodine are the same. Preliminary studies show that we can get reasonably accurate results with as low as 50 microcuries.

Although the ionization chamber is not sensitive enough to permit measurements at a distance, still we find it useful for making rapid measurements of the relative concentration of I^{131} in the thyroids of patients as a function of time. We are able to tell the percentage gain or loss of radioiodine in the thyroid with approximately 5 percent accuracy in a given patient. In order to make the curve of relative iodine concentration in the thyroid quantitative, the actual concentration can be measured by the Geiger counter at any one time interval. This one observation in microcuries of I^{131} in the thyroid, establishes the actual value at that point on the curve of relative concentrations. Hence, the actual concentration at other times can be determined.

During the preliminary studies, each ionization chamber measurement is being checked by an almost simultaneous Geiger counter measurement. A more complete description of results of the use of this instrument will be given in the next report.

In several patients with carcinoma of the thyroid, destruction of the thyroid has been achieved by radioiodine in doses on the order of 60 microcuries. Clinical and chemical myxedema has been produced. Destruction of the thyroid was carried out in order to see if the metastases would concentrate iodine when the normal thyroid ceased functioning. So far no success has been attained with this group of patients, most of whom has anaplastic growths. Studies are being continued on this.

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IV HEALTH CHEMISTRY AND PHYSICS

Health Chemistry

N. B. Garden

Personnel Monitoring in Areas Containing Radioactive Materials. During the summer quarter the monitoring requirements of the chemists in Bldgs. 4 and 5 were particularly heavy. This was due to the unusually large number of target bombardments during this period, which amounted to approximately 180. With the influx of many new chemists unfamiliar with techniques of handling radioactivity, it has been necessary to give more attention to the individual. A small handbook on the services and equipment available from the Health Chemistry Section is being prepared, which will include basic points in techniques of handling radioactive materials. This should prove of particular benefit to personnel to whom this field is new and it is hoped that it will assist in preventing health hazards and technical contamination.

Tests have been made with "band-aid" -type finger rings wherein the film is wrapped around the finger and held with a type of Scotch tape. The use of this will more accurately determine the radiation received on the hands and will supplement the information given by the regular film badges.

An improved ventilated glove for hot weather or for long periods of wearing has been used successfully.

Transportation, Disposal, Decontamination, Salvage. With the advent of heavy target work, mentioned above, the demand on the radioactive materials transportation group was very great, involving around-the-clock activities almost daily. An improved jig for more efficient and safe removal of the targets from the cyclotron probe is being developed.

Ten gloved boxes and many centrifuges have been decontaminated successfully. A decontamination chamber, now in mock-up stage, is being developed; among its proposed features will be a motorized electric dolly and a sump for decontaminating by electrolysis. The latter process has been tried on a small scale and appears to be extremely promising for the decontamination of certain types of objects.

Approximately 26 cement-packed, radioactive waste-containing drums and 6 to 8 packages per month were disposed of at sea.

Research and Development. The main projects under development or completed during this period are listed below:

1. The controlled atmosphere arc for spectroanalysis of radioactive materials by Dr. Conway was completed and tested.
2. Cow and spinner column developments were held inactive pending receipt of the latest techniques of chemically separating plutonium and hexone as developed in Chicago.

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3. A decontamination chamber was developed to the mock-up stage, plus the development of some of its accessories.
4. A strip coating study was carried out on 26 products; six are being further studied for aging tests, two of which seem highly satisfactory for the purposes to which they will be put. Numerous strip-coating jobs have been done on gloved boxes, hoods, and other equipment used in radiochemical area. Preliminary coatings have greatly facilitated dismantling and wrecking jobs in contaminated area.
5. Many gloved boxes have been assembled, in addition to the boxes sent to Los Alamos, for local use and one each for the New York City Golden Jubilee Exhibit and for Hanford.
6. Constant temperature baths for gloved boxes for microchemical work were developed and built.
7. A specially equipped box for Dr. Gofman for processing yttrium and strontium with minimum exposure has been almost completed.
8. The Webb 60-inch cyclotron target assembly for the S. G. Thompson interceptor run was completed and made ready for trial runs.
9. A portable 1-inch lead manipulator panel, to be used by itself or in connection with other panels, was designed and is under construction.
10. A target assembly for the 60-inch cyclotron for bombardment of recoil samples is under construction.
11. A continuous air sampler with alarm counter for alpha-contaminated air is being built.

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Health Physics

B.J. Moyer

Survey of Fast Neutron Field Outside 184" Cyclotron Shielding. A study is in progress in the evaluation of the fast neutron component of the radiation field outside the 184 inch cyclotron shielding.

As a first approach to the problem, measurements were made with an argon-CO₂-filled, brass proportional counter lined with 1/16" of polyethylene. The discriminator was set to reject proton recoil pulses below about 0.1 mev., and this effectively eliminated contributions from secondary electrons due to γ -rays in a chamber of the dimensions used.

Due to the increasing thickness of polyethylene effective as the neutron energy increases, and due also to the decrease of the n-p cross section, the efficiency of such a counter is roughly proportional to neutron energy between 0.1 and 10 mev. Above 10 mev. the polyethylene thickness is less than the maximum range of recoil protons. Consequently, the counter measures a quantity roughly proportional to the product of neutron flux and energy, and the interpretation of the flux is dependent upon assumed mean energy. Using a neutron source of known yield, and of approximately known energy distribution, a numerical value could be found for the efficiency.

In Figure 1 is given the results of a survey under the beam conditions stated there. The r.m.s. errors, from statistical considerations, range from about 40% for fluxes as low as 2 neutrons/cm²sec to about 10% for fluxes above 15 n/cm²sec. A mean energy of 1 mev. applies to these interpretations of flux.

A coincidence counting system, with a thin, adjustable wall between the counting volumes which are in coincidence, is under construction. This may allow an estimate of the energy distribution of the neutrons outside the shielding, and as a function of depth within the shield material.

Slow Neutron Measurements. Using the improved slow neutron standardization equipment, which was involved in the measurements reported last quarter on the slow neutron flux within the shielding material, a recalibration of the neutron pocket dosimeters is in progress. Considering 2000 slow neutrons/cm² sec to be a tolerance flux over 8 hours, the typical pocket chamber gives from one to two daily doses per full scale deflection when using a particular projection-type minometer assigned to this work.

A balanced-chamber electrometer, utilizing one chamber filled with enriched BF₃ and the other filled with argon has been constructed and calibrated. The slow neutron flux it indicates for the general building floor area is 50 to 100 neutrons/cm²sec. This is to be compared with a value of 60-70 determined by previous work with indium foils. Changes in disposition of large blocks of concrete, which are waiting on the floor for use on the shielding roof, cause variations in the values in localities involved.

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Studies of Film Dosimetry. In order to give a better understanding of our photographic dosimetry results, in which film blackening may be due to radiations of widely varying energies, a study of the dosage response vs. quantum energy has been begun. Results are not yet ready for publication.

Statistical Summary of Personnel Monitoring Service Provided..

A. Survey Instruments Maintained

1. β - γ Ionization Chambers	28
2. Victoreen 263 Meters	19
3. I.D.L. Portable Survey Meters	6
4. Cutie Pies	2
5. Recording γ -Intensity Meters	10

B. Personnel Meters in Use

Number of people covered with film badges	600
Total man days coverage with pocket chambers	818
Total man days coverage with pocket dosimeters	1164
Total man days coverage with pocket chambers, s.n	1067

C. Cases of Above Weekly Tolerance Dosage

1. 184 inch area	4*
2. Linear Accelerator Area	0
3. 60 inch area	0

* These people are chemists and received their exposures working on radioactive samples. The exposures are respectively as follows:

- 1.6 x weekly tolerance dose
- 1.5 x weekly tolerance dose
- 1.2 x weekly tolerance dose
- 7.0 x weekly tolerance dose

FIG. 1

SURVEY OF FAST NEUTRONS OUTSIDE OF CYCLOTRON SHIELDING
FOR A BEAM STRENGTH OF 1 r. ON $\frac{1}{2}$ " Be. TARGET @ 81" RADIUS
INTENSITIES IN NEUTRONS/cm²sec. (ASSUMED ENERGY 1 MEV) AT
SHOULDER HEIGHT

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ABSTRACT