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I THE METABOLIC PROPERTIES OF PLUTONIUM AND ALLIED MATERIALS

J. G. Hamilton

Project 48 A-I

Radioautographic Studies

The project for the comparison of the differences in localization of Ca, Sr, promethium, Am, and Pu in the costachondial junction of adult rats 1 and 24 hours after injection is nearing completion. At present Ca and promethium autographs are complete as is Sr at the longer interval. Preliminary autographs of Pu have been examined. The Sr at 1 hour interval is being repeated at a higher dose since thorough uptake was demonstrated after the 10 μc dose. The activity within sections was so slight as to require excessively long exposure time. As anticipated the deposition patterns of Ca and Sr are thus far similar. Promethium differed in that the element deposited only on surface of cortical bone and at the margin of calcified and non-calcified cartilage of the chondral segment of rib. It will be noted that spotty deposition of promethium in the cortical bone was not observed though the femur of the same animals showed this well known pattern, a characteristic which has been demonstrated to be associated with the vascular distribution within the bone. This behavior in the rib bone is to some extent confirmatory of the earlier findings since histological studies show the cortical bone to be very thin and virtually avascular as the consequence of this lack of vessels. Paravascular distribution in the spotting pattern would not be expected. Examination of available Pu autographs of costochondial junctions confirm the earlier observation of no appreciable localization in the cartilagenous part of rib even in the neighborhood of cartilage calcification by ordinary autographic techniques. More critical studies are to be performed using NTB plate techniques to determine whether any localization of these elements are taking place.

These studies have been delayed due to loss of the technical assistant and the necessity for training new personnel in the special technique necessary. They are, however, being resumed.

A study on any possible toxicity of I^{131} is being conducted using .4 dose levels ranging from 10 μc to 500 μc with post-injection intervals of 4, 30 and 180 days. This will form the basis for comparison of this element's effectiveness in destruction of thyroid tissue with that of astatine (At^{211}). Histology of endocrine and non-endocrine organs and hematopoesis are being studied.

The work on americium and praseodymium is still in progress.

Tracer Studies

Zirconium^{95} and Niobium^{95}. [1] The fate of Cb^{95} and Cb and Zr^{95} have been studied in normal and tumorous rats after it has been complexed with various agents which

prove to be capable of removing the plated out Zr and Zr and Cb on a straight chemical test. These data are summarized in Tables I to VI. It can be seen that all these materials, picolinic acid, nitrilo-tri-acetic acid, ascorbic acid, malonic acid, tartaric acid and lactic acid are all capable of influencing the distribution of Cb^{95} and $Cb\text{-}Zr^{95}$ in the rat regardless of the route of entry as compared to the uncomplexed and probably colloid-like materials, which has been published in earlier reports.[2] The salient point which stands out with this type of complexing is that (1) the uptake from an intramuscular injection site is greater with complexed than non-complexed material, (2) excretion is facilitated primarily via the urinary tract, (3) there is a lessened quasi-permanent deposition in skeleton as well as in the other tissues of the body. The uncomplexed carrier-free zirconium and columbium show skeletal depositions between 25 and 50 percent of the material absorbed, as compared to values for complexed material of less than 15 percent. Urinary excretion at 1 day after administration of uncomplexed material was less than 2 percent as compared to values of 7 to 20 percent when Cb was complexed. In consideration of the complexing agents themselves, it would appear that there is no variation between any of the items earlier mentioned with the exception that lactic acid does not reduce the final deposition in liver and skeleton to such low levels as citrates. It is interesting to not that of the tumor bearing rats investigated, the deposition in tumors approaches that or is higher than any other soft tissues in the body. The comparison of the various complexing agents with each other are summarized in Table VII.

Cerium^{144}. Attempts to complex Ce^{144} have not been successful when hexa-anti-pyrine and nitro-tri-acetic acid has been tried in rats following intravenous and intramuscular injection. The usually high liver and skeletal depositions which are characteristic of uncomplexed cerium were obtained. This indicates that the complex did not persist in the rat. There is a suggestion that nitrilo-tri-acetic acid facilitates the urinary excretion of Ce^{144} since these values are appreciably higher than those obtained with the use of hexa-anti-pyrine. Tables VIII, IX, X. Earlier data obtained at 4 days after intramuscular injection gave liver and skeletal values of 49 and 25 percent respectively when uncomplexed cerium was administered. These values agree with those summarized in Table VIII.

Samarium^{153}. The fate of Sm^{153} plus 35 micrograms of carrier per rat, complexed with ammonium citrate and administered to normal and tumor bearing rats has been studied up to 8 days following intramuscular and intravenous injection. Examination of Tables XI, XII, XIII and XIV show that this element is not different from the other lanthanide rare earths in that it seeks out the skeleton as well as liver, kidney and spleen. Samarium is excreted with difficulty. One experiment with BAL (Table XIV) did not alter the deposition of samarium in the rat.

Selenium^{75}. Deposition of carrier-free Se^{75} in rats has been completed 16 and 64 days after intramuscular injection. The 16 day group is summarized in Table XV. At this time period, the 99 percent of Se^{75} administered was absorbed from the injection site. 84 percent had been excreted in feces and urine. Values obtained in the tissues did not demonstrate selective localization to any extent. At 64 days after intramuscular administration, the values on the tissues of the animals were too low to count. 96.3 percent of the material administered was recovered in the urine and feces, this being 77.3 and 18.9 percent respectively.

Rhodium^{105}. Preliminary studies on carrier-free Rh^{105} have been carried out following intramuscular injection and summarized in Table XVI. The major organ of deposition is the kidney and liver. Examination of the intestinal contents indicated that fecal excretion would have contributed to the total excreted by the animals had the experiment been continued for longer than 18 hours following intramuscular injection. 67 percent of the absorbed dose was found in the urine at this time period.

TABLE I

THE DEPOSITION OF CARRIER-FREE Cb^{95} COMPLEXED WITH NITRILO TRI-ACETIC ACID AND PICOLINIC ACID* IN RATS 4 DAYS AFTER INTRAMUSCULAR INJECTION. DATA ARE EXPRESSED IN PERCENT OF DOSE ABSORBED AND CORRECTED FOR RECOVERY.

	NTA		Picolinic Acid	
	% per organ	% per gram	% per organ	% per gram
Heart	.39	.39	.33	.33
Lung	1.25	.70	.89	.49
Spleen	1.06	1.04	.65	.64
Blood	4.71	.34	6.77	.42
Liver	5.07	.62	4.30	.36
Kidney	2.58	1.24	1.77	.79
Adrenal	.01	-	.01	-
Thyroid	.01	-	.01	-
Lymph Gl.	-	.62	-	.39
Pancreas	.32	.56	.28	1.04
Brain	.11	.08	.10	.07
Fat	-	.23	-	.13
Stomach	.42	.23	.25	.06
Sm. Int.	1.33	.19	1.07	.11
Lg. Int.	3.62	.55	1.11	.14
Skeleton	12.6	.75	12.6	.65
Muscle	11.8	.11	14.9	.13
Skin	11.5	.34	17.8	.33
Eyes	.07	.22	.04	.18
Pituitary	<.01	-	<.01	-
Gonads	1.36	.50	1.11	.36
Urine	30.8	-	18.2	-
Feces	11.0	-	17.8	-

*Each rat received 25 milligrams of complexing agent.

TABLE II

THE DISTRIBUTION OF CARRIER-FREE Cb^{95} COMPLEXED WITH ASCORBIC ACID* IN THE GUINEA PIG 24 HOURS AFTER INTRAMUSCULAR INJECTION. VALUES ARE GIVEN IN PERCENT OF ABSORBED DOSE CORRECTED FOR RECOVERY.

	% per organ	% per gram
Heart	.35	.13
Lungs	.87	.11
Spleen	.11	.20
Blood	7.49	.25
Liver	2.00	.11
Kidney	2.17	.47
Adrenals	.05	-
Thyrodi	.07	-
Lymph Gl.	-	.20
Pancreas	.20	.17
Brain	.08	.03
Fat	-	.04
Stomach	.59	.16
Sm. Int.	4.26	.08
Lg. Int.	.58	.009
Skeleton	23.9	.54
Muscle	6.41	.03
Skin	11.4	.18
Gonads	.24	.05
Eyes	.08	.08
Pituitary	.08	-
Urine	37.7	-
Feces	1.47	-

* Guinea pigs received 50 milligrams of ascorbic acid.

TABLE III

THE DEPOSITION OF CARRIER-FREE Cb^{95} COMPLEXED WITH ORGANIC ACIDS* IN RATS 24 HOURS AFTER INTRAVENOUS INJECTION. DATA ARE EXPRESSED IN PERCENT OF ABSORBED DOSE CORRECTED FOR RECOVERY.

	Ascorbic Acid		Malonic Acid		Tartaric Acid		Citric Acid	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.50	.47	.32	.26	.47	.45	.63	.55
Lung	2.04	.76	.87	.42	1.98	.85	1.63	.97
Spleen	.69	.78	.32	.41	1.01	1.09	.71	1.19
Blood	14.3	.95	7.76	.44	15.5	.99	16.8	1.08
Liver	4.88	.61	2.19	.23	6.52	.76	7.56	.70
Kidney	1.61	.80	1.16	.50	1.50	.84	2.33	1.06
Adrenal	.06	-	.08	-	.05	-	.03	-
Thyroid	.01	-	.05	-	.02	-	.02	-
Lymph Gl.	-	1.01	-	.45	-	.76	-	.44
Pancreas	.36	.49	.14	.21	.26	.41	.15	.14
Brain	.11	.07	.06	.03	.08	.05	.04	.02
Fat	-	.10	-	.09	-	.14	-	.12
Stomach	.77	.44	.18	.11	1.01	.47	.57	.30
Sm. Int.	2.19	.29	1.19	.14	2.25	.30	2.92	.36
Lg. Int.	3.61	.87	19.6	3.21	2.39	.46	5.17	1.06
Skeleton	11.7	.60	9.49	.44	10.9	.63	17.7	.47
Muscle	13.7	.13	8.65	.06	16.7	.14	18.5	.16
Balance	.31	-	14.4	-	-	-	-	-
Skin	14.2	.34	11.5	.27	17.8	.35	11.4	.33
Eyes	.09	.38	.06	.30	.04	.22	.04	.12
Pituitary	<.01	-	.05	-	<.01	-	<.01	-
Gonads	1.34	.44	.83	.21	1.25	.37	1.27	.47
Urine	18.9	-	20.8	-	8.03	-	7.27	-
Feces	8.56	-	.32	-	12.2	-	5.24	-

* Each rat recieved 25 milligrams of complexing agent.

TABLE IV

THE DISTRIBUTION OF CARRIER-FREE Cb^{95} COMPLEXED WITH ASCORBIC ACID* IN FIBROSARCOMA BEARING RATS 24 HOURS AFTER INTRAVENOUS INJECTION. THE DATA ARE GIVEN IN PERCENT OF ABSORBED DOSE AND CORRECTED FOR RECOVERY.

	% per organ	% Per gram
Lung	1.25	.63
Spleen	1.05	.45
Blood	10.9	.86
Liver	7.55	.89
Kidney	1.75	.90
Adrenals	.06	-
Lymph Gl.	-	.89
Brain	.02	.01
G. I.	9.08	.58
Skeleton	8.30	.49
Muscle	8.69	.09
Balance	6.58	-
Skin	9.55	.32
Tumor	9.20	.68
Urine	23.3	-
Feces	2.67	-

* Each animal recieved 25 milligrams of ascorbic acid.

TABLE V

THE DISTRIBUTION OF CARRIER-FREE Cb^{95} COMPLEXED WITH AMMONIUM CITRATE* IN ADENOCARCINOMA AND LYMPHOSARCOMA BEARING RATS 2 DAYS FOLLOWING INTRAVENOUS INJECTION. DATA ARE GIVEN IN PERCENT OF ABSORBED DOSE CORRECTED FOR RECOVERY

	Adenocarcinoma		Lymphosarcoma	
	% per organ	% per gram	% per organ	% per gram
Heart	.12	.12	.42	.70
Lung	.51	.17	1.15	.59
Spleen	.16	.09	.28	.25
Blood	5.63	.35	6.75	.54
Liver	4.18	.33	4.25	.50
Kidney	.77	.31	1.60	.78
Brain	.01	.01	<.01	<.01
Stomach	.10	.06	.48	.19
Sm. Int.	.52	.08	2.59	.33
Lg. Int.	.47	.14	1.33	.29
Skeleton	16.4	.56	10.6	.41
Muscle	27.8	.22	7.45	.08
Balance	8.52	-	7.55	-
Tumor	14.4	.61	15.0	.78
Urine	18.0	-	38.3	-
Feces	2.50	-	2.19	-

* Each animal received 25 milligrams of ammonium citrate.

TABLE VI

THE DEPOSITION OF CARRIER-FREE Zr^{95}-Cb^{95} COMPLEXED WITH LACTIC ACID* IN RATS FOLLOWING INTRAMUSCULAR AND INTRAVENOUS INJECTION. DATA ARE GIVEN IN TERMS OF PERCENT OF ABSORBED DOSE, CORRECTED FOR RECOVERY.

	2 Days Intramuscular		2 Days Intravenous		2 Days Intravenous (adenocarcinoma)		4 Days Intravenous	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.46	.68	.39	.60	.23	.37	.41	.47
Lung	1.13	.91	1.13	.92	.87	.50	.99	.68
Spleen	.81	1.65	.93	1.54	.55	.84	.93	1.72
Blood	10.2	.93	12.2	1.22	5.67	.57	2.78	.27
Liver	8.11	1.04	8.83	1.03	5.36	.72	7.32	.94
Kidney	3.03	1.85	2.96	1.84	2.23	1.69	3.78	2.62
Adrenal	.09	-	.05	-	.06	-	.07	-
Thyroid	.03	-	<.01	-	.02	-	.02	-
Lym. Gl.	-	1.28	-	2.25	-	.84	-	1.67
Pancreas	.48	.72	.41	.71	.36	.50	.51	.74
Brain	.05	.05	.04	.03	.05	.03	.08	.04
Fat	-	.27	-	.19	-	.16	-	.25
Stomach	.65	.27	.52	.28	.27	.12	.45	.10
Sm. Int.	2.86	.30	2.83	.32	1.55	.22	2.51	.28
Lg. Int.	3.43	.37	2.65	.33	1.36	.22	1.99	.30
Skeleton	28.1	1.38	26.4	1.48	29.2	1.92	31.4	1.85
Muscle	15.2	.19	13.7	.19	18.5	.24	13.4	.18
Skin	6.64	.29	9.30	.41	13.9	.60	9.45	.40
Eyes	.07	.23	.04	.11	.04	.16	.05	.16
Pituitary	.02	-	<.01	-	<.01	-	<.01	-
Gonads	.38	.97	.38	.98	.11	.47	.19	.80
Tumor					4.97	1.02	-	-
Urine	10.2	-	8.12	-	9.66	-	11.5	-
Feces	8.00	-	9.14	-	5.18	-	12.2	-

* Each animal received 2.2 milligrams of lactic acid.

TABLE VII

COMPARISON OF LIVER AND SKELETAL DEPOSITION OF CARRIER-FREE Cb^{95} AND Zr^{95}-Cb^{95} IN RATS AND GUINEA PIGS USING VARIOUS COMPLEXING AGENTS. VALUES GIVEN AS PERCENT OF DOSE PER GRAM TISSUE WET WEIGHT.

Complexing Agent	Picolinic	N.T.A.	Ascorbic	Ascorbic	Malonic	Tartaric	Citric	Lactic	Lactic	Lactic
Animal	Rat	Rat	Guinea Pig	Rat	Rat	Rat	Rat	Rat	Rat	Rat
Route	I.M.	I.M.	I.M.	I.V.	I.V.	I.V.	I.V.	I.M.	I.V.	I.V.
Time	4 D	4 D	1 D	1 D	1 D	1 D	1 D	2 D	2 D	4 D
Element	Cb	Cb	Cb	Cb	Cb	Cb	Cb	Zr-Cb	Zr-Cb	Zr-Cb
Liver	.36	.62	.11	.61	.23	.76	.70	1.0	1.0	.94
Skeleton	.65	.75	.54	.60	.44	.63	.47	1.3	1.4	1.85

COMPARISON OF LIVER AND SKELETAL AND TUMOR DEPOSITION OF CARRIER FREE Cb^{95} AND Zr^{95}-Cb^{95} IN TUMOR BEARING RATS USING VARIOUS COMPLEXING AGENTS

Tumor	Fibrosarcoma	Adenocarcinoma	Lymphosarcoma	Adenocarcinoma
Complexing Agent	Ascorbic	Citrate	Citrate	Lactic
Route	I.V.	I.V.	I.V.	I.V.
Time	1 Day	2 Days	2 Days	2 Days
Element	Cb	Cb	Cb	Zr-Cb
Liver	.89	.33	.50	.70
Skeleton	.49	.56	.41	1.9
Tumor	.68	.61	.78	1.0

TABLE VIII

THE DISTRIBUTION OF CARRIER-FREE Ce^{144} COMPLEXED WITH ANTI-PYRINE* IN THE RAT 4 DAYS FOLLOWING INTRAMUSCULAR AND INTRAVENOUS INJECTION. THE DATA ARE GIVEN IN PERCENT OF ABSORBED DOSE CORRECTED FOR RECOVERY.

	Intramuscular		Intravenous	
	% per organ	% per gram	% per organ	% per gram
Heart	.11	.14	.07	.09
Lungs	.18	.11	.84	.47
Spleen	.14	.25	.84	1.85
Blood	.21	.02	.06	.005
Liver	46.4	4.99	52.0	5.34
Kidney	1.17	.64	.86	.46
Adrenal	<.04	-	<.01	-
Thyroid	<.04	-	<.01	-
Lymph Gl.	-	.88	-	.12
Pancreas	.04	.04	.02	.04
Brain	<.04	<.04	.01	.006
Fat	-	.04	-	.01
Stomach	.60	.78	.59	.22
Sm. Int.	.81	.11	.95	.10
Lg. Int.	1.24	.14	1.57	.15
Skeleton	29.5	1.88	27.4	1.62
Muscle	4.92	.05	2.15	.02
Balance	-	-	1.85	-
Skin	1.66	.07	1.77	.05
Eyes	<.04	.11	.01	.04
Pituitary	<.04	-	<.01	-
Gonads	.18	.07	.06	.03
Urine	5.63	-	3.87	-
Feces	7.22	-	5.10	-

* Each rat received 5 milligrams of hexa-anti-pyrine iodide.

TABLE IX

THE DISTRIBUTION OF CARRIER-FREE Ce^{144} COMPLEXED WITH NITRILO TRI-ACETIC ACID* IN RATS 1 and 4 DAYS AFTER INTRAMUSCULAR INJECTION. VALUES ARE GIVEN IN PERCENT OF ABSORBED DOSE AND ARE CORRECTED FOR RECOVERY.

	1 Day		4 Days	
	% per organ	% per gram	% per organ	% per gram
Heart	.06	.06	.05	.06
Lung	.23	.09	.22	.11
Spleen	.07	.09	.02	.02
Blood	.09	.005	.02	.001
Liver	20.1	2.13	16.4	1.50
Kidney	1.42	.67	1.06	.46
Adrenal	<.01	-	<.01	-
Thyroid	<.01	-	<.01	-
Lymph Gl.	-	.26	-	.33
Pancreas	.02	.04	.02	.02
Brain	<.01	<.01	<.01	<.01
Fat	-	<.01	-	<.01
Stomach	.16	.07	.10	.05
Sm. Int.	.31	.04	.33	.02
Lg. Int.	.34	.05	.70	.08
Skeleton	30.0	1.58	29.8	1.51
Muscle	1.46	.01	1.50	.01
Skin	1.55	.04	1.56	.04
Eyes	<.01	.02	<.01	.02
Pituitary	<.01	-	<.01	-
Gonads	.05	.01	.05	.02
Urine	43.0	-	44.1	-
Feces	1.22	-	4.00	-

* Each rat received 19 milligrams of nitrilo tri-acetic acid.

TABLE X

THE DISTRIBUTION OF CARRIER-FREE Ce^{144} COMPLEXED WITH NITRILO TRI-ACETIC ACID* IN RATS 1 and 4 DAYS AFTER INTRAVENOUS INJECTION. THE DATA ARE GIVEN IN PERCENT OF ABSORBED DOSE AND ARE CORRECTED FOR RECOVERY.

	1 Day		4 Days	
	% per organ	% per gram	% per organ	% per gram
Heart	.09	.11	.05	.06
Lung	.51	.22	.19	.09
Spleen	.07	.13	.06	.11
Blood	.05	.003	.01	.001
Liver	34.0	3.86	26.9	2.88
Kidney	.91	.44	.81	.42
Adrenal	<.01	-	<.01	-
Thyroid	<.01	-	<.01	-
Lymph Gl.	-	.12	-	.07
Pancreas	.03	.04	.03	.03
Brain	<.01	<.01	<.01	<.01
Fat	-	<.01	-	<.01
Stomach	.57	.28	.44	.15
Sm. Int.	.90	.11	.40	.04
Lg. Int.	1.64	.24	1.17	.11
Skeleton	40.9	2.05	40.4	2.08
Muscle	3.20	.03	1.14	.01
Balance	2.42	-	2.71	-
Skin	1.73	.05	1.64	.04
Eyes	.01	.04	<.01	.03
Pituitary	<.01	-	<.01	-
Gonads	.07	.03	.04	.02
Urine	12.3	-	16.7	-
Feces	.61	-	7.20	-

* Each rat received 2 milligrams of nitrilo-tri-acetic acid.

TABLE XI

THE DEPOSITION OF Sm^{153}* COMPLEXED WITH AMMONIUM CITRATE IN THE RAT FOLLOWING INTRAMUSCULAR INJECTION. VALUES ARE GIVEN IN PERCENT OF ABSORBED DOSE CORRECTED FOR RECOVERY.

	1 Day		4 Days		8 Days		4 Days Carrier	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.67	.73	.31	.38	.27	.33	.17	.17
Lung	.47	.24	.33	.22	.27	.15	.85	.68
Spleen	.59	.87	.55	.60	1.49	2.15	1.53	2.21
Blood	.16	.02	.02	.002	<.03	<.003	.09	.009
Liver	50.6	8.45	39.5	5.19	34.6	4.21	49.6	6.45
Kidney	11.9	7.05	8.70	4.61	1.88	1.04	4.24	2.72
G.I.	5.84	.84	3.82	.23	4.89	.24	3.74	.17
Skeleton	16.9	1.04	13.7	.79	16.2	.92	9.85	.59
Muscle	4.28	.06	4.18	.05	3.37	.04	8.15	.10
Skin	3.37	.12	2.32	.09	1.16	.06	3.74	.17
Urine	4.45	-	21.3	-	5.64	-	4.24	-
Feces	.51	-	4.93	-	30.3	-	13.8	

* One and four day rats each received 35 micrograms of stable Sm and the eight day rats received 150 micrograms of stable Sm. The above rats each received 3 milligrams of ammonium citrate. The four day carrier animals each received 1 milligram of stable Sm complexed with 3 milligrams of ammonium citrate.

TABLE XII

THE DEPOSITION OF Sm^{153}* COMPLEXED WITH AMMONIUM CITRATE FOLLOWING INTRAVENOUS INJECTION. THE DATA ARE GIVEN IN PERCENT OF ABSORBED DOSE AND ARE CORRECTED FOR RECOVERY.

	1 Day		4 Days		4 Days (adenocarcinoma)	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.25	.31	.16	.19	.08	.11
Lung	.44	.29	.40	.31	.50	.15
Spleen	.56	1.07	1.20	1.05	1.50	1.87
Blood	.45	.05	.05	.005	.15	.013
Liver	69.7	11.3	62.7	7.61	63.4	7.82
Kidney	3.43	2.42	.77	.43	2.02	1.34
G. I.	5.18	.33	3.42	.14	1.03	.12
Skeleton	11.6	.80	9.90	.59	9.90	.59
Muscle	1.70	.02	1.31	.01	3.93	.045
Skin	1.57	.06	1.95	.07	3.04	.10
Tumor					5.90	.34
Urine	.71	-	6.59	-	4.13	-
Feces	$<$.01	-	11.5	-	3.97	-

* Each rat received 35 micrograms carrier Sm complexed with 3 milligrams ammonium citrate.

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TABLE XIII

THE DISTRIBUTION OF Sm^{153}* COMPLEXED WITH VARYING AMOUNTS OF AMMONIUM CITRATE IN RATS 4 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE GIVEN IN PERCENT OF ABSORBED DOSE CORRECTED FOR RECOVERY.

	15 mg. Citrate		3 mg. Citrate	
	% per organ	% per gram	% per organ	% per gram
Heart	.04	.05	.33	.48
Lung	.07	.06	.61	.48
Spleen	.04	.07	.83	1.74
Blood	.04	.004	.04	.004
Liver	43.5	6.18	56.8	8.33
Kidney	.91	.61	2.82	2.20
G. I.	3.30	.17	4.19	.28
Skeleton	20.0	1.16	15.0	.99
Muscle	1.85	.02	4.07	.06
Skin	.78	.04	2.27	.11
Urine	16.4	-	2.58	-
Feces	13.1	-	10.5	-

* Each rat received 35 micrograms carrier Sm.

TABLE XIV

THE EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF BAL* ON THE DISTRIBUTION IN RATS OF Sm^{153}** COMPLEXED WITH AMMONIUM CITRATE 4 DAYS AFTER INTRAVENOUS INJECTION. THE DATA ARE GIVEN IN PERCENT OF ABSORBED DOSE CORRECTED FOR RECOVERY.

	Control		BAL	
	% per organ	% per gram	% per organ	% per gram
Heart	.04	.05	.04	.07
Lung	.07	.06	.11	.11
Spleen	.04	.07	.06	.09
Blood	.04	.004	.02	.002
Liver	43.5	6.18	37.1	5.60
Kidney	.91	.61	.67	.57
G. I.	3.30	.17	1.14	.04
Skeleton	20.0	1.16	17.8	1.12
Muscle	1.85	.02	4.99	.07
Skin	.78	.04	4.24	.20
Urine	16.4	-	21.9	-
Feces	13.1	-	11.9	-

* One-tenth of a cc. of 10 percent BAL in oil was administered intramuscularly to each rat at the time of the injection of the Sm^{153}.

**Each rat received 35 micrograms of stable Sm complexed with 15 milligrams of ammonium citrate.

TABLE XV

THE DEPOSITION OF CARRIER-FREE Se^{75} IN RATS 16 DAYS AFTER INTRAMUSCULAR INJECTION. VALUES GIVEN ARE EXPRESSED IN PERCENTAGE OF ABSORBED DOSE AND ARE CORRECTED FOR THE DEVIATION OF RECOVERY FROM 100 PERCENT.

	% per organ	% per gram
Heart	.10	.12
Lung	.20	.10
Spleen	.13	.15
Blood	1.03	.07
Liver	1.49	.17
Kidney	1.04	.52
Pancreas	-	.12
Brain	.07	.05
Stomach	.19	.14
Stomach, cont'd.	.04	.08
Sm. Int.	.31	.08
Sm. Int. cont'd.	.09	.03
Lg. Int.	.19	.10
Lg. Int. cont'd.	.27	.06
Skeleton	1.36	.056
Muscle	6.09	.056
Skin	1.33	.04
Testes	1.63	.47
Urine	66.3	-
Feces	18.2	-

TABLE XVI

THE DEPOSITION OF CARRIER-FREE Rh^{105} IN RATS 18 HOURS FOLLOWING INTRAMUSCULAR INJECTION. VALUES GIVEN ARE EXPRESSED IN PERCENT OF ABSORBED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PERCENT.

	% per organ	% per gram
Heart	.07	.09
Lungs	.29	.14
Spleen	.16	.20
Blood	2.70	.23
Liver	3.44	.47
Kidney	2.65	1.65
Pancreas	.21	.36
Brain	<.02	<.02
Stomach	.27	.16
Sm. Int.	1.47	.21
Lg. Int.	2.36	.75
Int. Cont.	11.2	3.17
Skeleton	2.47	.13
Muscle	3.49	.04
Skin	2.27	.21
Eyes	<.02	<.02
Gonads	<.02	<.02
Urine	66.9	-
Feces	<.02	-

Decontamination and Bone Metabolism Studies

Decontamination Studies. Effect of Zirconium citrate and Parathormone on the Distribution and Excretion of Y^{90} and Pu^{239}(VI). The value of prompt treatment with massive doses of zirconium citrate for decontamination of plutonium[1] and radioyttrium[2] has been reported by Schubert, and has been confirmed by work in this laboratory. However, the treatment is much less effective if commenced even a few days after the yttrium or plutonium has been administered. It would appear that once these metals are fixed in the skeleton, subsequent removal is very limited.

This experiment was undertaken to determine whether active bone resorption might expose the radioyttrium or plutonium previously fixed in bone to the decontaminating action of zirconium citrate. As one of the most potent physiological agents with this action, massive doses of parathormone were administered to effect bone resorption. Since histological studies have shown greatest resorptive activity from 24-48 hours following injection of the hormone, zirconium citrate treatment was given at this time.

Experiment. Young female rats (weight range 120-150 grams) were used for this experiment. They were reared and maintained on the regular stock diet. Each was injected in the dorsal vein of the foot with 0.25 cc. of a solution containing 40 microcuries of Y^{90} and 10 micrograms of hexavalent Pu^{239} in isotonic saline at pH 6. The animals were grouped as follows according to treatment:

Group	No. of Rats	Treatment
1	6	Controls - no treatment
2	4	"Pretreated" - 20 mg. of zirconium as citrate injected intraperitoneally 48 hours before the administration of Y^{90} and Pu^{239}.
3	5	"Immediate Treatment" - 20 mg. of zirconium as citrate injected intraperitoneally immediately after the administration of Y^{90} and Pu^{239}.
4	5	"Parathormone" - 500 units of parathormone (Lilly) injected intraperitoneally 24 hours after the Y^{90}-Pu.
5	5	"Late Zr Treatment" - 20 mg. of zirconium as citrate injected intraperitoneally 48 hours after the Y^{90}-Pu.
6	5	"Parathormone-Zr. Treatment" 500 units of parathormone IP 24 hours after the Y^{90}-Pu. 20 mg. Zr as citrate IP 48 hours after the Y^{90}-Pu.

Following administration of the Y^{90} and the Pu^{239}, the rats were placed in individual metabolism cages, and urine and feces were collected separately for 6 days, at which time the animals were sacrificed. Urine, feces, femur, liver, kidney and residual carcass were analyzed for Y^{90} (using a Geiger counter) and Pu^{239} (using an alpha counter). Tissues were dried and ashed before analysis. The total recovery for both Y^{90} and Pu^{239} averaged 99.3 ± 1.8 percent of the administered dose.

Results. The results of the analyses of tissues and excreta are given in Tables I and II (for Pu^{239}) and Tables III and IV (for Y^{90}). (The data for Y^{90} was presented in a previous Quarterly Report, but is included in this series for comparison).

The general distribution and excretion of Y^{90} and Pu^{239}(VI) was quite similar in the control animals. Pretreatment with zirconium citrate (-48 hours) reduced the amount of Pu and Y^{90} deposited in the femur. The urinary excretion of Y^{90} was higher, but there was no increase in the urinary plutonium. The actual differences, although statistically significant, were small.

As has been observed previously, prompt treatment with large amounts of zirconium citrate causes a sharp increase in urinary excretion of both elements, which was most marked in the case of Y^{90}. There was a corresponding reduction in the deposition in femur. The liver values were somewhat higher, but fecal excretion was unchanged.

Treatment with parathormone alone had no effect on the excretion of either element, nor was there any significant difference in the uptake by femur. It would appear that bone resorption so produced is not, per se, effective in decontamination. Late treatment with zirconium citrate (+48 hours) did increase the urinary excretion of Y^{90} and Pu, but the effect on the femur values was not significant, nor was its action enhanced by the previous administration of parathormone.

Conclusions: (1) "Pretreatment" with zirconium citrate 48 hours before administration of Y^{90} and Pu^{239} does appear to increase the urinary elimination of radioyttrium and reduce somewhat the retention in the femur, but the effect on plutonium, if any, is small. Since Schubert[2] has shown that zirconium excretion continues for several days following administration, this effect may have been due to residual Zr in the body.

(2) Prompt treatment with large doses of zirconium citrate does increase urinary excretion and reduce deposition in femur of both elements - a decontamination effect which may be due to "carrier" action of the zirconium.

(3) Active bone resorption induced by large doses of parathormone does not materially affect the excretion of bone retention of Y^{90} or Pu^{239}, even when followed by zirconium citrate treatment. This suggests that large amounts of plutonium are not mobilized from bone during bone resorption, and indicates that such treatment would be of little value in decontamination.

TABLE I

EFFECT OF TREATMENT WITH ZIRCONIUM CITRATE AND PARATHORMONE ON THE DISTRIBUTION AND EXCRETION OF PU(VI) INJECTED INTRAVENOUSLY
PERCENT OF THE INJECTED DOSE OF PU*

Treatment	Urine	Feces	Femur	Liver	Kidney	Carcass
1. Control	13.9±1.4	5.6± 0.4	5.4±0.8	6.2±1.7	1.8±0.1	61.5±1.7
2. Zr Citrate injected 44 hrs. before the Pu	10.6±1.0	9.4±0.3	3.7±0.2	11.4±1.6	1.7±0.5	62.4±2.0
3. Zr Citrate injected IP at same time as Pu	18.9±3.1	8.5±0.7	3.0±0.5	12.3±2.1	1.6±0.2	55.5±4.9
4. Parathormone 500 units 24 hrs. after Pu inject.	11.7±3.4	7.1±1.3	4.4±0.2	11.2±1.2	1.9±0.2	63.6±2.6
5. Zr Citrate injected IP at 48 hrs. after Pu	14.1±0.7	10.4±1.0	3.8±0.2	15.7±5.0	1.5±0.3	53.9±2.5
6. Parathormone 500 units at 24 hr. Zr Cit. at 48 hrs. after inject. of Pu	9.2±1.0	12.0±2.0	4.2±0.3	11.1±1.3	1.3±0.1	62.0±2.6

* Values given are averages ± the standard error.

TABLE II

EFFECT OF PARATHORMONE AND ZIRCONIUM CITRATE TREATMENT ON EXCRETION OF PU(VI)

A. Urine	Day following administration of Pu						
Group	1	2	3	4	5	6	Total
1. Control	12.6±1.4	0.5±0.03	0.3±0.03	0.2±0.0.3	0.2	0.1	13.9±1.4
2. Zr Citrate -48 hrs.	8.4	0.8	0.5	0.4	0.3	0.2	10.6±1.0
3. Zr Citrate 0 (immediately)	16.9	0.6	0.5	0.4	0.3	0.2	18.9±3.1
4. PTH* +24 hrs.	9.8	0.7	0.3	0.3	0.2	0.2	11.7±3.4
5. Zr Citrate +48 hrs.	6.5	0.5	1.3	0.4	0.3	0.2	14.1±0.7
6. PTH -+24 hrs. Zr Citrate+48 hrs.	11.0	0.8	1.4	0.4	0.3	0.2	9.2±1.0

B. Feces	Day following administration of Pu						
Group	1	2	3	4	5	6	Total
1. Control	1.0	0.9	0.7	0.8	1.0	1.2	5.6±0.4
2. Zr Citrate -48 hrs.	2.1	1.3	1.3	1.4	1.7	1.6	9.4±0.3
3. Zr Citrate 0 (immediately)	1.9	0.8	1.2	1.4	1.5	1.7	8.5±0.7
4. PTH + 24 hrs.	2.8	1.1	0.9	0.8	0.7	0.8	7.1±1.3
5. Zr Citrate +48 hrs.	2.3	1.2	1.5	1.9	1.9	1.6	10.4±1.0
6. PTH +24 hrs. Zr Citrate +48 hrs.	3.7	1.3	1.4	1.6	2.6	1.4	12.0±2.0

* -PTH-parathormone.

TABLE III

EFFECT OF TREATMENT WITH ZIRCONIUM CITRATE AND PARATHORMONE ON THE DISTRIBUTION AND EXCRETION OF RADIOYTTRIUM (Y^{90}) INJECTED INTRAVENOUSLY. PERCENT OF THE INJECTED DOSE OF RADIOYTTRIUM*

Treatment	Urine	Feces	Femur	Liver	Kidney	Carcass
1. Control	9.9±0.2	9.7±0.2	3.0±0.1	10.3±0.2	2.4±0.1	64.5±0.8
2. Zr Citrate IP 48 hrs. before Y^{90}	16.0±0.4	11.6±0.1	1.8±0.1	13.7±0.8	1.2±0.01	55.6±1.9
3. Zr Citrate IP at same time as the Y^{90}	28.0±1.4	7.4±0.7	1.6±0.1	17.3±0.9	1.0±0.1	43.8±1.7
4. Parathormone-500 units 24 hrs. after injection of Y^{90}	8.4±0.1	8.4±0.2	3.3±0.1	7.7±0.6	1.5±0.1	70.5±1.0
5. Zr Citrate IP at 48 hrs. after injection of Y^{90}	16.4±0.3	13.0±0.3	2.5±0.1	8.4±0.4	1.9±0.1	57.5±0.7
6. Parathormone-500 units 24 hrs after Y^{90}. Zr Citrate at 48 hrs. after Y^{90}	13.8±0.2	13.2±0.6	2.7±0.1	10.8±0.4	1.8±0.1	57.5±0.6

* Values reported are mean values ± standard error.

TABLE IV

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

A. Urine

Group	Days following administration of Y^{90} (daily excretion) 1	2	3	4	5	6	Total
1. Control	4.5	2.0	1.3	0.7	0.6	0.5	9.7±0.2
2. Zr Citrate -48 hrs.	7.5	2.3	2.0	1.6	1.3	1.1	16.0±0.4
3. Zr Citrate 0 (immediately)	21.7	1.4	1.6	1.3	1.0	1.0	28.0±1.4
4. PTH_1 +24 hrs.	4.1	1.8	1.2	0.6	0.5	0.4	8.6±0.1
5. Zr Citrate, +48 hr.	6.5	2.0	3.2	2.0	2.1	1.3	16.1±0.3
6. PTH_1 +24 hrs. Zr Citrate, +48 hr.	4.6	2.1	2.3	1.5	1.7	1.3	13.6±0.2

B. Feces

Group	Days following administration of Y^{90} (daily excretion) 1	2	3	4	5	6	Total
1. Control	2.7	1.9	1.4	1.4	1.3	1.2	9.5±0.2
2. Zr Citrate, -48 hrs.	2.5	2.6	1.8	1.8	1.6	1.3	11.6±0.1
3. Zr Citrate 0 (immediately)	2.2	2.2	1.1	1.1	0.9	0.9	7.4±0.7
4. PTH_1 +24 hrs.	2.5	1.3	1.3	1.3	1.2	0.9	8.5±0.1
5. Zr Citrate, +48 hrs.	2.9	2.3	2.2	2.2	1.8	1.1	12.7±0.3
6. PTH_1 +24 hrs. Zr Citrate, +48 hrs.	2.3	1.5	2.7	2.5	2.7	1.4	13.1±0.6

Decontamination Studies. Effect of Zirconium Citrate Treatment on the Initial Uptake and Excretion of Ce^{144}. The value of massive injection of zirconium citrate on decontamination of radiocerium was studied in the following experiment. The zirconium citrate was administered by intraperitoneal injection immediately after intravenous injection of Ce^{144}, and the amount in tissues and urine was determined at various time intervals up to 1 hour.

Experiment: Female rats in the weight range 175-225 grams were injected I.V. with 0.25 cc. of a solution of isotonic saline containing 5 microcuries of carrier-free Ce^{144}(pH-5), in the dorsal vein of the foot. Immediately thereafter, they were given 40 mg. of Zr as citrate by intraperitoneal injection. The animals were sacrificed at 5, 10, 15, 30 or 60 minutes following injection. The radiocerium in blood, plasma, femur, liver and urine was determined. The values for the 2-3 animals sacrificed at each time interval have been averaged and are given in Table V.

A similar group of rats injected with radiocerium, but without zirconium citrate treatment, served as untreated controls.

Results: The results of this experiment are given in Table V. (Curves for Ce^{144} concentration in blood and in femur are shown in Figures 1 and 2.) It will be noted that the blood levels of Ce^{144} are lower in the treated animals, and the uptake by the femur is less. There appears to be no significant effect on the radiocerium in liver (Fig. 3). However, there is a tremendous increase in the urinary excretion of Ce^{144}, which is apparent even after 5-10 minutes. This is particularly interesting, since the urinary excretion of radiocerium is usually low.

Conclusions: As with radioyttrium and plutonium, prompt zirconium citrate treatment causes a marked increase in urinary excretion of radiocerium within 5 minutes after injection. Skeletal uptake of Ce^{144} was reduced, but there was no apparent effect on liver Ce^{144}. It would appear that zirconium citrate should be of value in radiocerium decontamination.

Bone Metabolism Studies. Study of Normal and Rachitic Animals with Radiophosphorus. Radiophosphorus (P^{32}) was used in the following experiments to: (a) compare the metabolism in young growing rats with that in skeletally mature adults, and (b) to compare normal rats with animals suffering from low phosphorus rickets. The experiments were carried out to investigate further the changes in bone metabolism with age, and with rickets.

The Effect of Age. Mature adult rats and young growing rats that had been raised on the supplemented control diet were used in this experiment. They were injected with five microcuries of P^{32} and sacrificed twenty-four hours later. The tissues and excreta were assayed for their content of radioactive phosphorus. The average recovery of P^{32} was 99.00 ± 0.73 percent for these experiments. Table VI gives the distribution of P^{32} as the percent of the total administered dose of radioactive phosphorus. Table VII gives the percent distribution of the P^{32} actually retained within the body after allowing for excretion.

The young growing animals deposited twice as much radioactive phosphorus in the femur and skeleton as did the adult animals. At the same time they excreted

TABLE V

EFFECT OF ZIRCONIUM CITRATE ON DISTRIBUTION AND EXCRETION OF INTRAVENOUS Ce^{144}

A. Control Group (no treatment)

Values expressed as % administered dose of Ce^{144}/gm.

Time (mins)	Urine	Femur	Liver	Whole Blood	Plasma
5	0.2±0.1	0.66±0.05	3.24±0.59	1.63±0.31	2.32±0.28
10	0.6	1.03	3.74	0.95	1.21
15	0.3±0.1	0.82±0.07	4.87±1.08	0.75±0.03	1.13±0.07
30	1.1±0.6	1.05±0.14	5.59±0.40	0.66±0.08	0.83±0.04
60	0.9±0.1	1.45±0.05	5.82±0.05	0.49	0.47±0.18

B. Treated Group (40 mg. Zr. Citrate)

Values expressed as % administered dose of Ce^{144}/gm.

Time (mins)	Urine	Femur	Liver	Whole Blood	Plasma
5	5.9±1.0	0.65±0.01	2.89±0.33	1.50±0.08	2.25±0.08
10	10.4	0.52	3.57	0.71	1.24
15	7.7	0.75	5.45	0.54	0.78
30	14.8±4.1	0.95±0.12	5.15±0.26	0.36±0.03	0.62±0.02
60	9.2±0.6	0.86±0.19	5.48±0.16	0.23±0.01	0.40±0.02

TABLE VI

PERCENT DOSE OF ADMINISTERED P^{32} IN TISSUES AND EXCRETA

	Adult	Young Normal
Femur	0.90-0.06	1.99-0.25
Muscle*	27.40±0.77	14.21±0.56
Skeleton**	16.84±1.28	40.54±1.28
Skin	6.86±0.44	4.94±0.27
Viscera	23.96±0.46	8.84±0.48
Urine	20.43±0.94	13.22±0.68
Feces	2.35±0.94	0.713±0.21

In all of the tables:

* Total muscle weight calculated on the basis of figures of Donaldson, 1924.

**Amount of P^{32} in total skeleton calculated as the amount in the carcass minus that in the muscle. This figure was consistent for normal animals but was not used for rachitic rats.

All values given represent the average of 5-6 animals plus or minus the Standard Error (σM).

$$\sigma M = \frac{\sigma}{\sqrt{n - 1}}$$

TABLE VII

PERCENT OF P^{32} RETAINED IN THE BODY

	Adult	Young Normal
Femur	1.16±0.06	2.32±0.25
Skeleton	21.80±1.28	47.10±1.28
Muscle	35.42±0.77	16.50±0.56
Skin	8.90±0.44	5.74±0.27

only half as much P^{32} in the urine as did the adults. The muscles of the mature animals also contained much more P^{32} than was the case for the young animals. This reflects the positive phosphorus balance and greater deposition in the growing bone of the young animals.

The Effect of Rickets. Rats reared on the low phosphorus diet described above all showed gross evidence of severe rickets when used for this experiment. Pair-fed control mates and rats of the same age fed on the control diet ad libitum were used for comparison. Twenty-four hours after injection with five microcuries of P^{32} they were sacrificed and the tissues and excreta analyzed for radioactive phosphorus. The results given in Table VIII give the distribution of P^{32} as the percent of the total administered dose. Table IX gives the percent distribution of the P^{32} actually retained within the body after allowing for excretion.

The rachitic animals deposited less P^{32} in the femur than the pair-fed controls or normal young rats. They excreted only one-sixth as much P^{32} in the urine as their controls. However, considerably more radioactive phosphorus was located in the muscle and other soft tissues of the deficient animals. This evidence supports the view of Day and McCollum that phosphorus is shifted from the skeleton to the soft tissues in phosphorus deficiency to meet the essential requirements of the cells.

Bone Metabolism Studies. Localization of P^{32} in the Tibia of Normal and Rachitic Rats. At autopsy one tibia was removed and immediately frozen. It was then cut logitudinally into two equal halves in order to show clearly the proximal head of the tibia. Half of the bone was stained with silver nitrate after the method of von Kossa to show the areas of calcified material ("silver line test"). The other half was used for a radioautograph to show the areas of localization of the P^{32} within the bone. (Fig. 4)

The radioautographs were made by placing a sheet of chilled Eastman "Industrial Type" x-ray film in contact with the cut surface of the frozen tibias. The bone had been laid in rows on a sheet of absorbent cotton. The film, bones, and backing were then enclosed in a cardboard x-ray cassette and replaced in the freezer. Some weight was placed on top of the cassette to insure contact of the bones with the film. The film was removed and developed after being exposed for three or four days. Radioautographs of the head of a tibia of a rachitic and a pair-fed control rat are shown along with the silver line test of the same bones in Fig. 4.

In the rachitic animals the silver line test showed a very broad uncalcified cartilage plate with sparse areas of calcified material. In the normal pair-fed controls the epiphyseal cartilage was narrow and there was an area of calcified trabeculae beneath it. There appeared to be little or no P^{32} deposited in the broad epiphyseal cartilage of the rachitic bone. Indeed there was very little in the epiphyseal region as compared with that found in the control. In contrast the heaviest deposition of P^{32} in normal bone occurred in the region of active bone growth at the epiphysis.

Work in Progress:

1. Decontamination studies with zirconium citrate involving the effect on time curves of blood level, tissue concentration and urinary excretion of Y^{90} and Cb^{95}.

TABLE VIII

PERCENT OF ADMINISTERED P^{32} IN TISSUES AND EXCRETA

	Rachitic	Pair-fed Control	Normal (ad lib)
Femur	1.57±0.13	2.03±0.15	1.99±0.25
Muscle	22.13±0.59	18.94±0.50	14.21±0.56
Liver	10.16±0.42	5.85±0.22	5.72±0.33
Skin	7.56±0.50	4.69±0.27	4.94±0.27
Viscera	19.18±0.41	10.02±0.25	8.84±0.38
Urine	1.76±0.14	11.85±0.71	13.22±0.68
Feces	0.344±0.22	0.227±0.18	0.731±0.21

TABLE IX

PERCENT OF P^{32} RETAINED IN THE BODY

	Rachitic	Pair-fed Control	Normal (ad lib)
Femur	1.60±0.13	2.31±0.15	2.32±0.25
Muscle	22.60±0.59	21.60±0.50	16.50±0.56
Liver	10.39±0.42	6.66±0.22	6.65±0.33
Skin	7.74±0.50	5.34±0.27	5.74±0.27

2. Modification of the low phosphorus diet to obtain more consistent rickets in older animals suitable for bone studies.

3. Analysis of Ca^{45} in samples from the series to study the effect of age and rickets on the metabolism of radiocalcium.

4. Development of methods for measurement of phosphatase activity in small blood samples and bone, to study the effect of age, diet, hormones and radiation on phosphatase activity.

Radiochemistry

A procedure has been developed for isolating carrier-free radiocobalt from deuteron bombarded iron and from milligram amounts of Cu, Al, Cd and Zn impurities introduced from the target plate, solder, etc. The isolation is based on the observation that carrier-free radiocobalt does not carry on $Fe(OH)_3$ precipitated from NH_4Cl-NH_4OH solution, but is carried if $Fe(OH)_3$ is precipitated with NaOH. The isolated activity showed the 72-day period of $Co^{56,57}$ and the 1.2 Mev beta reported for Co^{56}. The gamma radiation had a half-thickness in lead of approximately 10 gm/cm^2. Two millicuries of $Co^{56,57}$ were isolated.

Three millicuries of Rh^{105} were isolated from neutron irradiated RuO_2 using a procedure which involved the volatilization of ruthenium from H_2SO_4-$NaBrO_3$ solution. The Rh^{105} fraction was found to contain an unidentified long-lived radioactive impurity presumably produced from contaminants in the bombarded RuO_2.

Several methods of separating carrier-free Cb^{95} from Cb-Zr mixture have been investigated to obtain a more rapid procedure. Precipitation of Cb^{95} on MnO_2 from 10 N HNO_3 and separation of the carrier-free Cb from manganese by ion exchange resin has been found satisfactory.

A procedure for recovering Np^{227} from large amounts of animal ash has been developed.

Several isotonic saline solutions of radio-columbium and rare earths have been prepared for injection. Additional information has been obtained on the complexing of carrier-free radioisotopes with organic agents.

A manual on the preparation and isolation of carrier-free radioisotopes is being prepared. Seven individual separation procedures have been written for publication.

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1 Schubert, J., Science 105:389, 1947.
2 Schubert, J., J. Lab. Clin. Med., 34: 313, 1949.

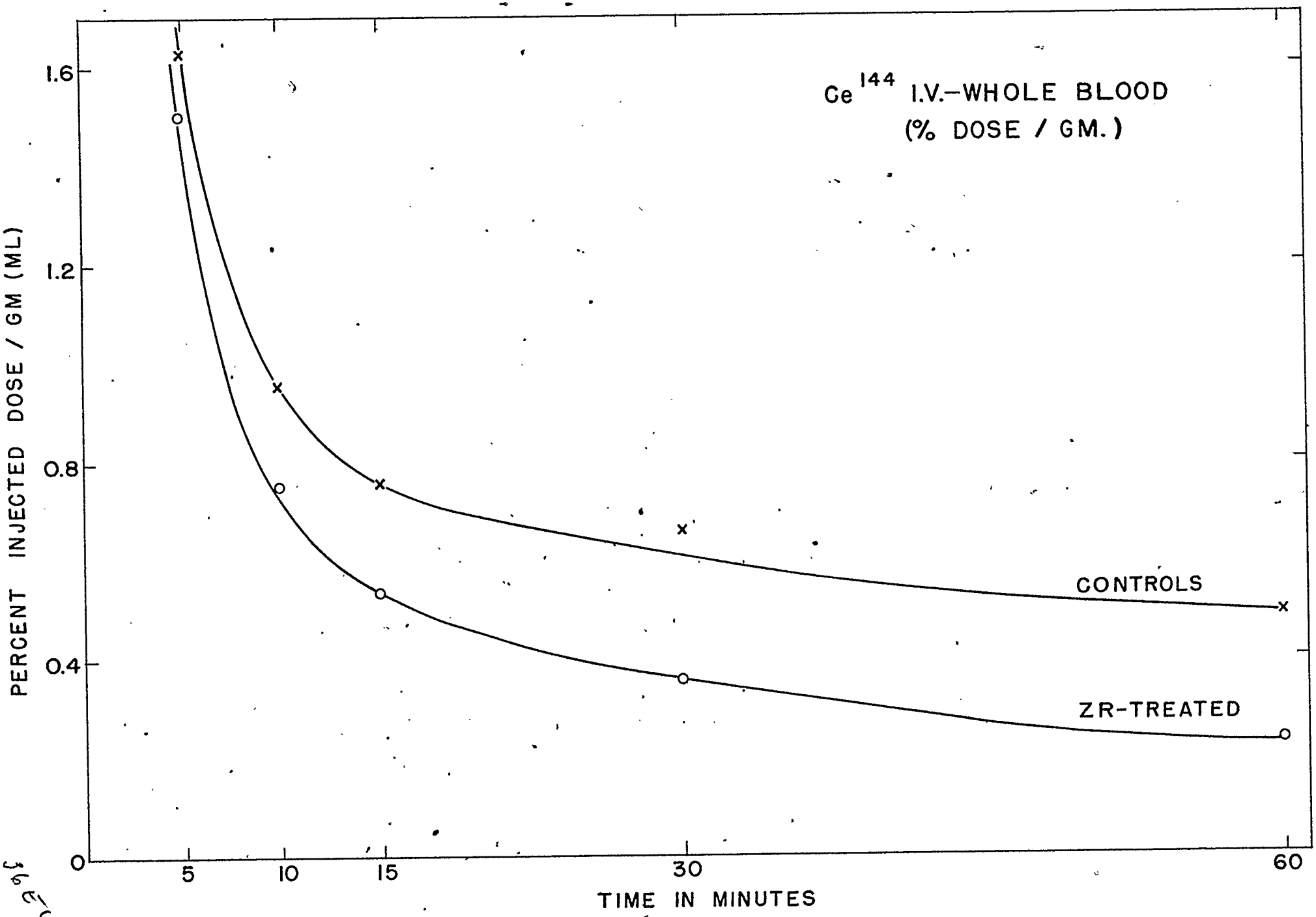

Ce 144 I.V.—WHOLE BLOOD
(% DOSE / GM.)
PERCENT INJECTED DOSE / GM (ML)
1.6
1.2
0.8
0.4
0
5
10
15
30
60
TIME IN MINUTES
CONTROLS
ZR-TREATED

FIG. 1

14230-1

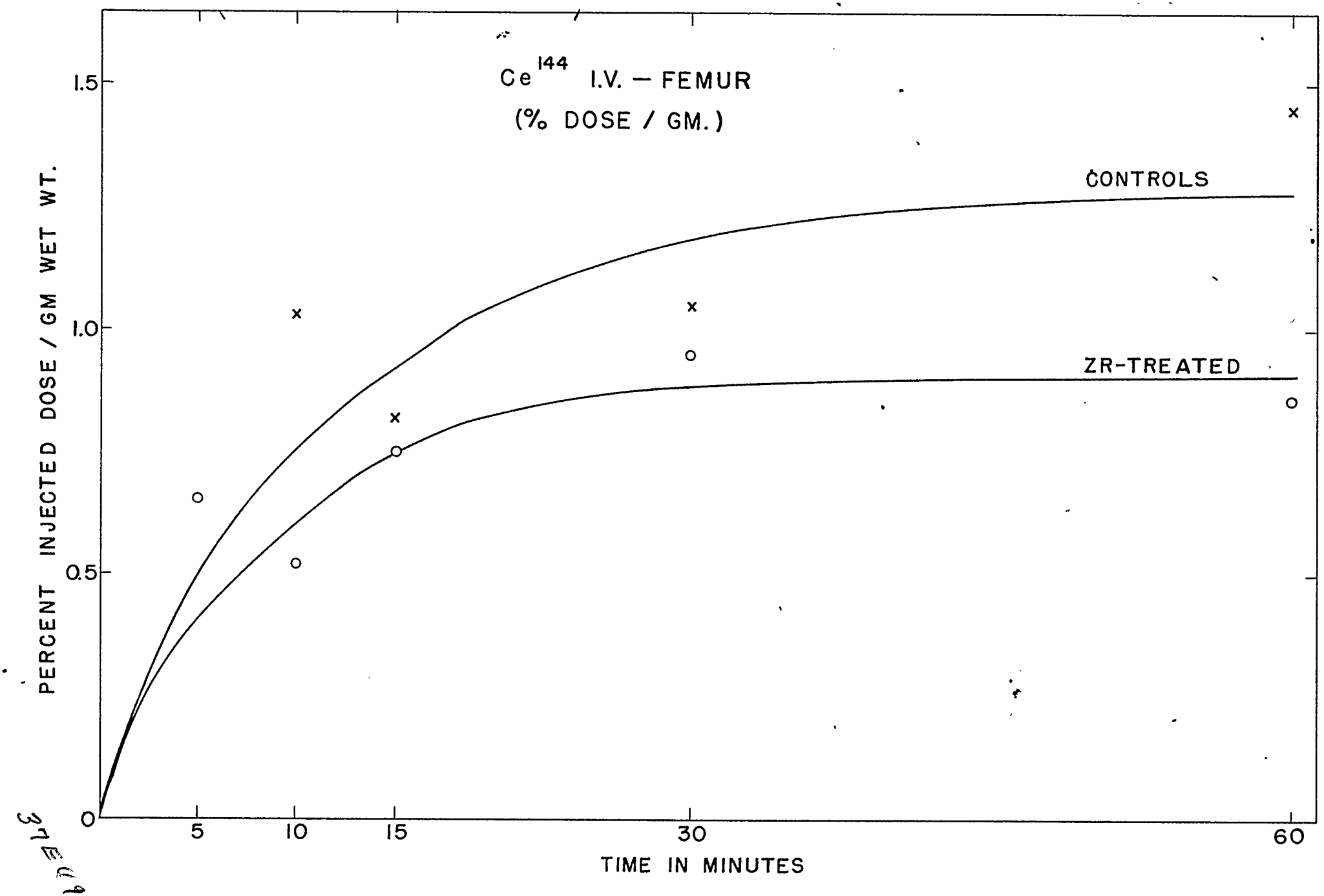

Ce 144 I.V. — FEMUR
(% DOSE / GM.)
CONTROLS
ZR-TREATED
PERCENT INJECTED DOSE / GM WET WT.
1.5
1.0
0.5
0
5
10
15
30
60
TIME IN MINUTES

FIG. 2

14231-1

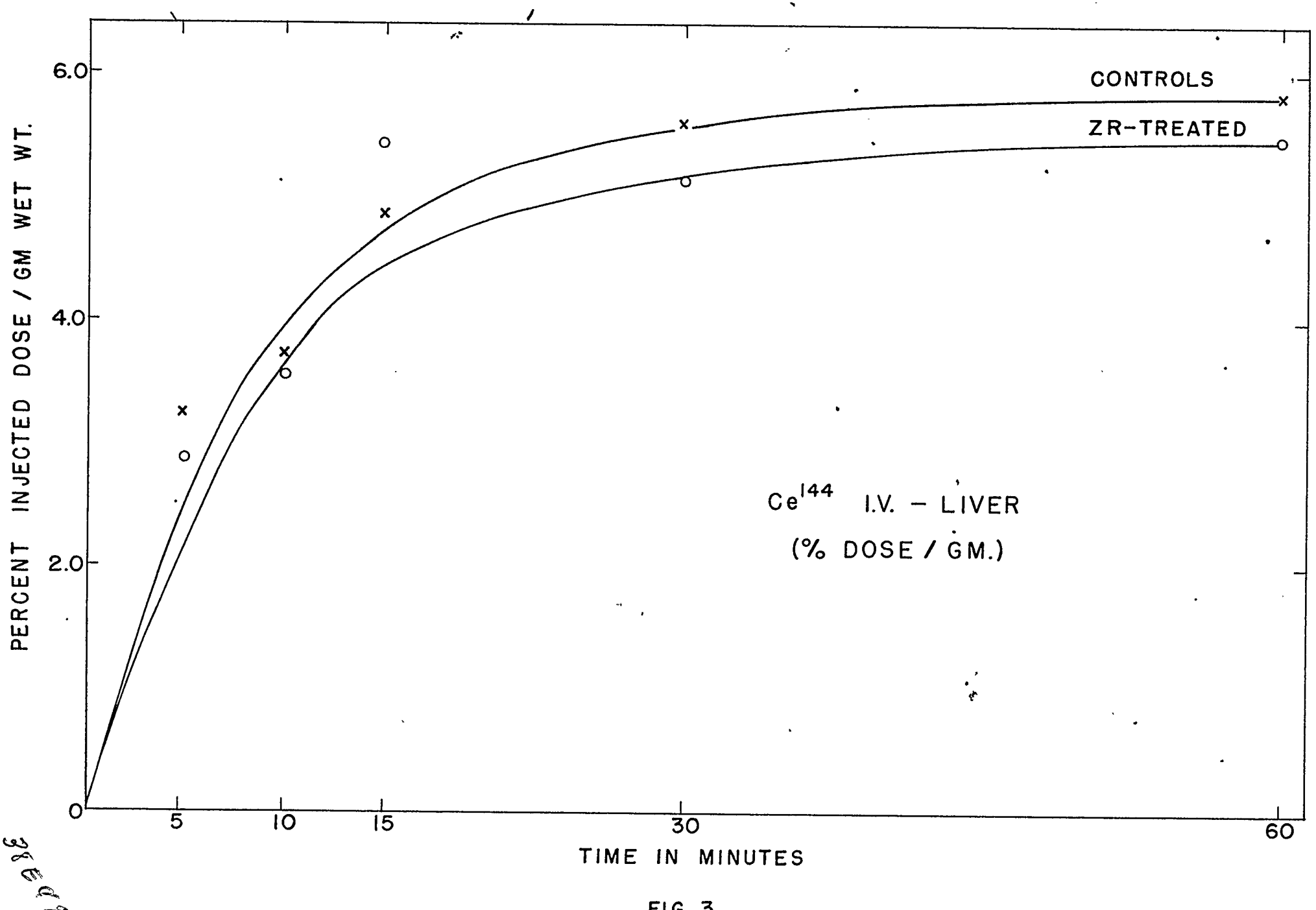
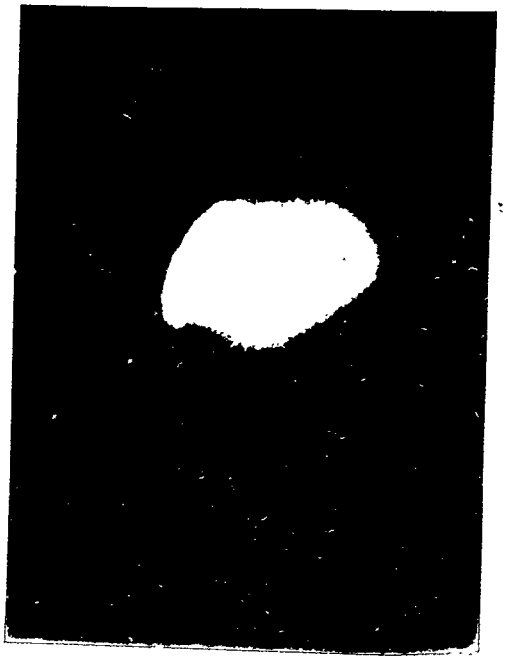

6.0
4.0
2.0
0
PERCENT INJECTED DOSE / GM WET WT.
CONTROLS
ZR-TREATED
Ce144 I.V. – LIVER
(% DOSE / GM.)
5
10
15
30
60
TIME IN MINUTES

FIG. 3

14232-1

Radioautographs of Rat Tibiae

Normal



Rachitic

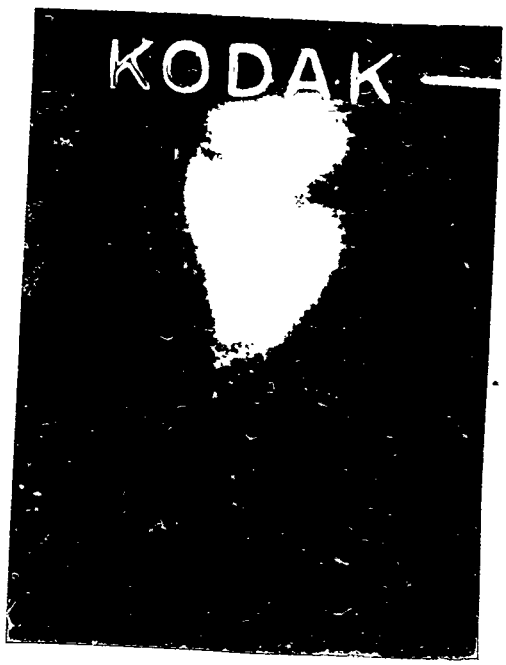


FIG. 4

OZ-708

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II BIOLOGICAL STUDIES OF RADIATION EFFECTS

J. H. Lawrence - in charge

Project 48A-II

Activation Trace Analysis

Ray Wolfe, Rayburn W. Dunn and Cornelius A. Tobias*

Determination of turnover rates of trace elements in metabolic processes by use of the isotopic technique requires accurate knowledge of their absolute concentration. For many elements activation analysis[1] presents a method of increasing the accuracy and sensitivity over that of microchemical methods. Future increases in neutron pile fluxes promise to enhance further the desirable advantages of this technique. Development of a method having high sensitivity may reveal heretofore unknown biological trace elements, and lead to the discovery of possible biological functions.

In a previous communication we established the feasibility of employing activation analysis in biological studies[2].

Briefly, activation analysis is applied as follows: the material to be assayed is placed in quartz tubes and neutron bombarded. The neutron energy is of the thermal level thus reducing nuclear transmutation reactions and resulting predominantly in neutron capture. Several elements in this sample may become radioactive, and, after a known exposure, the amount of each newly formed radioactive isotope may be measured by standard radiochemical techniques. If a radioactive isotope A is formed from trace element B, the mass of element B originally present in the sample may be calculated from the formula:

$$x = \frac{d.a.\varepsilon\lambda\tau}{6.02.10^{23}.\sigma.F\,(1-\varepsilon^{-\lambda t})}$$

where:

x = unknown mass.
A = atomic weight.
F = particle flux.
λ = decay constant of induced radioactivity.
σ = cross section of B to form A.
t = length of time of exposure to neutrons.
τ = length of time elapsed between neutron exposure and time of measurement of rate of disintegration of the separated isotope A.
d = rate of disintegration of sample A measured at time T.

The above formula will hold true if the neutron flux remains constant throughout irradiation time, if the sample is not large enough to depress appreciably the

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* With the technical assistance of Mrs. Jean Luce.

the neutron flux, and if the irradiation is not intense enough to decrease significantly the amount of isotope B to form A. One does not always have to determine the absolute neutron flux in such a case. It is more convenient to measure the radioactivity induced after silultaneous irradiation of a known amount of element B. A list of some of the elements appreciably activated is available in an article by Tobias and Dunn[2]. Sensitivity and accuracy for a given element will be a function of the half life, thermal neutron cross section, and the neutron flux to which it is subjected.

Many elements in biological tissue ash may become activated. It thus becomes necessary to separate these elements to identify them and determine their radioactivity. Except in special cases[4] the chemical technique of separation in this problem generally has been difficult to apply, as the large amount of phosphorus and sulphur (99.9 percent of total radioactivity shortly after bombardment) is unwieldy and the problem of occlusion of trace elements to added carrier is difficult to overcome. Chemical methods also require trial and error addition of carrier amounts of each element whose presence is deemed possible or probable and development of suitable radiochemical methods of separation - a monumental task. Regardless of the difficulties involved some progress has been made with chemical separations[3]. In view of the recent advances in the application of ion exchange, such a technique seemed promising as a possible method of separating the radioisotopes produced by neutron activation.

Briefly, ion exchange column separations are carried out as follows: In Dowex 50 we have a strongly acid cation exchanger of synthetic origin containing only one type of exchange group- sulfonic acid. The synthetic, organic anion moiety is insoluble and highly resistant to corrosive action by virtue of its chemical composition and high degree of polymerization. If we consider the condition where cation C^+ is allowed to exchange with the hydrogen form resin HR, the reversible equilibrium exchange reaction can be expressed as follows: $C^+ + HR \leftrightharpoons H^+ + CR$. If a small amount of resin containing a cation (CR) is placed at the top of a column of hydrogen form resin and an aqueous solution of H^+ is passed through the column, the cation will develop down the column at a rate which is a function of its distribution coefficient K_d (solid over liquid concentration ratio). If the linear rate of flow is slow enough that the condition of equilibrium is essentially maintained, and the distribution coefficient K_d has a relatively high value (cations are predominantly associated with the resin) the cation will be developed down the column in a band which does not greatly increase in width. The rate of development will be a function of the distribution coefficient so that cations having different K_d values will develop down the column at different rates. If the rates are different enough and the column long enough, a complete separation can be produced. If volumne segments are collected and cation concentrations determined in each volume, a plot of concentration against volume eluted yields the elution curve. The K_d can be controlled for a specific ion by the concentration of the eluting ions because of the mass effect. The ion exchange affinities of various ions differ because of charge, ionic radii and hydration radii differences.

Several theoretical advantages of ion exchange in this procedure are as follows: Anions and cations can be separated. Cations as a group and anions as a group can be further separated on the basis of ionic charge or valence. Certain separations of cations or anions of like charge, based on differences in ionic radii or radius of hydration are possible. For ions similar in, or mutually

compensating in all of the above respects, separations are possible by use of suitable complexing agents. The value of complexing agents in the ion exchange separation of rare earths and fission products has been well established[5].

The procedures used have been developed on the basis of experience gained from previous bombardments and after preliminary ion exchange studies which will be described later in the text.

Methods and Procedure. Method of Preparing and Handling Samples Before and After Irradiation.

Tissue Preparation - the biological samples were prepared as follows:

Tissues are weighed immediately after removal from the sacrificed animal. Wet ashing was then carried out in 125 ml. Erlenmeyer flasks using HNO_3 and a few drops of 30 percent H_2O_2. Emphasis was placed on utilizing glassware of the highest possible cleanliness. Any contamination not present uniformly between control and samples will produce errors in ultimate quantitative determinations. Since we are dealing with sub-micro quantities the ordinarily overlooked sources of contamination, i.e. dust, splash contamination by breaking gas bubbles in adjacent samples on the hot plate, and contamination from the hood itself, become quite important. Suitable reagent controls were employed to check on contamination introduced with reagents and in the glassware. After tissues had been digested down to their ash content the flasks were taken to dryness. The ash contents of each flask was then transferred to quartz tubes 10 mm in diameter and 6 cm long by taking it up in a minimum volume of 1 N HCl and transferring it to the quartz tubes by use of a transfer pipette. Quartz tubes are used as the sample containers for several reasons: The container should not absorb many neutrons. It should not contribute appreciable amounts of activity (i.e. glass has a high boron content). It should be capable of withstanding somewhat elevated temperatures. It should be relatively unreactive chemically and incapable of exchanging or adsorbing material. In view of the above considerations quartz was deemed the best material for the purpose. Contents of the quartz tubes were then evaporated to dryness at 70°C in an oven. Several transfers were necessary to effect complete and quantitative tranfer of the sample. The importance of complete drying is to be emphasized. Since the quartz tubes are fire sealed before irradiation, a closed system is established. It is well known that H_2O_2, hydrogen, and oxygen are produced upon irradiation of aqueous solutions by ionizing particles. Under conditions of high thermal neutron flux for a considerable period of time high positive O_2 and H_2 pressures could be produced forming an explosion and radioactive contamination hazard.

Packing and Irradiation. After being sealed the quartz tubes are placed in aluminum cylinders of slugs designed especially for pile irradiation. To prevent breakage of quartz tubes the unfilled space in each tube is packed lightly with special high purity graphite. A small amount of graphite is placed in the aluminum tube followed by a sealed quartz tube. After more graphite has been added, a second quartz tube is placed in each aluminum container. A small amount of graphite is then placed between the last quartz tube and the screw plug at the end of the aluminum slug. The graphite is packed firmly by tapping the aluminum slug lightly on the table. Irradiation times can be varied to suit the particular requirements of the experiment. At a given constant neutron flux 99 percent of the equilibrium

activity will be attained after seven half lives irradiation time (equilibrium activity is attained when the rate of decay equals the rate of activation). The equilibrium activity level will be a function of the neutron flux. Longer irradiation favors determination of the long lived isotopes whereas a shorter irradiation will be sufficient to bring short lived isotopes up to their equilibrium activity. Irradiation or bombardment times have been of the order of two weeks to one month thus far.

Post Irradiation Technique. Following irradiation the aluminum slugs were received in a large lead shield. The slugs were removed, one by one, using long handled clamps, and placed in a lead brick with a locking set screw. The set screw is tightened to allow the plug in the end of the aluminum slug to be removed with a screw driver. Adequate shielding is important because samples measure up to lr/hr at 6 in. with a Zeuss meter. Attempts to use a rubber tube with suction to remove the quartz tubes from the aluminum slugs have failed in several cases. Stuck samples are removed by sawing with a hack saw using a lead shield with a narrow slit through which the saw is placed. Mr. Aldo Azzalini, under the direction of Mr. Nelson Garden, Health Chemistry Division, U.C.R.L., is now cooperating in the construction of apparatus suitable for dissolving off the aluminum slug in HCl. This modification will greatly simplify the process and cut down the radioactive exposure by a large factor. After the quartz tubes have been removed, they are washed and opened. Opening by use of a glass saw was found to be unsatisfactory because of the quartz dust introduced into the sample. In the future one end of the quartz tube will be drawn and narrowed down before sealing to facilitate breaking of the tube after irradiation. The samples were identified by numbers scratched on the quartz tubes with a diamond pencil. The ash is taken up in 1 ml of 1 N HCl and transferred to a labeled 10 ml volumetric flask. Finally the contents of the flask are diluted up to volume with distilled water.

Materials and Procedure. Apparatus Developed or Obtained for the Ion Exchange Study. Fig. 1 illustrates a preliminary apparatus developed to check the feasibility of using this technique. A model AW Esterline Angus graphic instrument was modified to carry the graphing paper roll out horizontally 12 inches, then back to the take up reel. A lucite rack was made to support micro-ion exchange columns (bore 2 mm) over the paper and the gravity feed eluting solution fell in drops and was dried as it passed slowly beneath a heating element. The rate of movement of the graph paper was adjusted so that drops fell about 1/2 inch apart. The graphing paper was later pulled through a slit under a shielded end window Geiger counter tube which was hooked to a counting rate meter. The counting rate meter response was graphed on an unmodified Esterline Angus graphic instrument. The various elution activity curves are plotted automatically in this manner. The elution, drying, counting, and graphing operation could have been incorporated into a single operation. By using two stages in the process, it was possible to run three columns at once, later taking activity curves by running the paper roll through the counting set up at a faster rate, once for each elution track.

The glass ion exchange columns illustrated in Fig. 2 were constructed by U.C.R.L. Glass Shop. Fig. 2 illustrates the micro ion exchange column used. Specifications for this piece of apparatus were obtained from Dr. K. Street, a member of Dr. Glenn Seaborg's group, U.C.R.L. Fig. 2 also illustrates the larger columns used in this work. The basic design is a modification of that used by E. R. Tompkins[6].

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Fig. 3 illustrates the sample changer designed for U.S.R.L. by Mr. Herman Robinson. This apparatus was later modified to accommodate 1 in. counting cups, as well as test tubes.

To assure a constant rate of flow a syringe feed apparatus was constructed. Eight Luer type all glass syringes were mounted vertically in a rack. A horizontally mounted bar was used to carry the pistons downward by a rotating lead screw. The lead screws were geared off a drive bar by worm gears. The power was supplied by a 1 RPM synchronous motor having 120 inch ounces of torque. Two syringes were operated in parallel giving a volume rate of flow of 10 ml/hr for four columns simultaneously. Fluid leakage around bearing surfaces was eliminated by use of a silicone grease.

Samples were counted in 1 in. plastic (polyethylene) cups using an automatic sample changing, recording counter. This device will accommodate 25 samples per lead and records on a paper tape the time required for a predetermined number of counts together with the sample number.

Methods and Procedure.

Developmental Considerations and Methods in Ion Exchange. The following considerations were studied in deciding on the conditions to be used:

Elution Conditions. Solubility of the salts of the anion in the eluting solution and the various cations encountered. Volatile eluate solution as a means of eliminating self absorption in the counted samples. Oxidizing and reducing influence of the eluate. The best substance in view of the above considerations is HCl.

Rate of Flow of Eluate. Studies by E. R. Tompkins[6] have indicated the importance of operating columns as near to equilibrium conditions as possible. A practical rate of flow was found to be 0.2 ml/cm^2/min. Consequently a standard rate of flow of 10 ml/hr was used with the understanding that later modification might be necessary for this particular application. The rate of flow was adjusted by the syringe feed apparatus described under apparatus.

Cross Sectional Area of Columns. The cross sectional area of the columns will of necessity be adjusted to the number of equivalents to be developed down the column. Harris and Tompkins[7] found that a maximum of 0.5 - 1.0 grams/cm^2 cross sectional area could be accommodated in a rare earth separation. This would be approximately 0.3 milliequivalents per square centimeter area. Consideration of the estimated average molecular weight of tissue ash and the minimum measurable activity led to a size of the order of one square centimeter cross sectional area as a practical size. Consequently 1.4 cm^2 was used as the standard size column.

Column Length. Various column lengths (6-24 cm) were used in preliminary experiments. It was determined that a 12 cm column was long enough to allow separations of several more rapidly eluting cations and short enough to elute most cations in a practical period of time.

Pre-treatment of Resin. The commercially available Dowex 50 (hydrogen form 250-500 mesh) was found to contain impurities, the most obvious of which is iron.

Contaminating cations were removed by passing 6 N HCl through a column of the resin. This procedure assures that the resin is all converted to the hydrogen form. The resin is washed free of acid with distilled water, and stored in the wet state until used in packing the ion exchange column. Columns were packed by placing a thin mat of glass wool at the bottom of the column. The wet resin was then slurried into a column about half full of water. The heavier particles settle to the bottom first forming a mat which prevents the smaller particles from working through. Distilled water is passed through the column for twenty-four hours to pack the resin down the column, which if packed at first to about 12.5 cm packs down to 12 cm length.

Method of Applying Activity to the Column. Two methods of application are possible. First cations can be adsorbed on a narrow band at the top of the resin column by passing a near neutral solution very slowly through the column. Secondly the activity can be preadsorbed on resin in a separate container and transferred to the top of the column. The latter method was chosen because samples were received in dry quartz tubes, and it was necessary to dissolve them in 1.0 N HCl, which is later diluted giving 0.2 N acid. The latter method is also better suited to washing or removal of anions before placing activity on the column, should such a modification be desirable.

Equilibrium Studies. Equilibrium studies were conducted by allowing a given weight of hydrogen form resin (air dried at 80° C) to come to exchange equilibrium with a known amount of activity (Co^{60}) under varying conditions of acidity. Activity concentration of the solution was determined by withdrawing an aliquot after equilibration, and acidity was determined on a second aliquot by titration. Concentration of acitivity on the resin was determined by difference of known total and amount found in solution. The time required for the same activity to elute from a standard 12 cm column under varying conditions of acidity was also determined.

Elution Studies. Elution studies were carried out by using known activities under the same conditions as thos proposed for biological samples. Inert ash was eluted with a known amount of activity and recovery checks were made. In one case two important elements, Fe and Co, eluted together and a method of separating by eluting with oxalate complexing agent was developed. Elution characteristics of Fe, Co, Zn, Na, K, Rb, P, Ca and Sr were studied using known radioisotopes. On the basis of these studies conditions were modified to compromise all the conflicting factors.

Biological Samples. Two sets of biological samples have been and are still being studied. The first was separated red blood cells, white blood cells, and plasma from humans. The second set consisted of the tissues of mice. Some of the conditions were varied slightly as the work progressed, but the following is the general procedure. Aliquots of the samples were adsorbed in separate containers on Dowex 50 hydrogen form resin, as described previously. Three separate batches of resin were used to adsorb each sample. The resin was then placed on the top of previously prepared columns and eluted with HCl at a rate of 10 ml/hr. The acidity was 0.9 N for 30 hours, 1.5 N for 30-50 hours, 6 N after 50 hours, and later it was found necessary to use 12 N HCl after 75 or 80 hours. through 100 hours. Samples were first collected in test tubes but later this step was modified so that counting samples of up to 10 elution aliquots could be collected and in alternate positions on the sample changer the eluate was allowed to be collected in a flask for periods up to 20. Definite volumes could be collected in these flasks for late manipulation after adding small amounts of carrier. Samples were dried 24 per holder (1/4 in. plywood with 1 in. depressions drilled on one side) in a corrosion resistant electric oven at 70° C in a hood. After drying they were counted in the automatic sample changing counter described previously. Curves of elution were plotted on similog paper for the convenience of the resultant size of the graph. Aliquots of elution curves between 20-45 hours were collected and re-eluted in an attempt to separate the activities superimposed in the HCl elution curve in the range. Since several activities are known to come off

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simultaneously, volume segments of special columns were collected in carrier solutions and separated by chemical methods. The volume segments collected were known to contain the Cu, Zn, Fe, Co and Rb sections of the elution curve, plus possible unknown activities. The chemical separation technique follows.

Methods and Procedure.

Method of Separation and Recovery of Copper, Iron, Zinc, Cobalt, Rubidium and Cesium.

Preliminary Elution from Ion Exchange Columns. For the purpose of chemical separation of Cu, Fe, Zn, Co, Rb and Cs, two consecutive fractions were collected from each of the eight ion exchange columns. Fraction one contained all of the Cu, and possibly small quantities of some of the elements listed above. This portion was collected in a container in which was previously placed a small amount of solution containing milligram amounts of carrier Cu and microgram amounts of the remaining five elements for holdback. The elution acidity was 0.9 N.

Fraction two was 1.5 N in acid and contained the balance of those elements listed, possibly contained Cs, but was free of Cu. This portion was collected in a flask containing 1 milligram each of carrier Fe, Zn, and Co, plus microgram amounts of holdback Rb and Cs.

Separation of Copper. The Cu fraction was immediately treated with H_2S to saturation and the precipitate removed by centrifugation. The supernatant liquid, together with the dilute HCl-H_2S saturated wash solution, was set aside for later combination with the second ion exchange fraction.

The washed CuS precipitate was dissolved in concentrated HCl plus successive small portions of HNO_3. The resulting solution was diluted with 0.9 N HCl containing milligram amounts of holdback phosphate. This solution was again saturated with H_2S, etc., as above, saving the supernatant liquid plus wash solution.

A third precipitation, with holdback phosphate, is advisable, but was not performed. The CuS was redissolved and transferred to a standard plastic cup for counting. The elapsed time after removal from pile to beginning of decay curve data collection was approximately 107 hours.

Separation of Iron. The Cu-free solution was evaporated to dryness to remove H_2S and excess HCl, and the residue taken up in a few drops of HCl. This solution was transferred with a few ml of water to a centrifuge cone containing 0.1 mg each of carrier Fe, Zn, and Co. The volume after transfer was 6-8 ml, and to this was added NH_4OH to neutralize the acid; then 2 ml of freshly prepared $(NH_4)_2S$ solution. After centrifugation, the supernatant liquid, plus wash solution, was discarded. (Since the first fraction from the ion exchange column contained relatively larger amounts of P^{32} than did the second fraction, and little or no Rb, $(NH_4)_2S$ precipitation was resorted to with the Cu-free solution in order to decrease the undesirable activities before combination of the Fe, Zn, and Co with the second elution fraction.)

The second portion from the ion exchange columns was now evaporated to dryness and treated the same way as described in the paragraph immediately above, except that no additional carrier was added, and the supernatant liquid, plus wash, was filtered and saved for subsequent Rb and Cs analysis.

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The two sulfide precipitates were dissolved in aqua regia, using heat and additional small amounts of HNO_3 to oxidize the elemental sulfur, and then combined. After transfer the resulting solution had a volume of 5-6 ml, and was again treated to precipitate the sulfides. The supernatant liquid plus wash was filtered into the beaker already containing the Rb-Cs solution. (Filtration is necessary here in order to remove from the Rb-Cs solution any small amounts of precipitate remaining on the meniscus after centrifuging. No attempt was made to recover these small amounts of sulfide.)

A third precipitation of the sulfides was not considered necessary, in view of the specificity of the subsequent analytical procedure.

The reprecipitated sulfide was again dissolved, as outlined above, and the final solution evaporated nearly to dryness. Addition of successive small amounts of HCl and heating served to convert the metal salts to the chlorides.

Using a total of 20 ml of 8 N HCl, the active solution was transferred to a 60 ml separatory funnel and extracted once with an equal volume of thoroughly acid - and water-washed isopropyl ether. The aqueous layer was transferred to a second 60 ml separatory funnel to which had previously been added an additional 2 mg of carrier Fe. A second similar extraction was then performed in funnel number two and the aqueous layer removed to a beaker and saved for subsequent Zn and Co separations.

The first separatory funnel was twice shaken with 20 ml of 8 N HCl, each extraction wash being transferred in turn to the second separatory funnel, and shaken up before collection in the beaker.

The two ether portions were combined in the first separatory funnel. The second funnel was washed twice with 2 ml portions of water, each portion being transferred in turn to first separatory, shaken, and removed to a centrifuge cone. Finally the first funnel was extracted once more with a 3 ml portion of water.

The combined aqueous solution containing the Fe was precipitated with NH_4OH, centrifuged, and the supernatant liquid was discarded. The precipitate was dissolved in a small amount of HCl and transferred to an electroplating cell. The electrolyte was made up with 5 volumes of saturated $(NH_4)_2 C_2O_4$ plus one volume of saturated $H_2C_2O_4$. 30 ml of this mixture were used to affect the transfer. Electroplating was carried out at 0.8 ampere using a platinum anode and a 1 in. copper planchet cathode. At the end of 30 minutes an additional 2 mg of carrier Fe and 3 ml of saturated $H_2C_2O_4$ solution were added. Plating was completed at the end of another 30 minutes. Quantitative removal of Fe from solution was tested for by mixing a drop of the solution on a test plate with a drop of $(NH_4)_2S_x$ solution. After washing in water and acetone, and drying, the planchets were ready for counting.

Separation of Zinc. The ether-extracted solution, containing all the Zn and Co and a trace of Fe, was evaporated to dryness, and the residue was treated with HNO_3, then HNO_3-$HClO_4$ to oxidize organic material remaining from the ether.

The inorganic residue was dissolved in HCl, and tranferred to a 60 ml separatory funnel, using 20 ml of 0.5 M sodium acetate - 1 percent KCN solution to affect the transfer. Using bromcresol green indicator, the pH was adjusted to between 5 and 5.5 with NH_4OH.

The dithizone extraction procedure of Sandell[8] was followed for the Zn separation, with the exception that a half saturated solution of dithizone in carbon tetrachloride was used. Extractions were repeated with 20-25 ml portions of dithizone solution until the red color of zinc dithizonate was no longer apparent, and then once more. (The amount of carrier zinc used was predicated upon the assumption that a satisfactory separation of Zn from Co could be made if the former were precipitated with H_2S from a buffered citrate solution at pH 3.0. This procedure was tested but was not consistently satisfactory. A five-fold decrease in carrier Zn should be made if the dithizone extraction is to be employed.)

The combined dithizone solutions were next extracted with successive small portions of 0.01 N HCl until the original green color prevailed in the CCl_4 layer. The pooled aqueous portions were evaporated to dryness and treated with HNO_3, then HNO_3-$HClO_4$, in order to eliminate organic decomposition products from the dithizone. The inorganic residue was dissolved in a small amount of HCl and transferred, with the aid of 20 ml of 1 N NaOH-1 percent KCN solution, to an electroplating cell. Plating was carried out at 0.4 ampere for one hour, using the same setup as with the Fe. 1 mg. of additional Zn carrier and 10 ml of the electrolyte solution were then added, and plating continued for another hour, at which time deposition was complete. After washing, etc., as previously outlined, the planchets were ready for counting.

Separation of Cobalt. The Zn-free, dithizone-extracted aqueous solution containing the Co and a trace of Fe, was evaporated to dryness, and treated with acid in order to expel HCN. The residue was treated with HNO_3 and finally with HNO_3-$HClO_4$. The acetate was not completely oxididized, however, before transferring the material to a centrifuge cone and precipitating with $(NH_4)_2S$ solution. The precipitation was quantitative, nevertheless, since little or no activity could be detected in the material obtained after drying the supernatant liquid.

The CoS was dissolved in aqua regia, as previously outlined, and transferred with 20-30 ml of 10 percent hydroxylamine- conc. NH_4OH solution to an electroplating cell, as outlined for Fe and Zn. Plating was carried out at 0.4 ampere for three hours, without further addition of carrier cobalt.

Results.

Equilibrium Studies. Figure 4 shows a plot of the relationship between K_d and HCl normality for cobalt 60. The rapid increase in K_d with decreasing acidity shows why it is possible to adsorb cations on a very narrow band at the top of the column by passing through a nearly neutral solution.

Figure 5 illustrates a plot of log K_d against log of the rate of elution as a straight line. This indicates that the rate of elution has a reciprocal relationship to the distribution coefficient, or the rate is proportional to the amount of a given cation in the fluid phase. This data is helpful in interpreting equilibrium data in terms of column elution.

Elution Studies. Figure 6 illustrates an elution curve for P^{32}, Na, K, and Rb.

Figure 7 illustrates an elution curve for Na, Zn, Co, Ca, Sr and P. It was found that the presence of radioactive inert tissue ash had no detectable influence on the general elution characteristics. It was observed, however, that the Fe elution curve tended to drag out becoming assymetrical. This might possibly be explained by lack of equilibrium due to a slower rate of ion exchange peculiar to this ion. Use of smaller resin size (colloidal aggregate) and higher operating temperatures would possibly eliminate this assymetry.

Figure 8 illustrates the elution curve for the separation of Fe and Co, which elute together with HCl, using ammonium oxalate. The influence of pH in this elution was found to be quite critical.

As a result of these studies, it was found best to increase acidity of HCl during the elution of the biological samples as described under methods.

Activated Tissue and Blood Samples. Result of the elution of activated tissue and blood samples are not yet complete as decay curves are being made and further separations attempted. They are shown here mainly to indicate the power of the method, and readily detectable differences in tissue composition. Figure 9 shows the typical elution curves for various types of blood samples. The known cation elution curve is reproduced from Figure 7 for convenient comparison. The first activity apparent at the origin of the elution curve consists of all negatively charged ions present in the sample. These consist mainly of P^{32} and S^{35}. The activity peak apparent at eight hours in the RBC WBC elution curve is that of Na^{24}. Because of its short half-life, it was not obtained in the plasma sample which was eluted later. The activity peak at 24 hours in the RBC elution curve is that of K which had also decayed out in white cell and plasma elution curves which were made much later. Probably the outstanding difference is the large activity plateau evident in the red blood cell elution curve at 40 hours.

Figure 10 is a plot of the elution curve of a RBC sample two days after bombardment stopped. The second count of the samples two days later, as indicated by dashed lines, is reproduced on the same graph. Several short half-lived peaks, that of sodium (8 hours or 80 ml. elution volume) and that of potassium superimposed on copper (24 hours or 240 ml elution volumes) is quite marked in its rapid decay.

Figures 11 and 12 represent various counts of a RBC elution curve made at a later date under improved experimental conditions. Figure 11 is the primary count made on the samples together with the dashed in locations of several known elements. Several counts of the same samples were made at various time intervals after the first. The last count made after 174 days is reproduced in the lower part of Figure 12. The upper part of Figure 12 is the first minus the last activity measurement on the elution samples, or it represents the net decay.

Decay curves were made of important samples and absorption curves were made whenever the activity was great enough. Table I lists activity peaks along with sample number, approximate half-life, approximate beta energy if known, elements known to elute under same conditions at this particular place, and possible isotopes as obtained from Seaborg Perlman Tables[9] eliminating those not produced by neutron capture from stable elements. In the areas of the above three elution curve plots where several activities are superimposed, the shorter half-lived components have

49 49

decayed out. For example, active peak at elution volume 250 ml. Figure 11 has been isolated by decay from the large peak becoming a separate peak in Figure 12. A decay curve of active peak at elution volume 290 ml gives a straight line semi-log curve indicating a pure half life component. This activity has been identified as Rb on the basis of physical measurements and by eluting a known radioactive sample of Rb under similar conditions (see Table I). Work is underway to determine the relative amounts of Fe and Co and other activities in the activity peak at elution volume 320 ml. Activity peaks at 640 ml, 680 ml, 760 ml and 960 ml elution volumes. (Figures 8 and 9 are two low for accurate physical measurements, but approximate half lives are listed in Table I.) Two other low activity peaks at 130 ml and 200 ml elution volumes are as yet unidentified. Fluctuations in the general curve shape at below 25 CPM counting rate result from statistical variation and for this reason, plus the required greater activity for physical measurements, small peaks will of necessity be determined later only by increasing the activity. As later elution curves show some activities are eluted even after 1000 ml elution, consequently, the acidity of the eluate was increased to 12 N HCl.

Figure 13 is an elution curve plot of activated mouse kidney tissue ash. One of the striking observations is the consistent appearance of the small activity peak in blood and the tissue samples that follow at 140 ml elution volume. The general area from 200-300 ml elution volumes in this curve seems to be high in activity instead of showing a distinct cleavage in this area as shown in Figure 11. The general order of isotopes as a function of eluting volume is about the same as that discussed under red cell ash.

Figure 14 is a plot of the elution of activated mouse spleen ash. The elution curve for the reagent control (all mouse tissue) is included in this graph. The essential or outstanding difference here is the appearance of a quantitatively large peak at 330 ml elution volumes which is absent in the kidney curve. The second large peak at 600 ml is calcium and strontium and the last large peak at 930 ml elution volumes was identified as Au^{198} on the basis of half life and beta energy measurements. Unfortunately this activity had decayed out of the control sample before an elution curve was completed, so it is not certain whether this is actually present in tissue or whether it is a contaminant.

Figure 15 is a plot of the elution of activated mouse lung tissue. The general shape of this curve closely resembles that of Figure 13 with the exception of quantitative differences in 130 ml and 240 ml elution volume area. The gold peak is apparent at 750 ml elution volumes in this graph as this activity seems to come off immediately after changing from 6 N to 12 N HCl in the eluate. Some inconsistency in the time of change of acidity is responsible for this difference in location on the elution curve.

Figure 16 is the plot of the elution curve of mouse liver ash. The outstanding difference in this curve is the appearance of a strong unidentified activity at 690 ml elution volume. Identification of this activity awaits completion of half-life and absorption measurements and chemical tests.

The marked segment in Figure 16 was collected with Fe, Co and Zn carrier and re-eluted with NH_4 oxalate. Figure 17 shows the resulting second elution curve. It is known from previous data (Figure 8) that in this type of elution Fe is the first activity off. The second activity peak is Co and the last peak contains Rb, Zn and possibly other unidentified elements. It is our present aim to further

develop this re-elution technique to enable us to separate activities eluted together using HCl.

Integrated activity content of the peaks on the activated tissue ash curves are not comparable as various fractional aliquots were taken. When final data has been taken this factor will be accounted for in calculating the mass of each given isotope present.

One feature that is apparent in the above curves is the tendency for P^{32} to carry out into the elution curves. Several attempts have been made to eliminate this with only partial success. Pre-washing the column with water does not eliminate this tendency. Apparently some adsorption phenomena is responsible for this characteristic, and this is not surprising as it is well known that phosphorus is strongly adsorbed on glassware. Studies are now underway which will attempt to remove anions by adsorption on anion exchange resins before elution of the cations.

Chemical Analysis Combined With Ion Exchange Elution. An analysis of the elution segments containing K, Cu, Rb, Zn, Fe and Co was made using routine tracer method as described on page 43 regarding methods. The data obtained are given below in Table II. They should be regarded as of preliminary nature only.

In conclusion we wish to emphasize that considerable refinement is desirable, but that on the basis of developments thus far, the ion exchange method shows promise of being a very powerful tool in its application to the separation of elements after neutron activation. As more knowledge is gained on the ion exchange separation of isotopes, quantitative determinations of micro-quantities and qualitative identification of new elements in various biological tissue, tissue fractions, and cellular components should be greatly facilitated. The work is being continued.

Summary. The method of irradiation of tissue ash in the chain reacting pile is described. Ion exchange and chemical separation methods are given for some elements. Partial results are given for activation analysis of blood and various animal organs, with preliminary data on iron, copper, zinc and cobalt.

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5 ACS Symposium on Ion Exchange, JACS (1947).
6 Tompkins, E. R. and S. W. Moyer, JACS, 69, 2859 (1947).
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TABLE I

HUMAN RED BLOOD CELL ELUTION DATA

Summary of Radioactive Peaks in Elution Curves (see graphs 7 and 8)

Elution Time hrs.	Approx. measured half life	Measured beta energy Emax Mev	Probable isotope	Known element ion exchange elution test available
0	short 14.3 days	1.7	Anions	Yes
	87 days	0.17	P^{32}	Yes
			S^{35}	
8	14.8 hours	1.5	Na^{24}	Yes
13	> 15 days	?	Unknown	No
20	105 days	0.17	Unknown	No
25	14.8 hours	3.0	K^{42}	Yes
25	14.8 hours	0.6	Cu^{64}	Yes
26	250 days	0.4	Zn^{65}	Yes
30	18 days	1.7	Rb^{86}	Yes
32	> 1 year	~0.3	Co^{60}	Yes
38	56 days	0.3	Fe^{59}, possibly, superimposed by another element.	Yes Fe^{59}
42	46 days	0.45		
50	170 days	0.24	Ca^{45}	Yes
54	?	?	Sr^{89}	Yes
58	?	not known	not known	No
63	?	not known	not known	No
68	4 days	not known	Ytterbium^{169} ?	No
	33 days			
76	84 days	~0.17	?	No
96	66 days	~0.4	Sr^{95} (?)	No

TABLE II

RECOVERY AND CONCENTRATION OF SEVERAL ELEMENTS FROM ASHED BIOLOGICAL MATERIAL AFTER NEUTRON ACTIVATION AND CHEMICAL SEPARATION

Duplicate results are on tissues from different animals.

	Iron		Zinc		Copper		Cobalt	
Sample	Found in sample gms x 10^{-4}	Micrograms per gram wet tissue	Found in sample gms x 10^{-5}	Micrograms per gram wet tissue	Found in sample gms x 10^{-6}	Micrograms per gram wet tissue	Found in sample gms x 10^{-9}	Micrograms per gram wet tissue
Liver 2	9	250	lost	lost	25	6.9	lost	lost
Spleen 1	7.2	570	1.1	9	4	3.1	10	$0.8 \cdot 10^{-2}$
2	6.5	860	0.2	3	1.5	2.0	7.3	$1.0 \cdot 10^{-2}$
Kidney 1	2.1	180	lost	lost	7.2	6.0	8	$0.7 \cdot 10^{-2}$
2	2.7	210	2.2	17	12.4	9.5	70	$5.4 \cdot 10^{-2}$
Lung 1	2.0	430	lost	lost	3.1	6.6	5.5	$1.2 \cdot 10^{-2}$
2	1.8	260	1.3	19	2.9	4.1	8	$1.1 \cdot 10^{-2}$
Reagent control (subtracted)	0.25	-	0.54	-	1.1	-	4.2	-

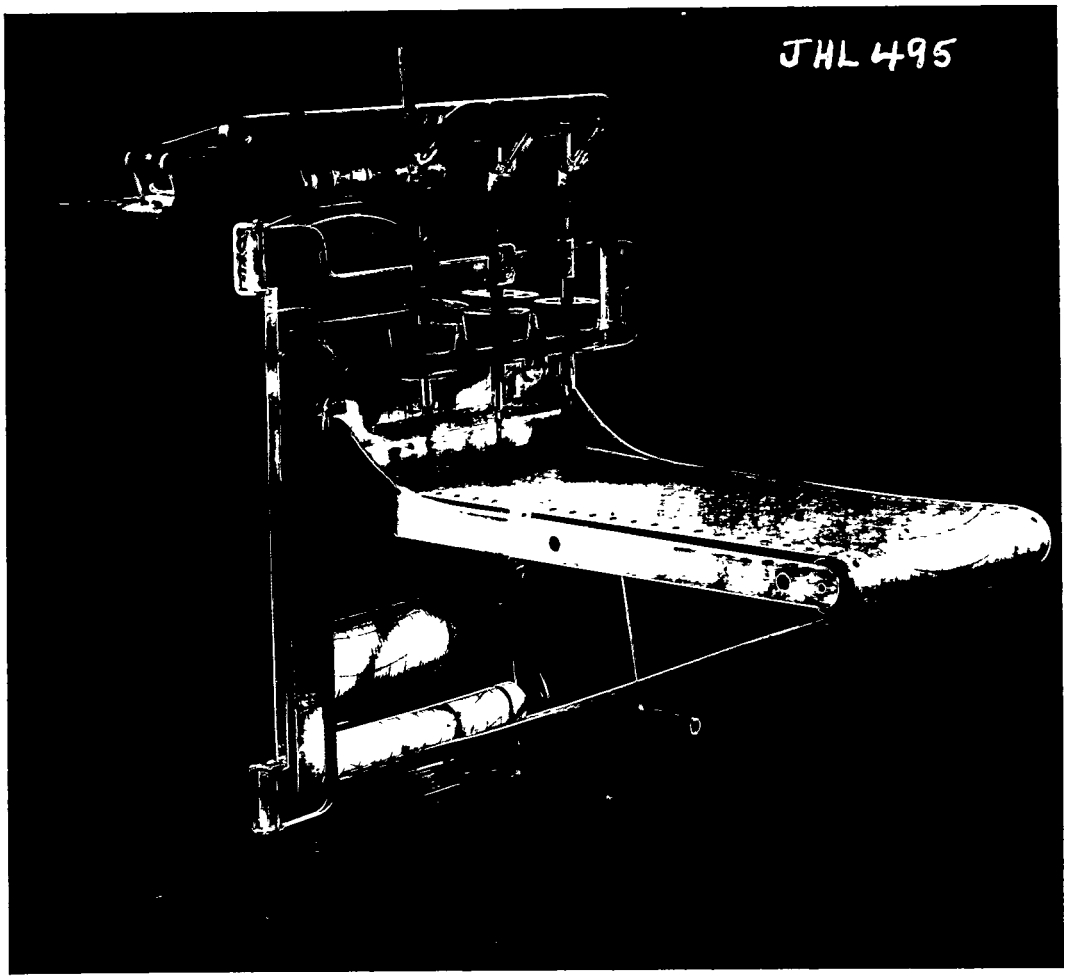

FIG. 1

OZ 742

54 E 49

MACRO ION EXCHANGE COLUMN

MICRO ION EXCHANGE COLUMN

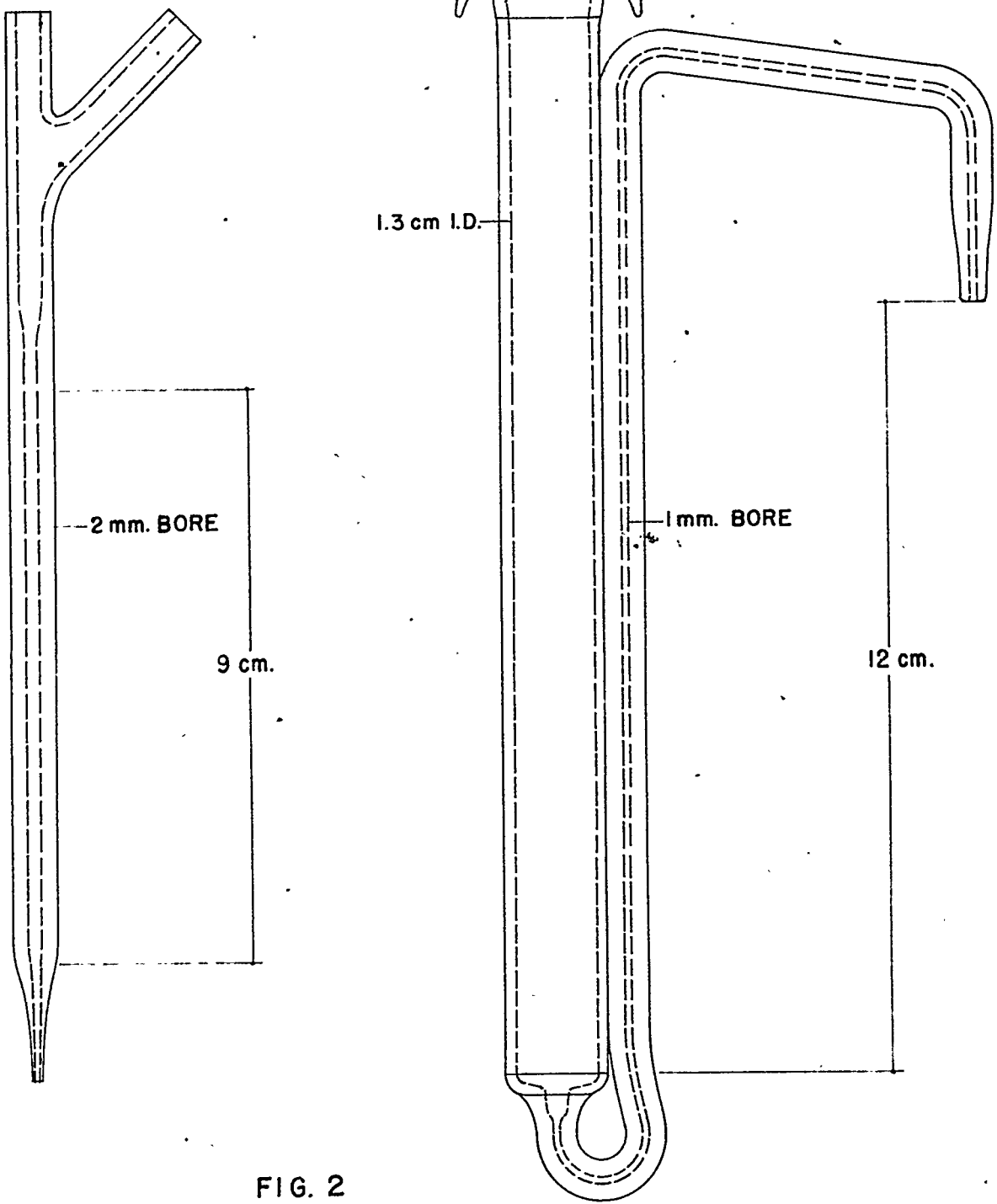

FIG. 2

55649

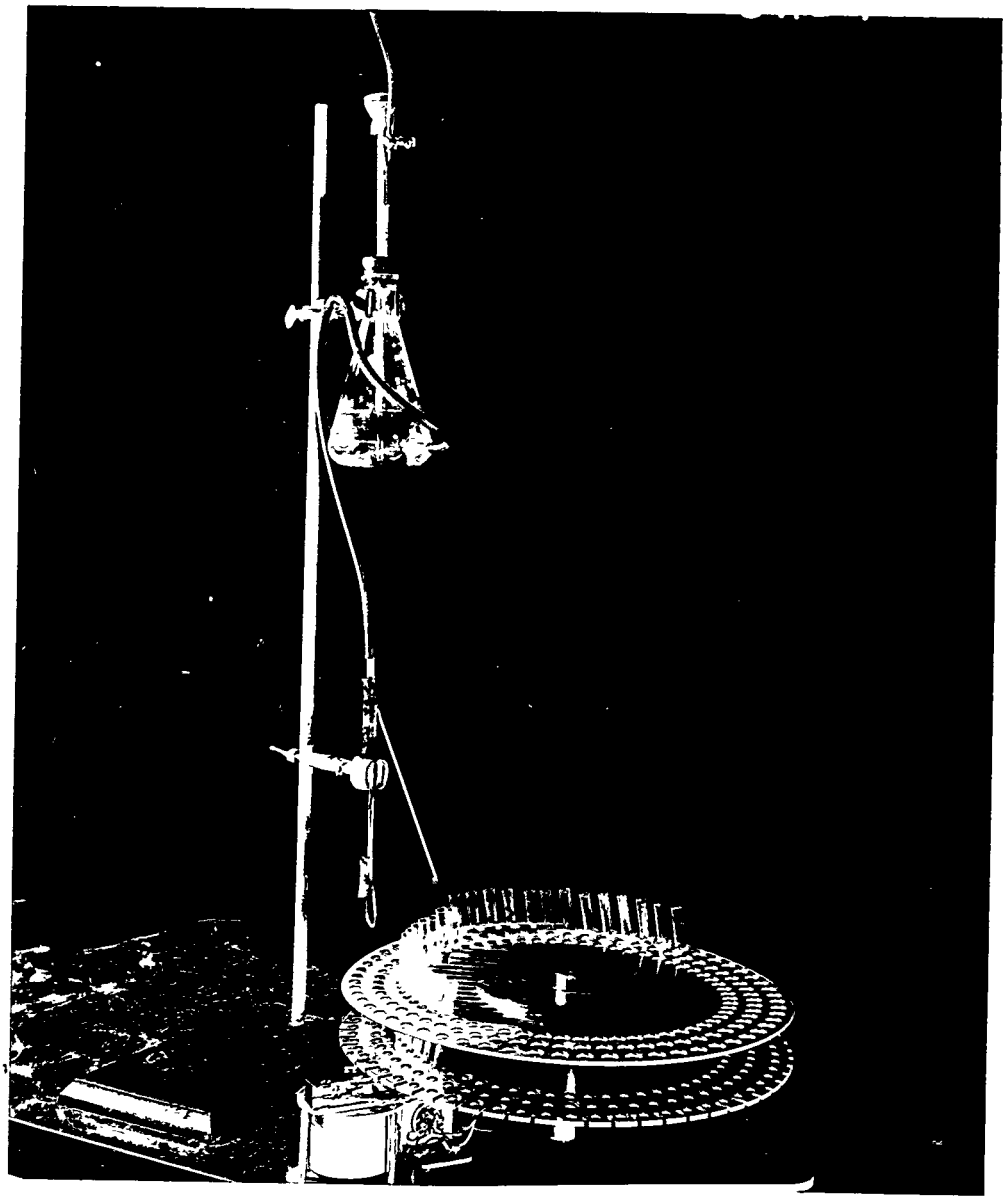

FIG. 3

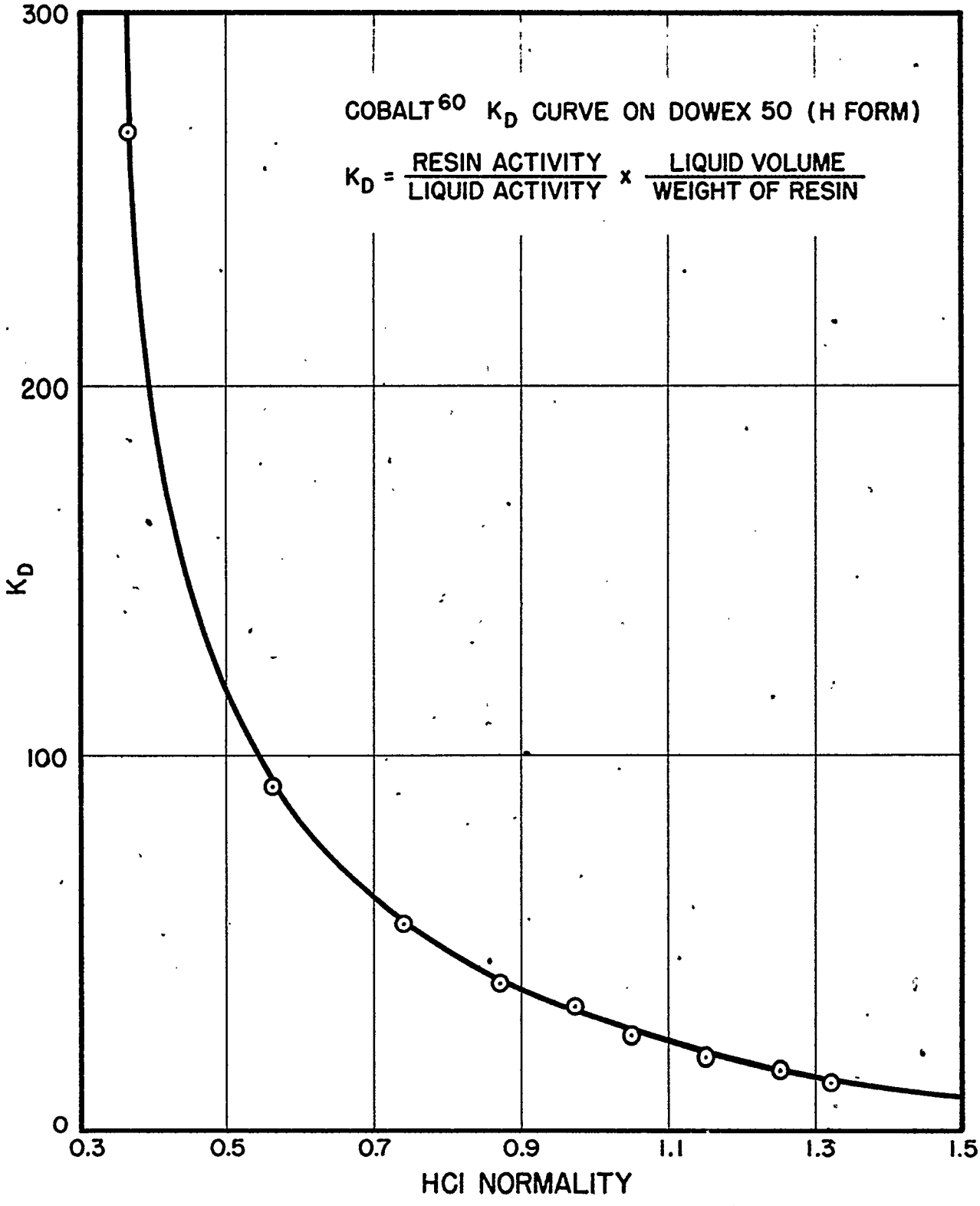
COBALT 60 K_D CURVE ON DOWEX 50 (H FORM)
$K_D = \frac{\text{RESIN ACTIVITY}}{\text{LIQUID ACTIVITY}} \times \frac{\text{LIQUID VOLUME}}{\text{WEIGHT OF RESIN}}$
300
200
100
0
K_D
0.3
0.5
0.7
0.9
1.1
1.3
1.5
HCl NORMALITY

FIG. 4

5 7E 49

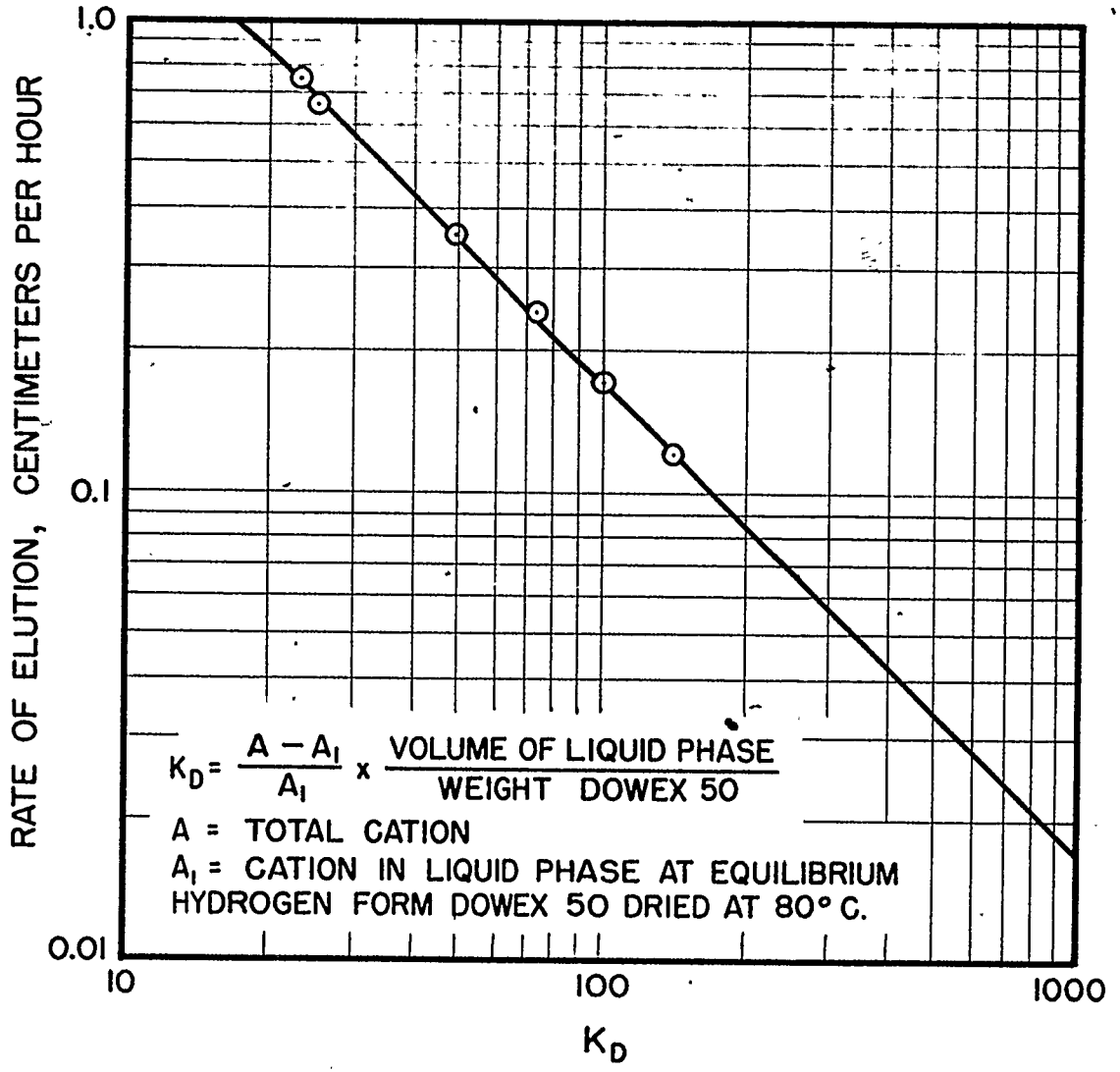
1.0
0.1
0.01
RATE OF ELUTION, CENTIMETERS PER HOUR
10
100
1000
K_D
$K_D = \frac{A - A_l}{A_l} \times \frac{\text{VOLUME OF LIQUID PHASE}}{\text{WEIGHT DOWEX 50}}$
A = TOTAL CATION
A_l = CATION IN LIQUID PHASE AT EQUILIBRIUM
HYDROGEN FORM DOWEX 50 DRIED AT 80° C.

FIG. 5

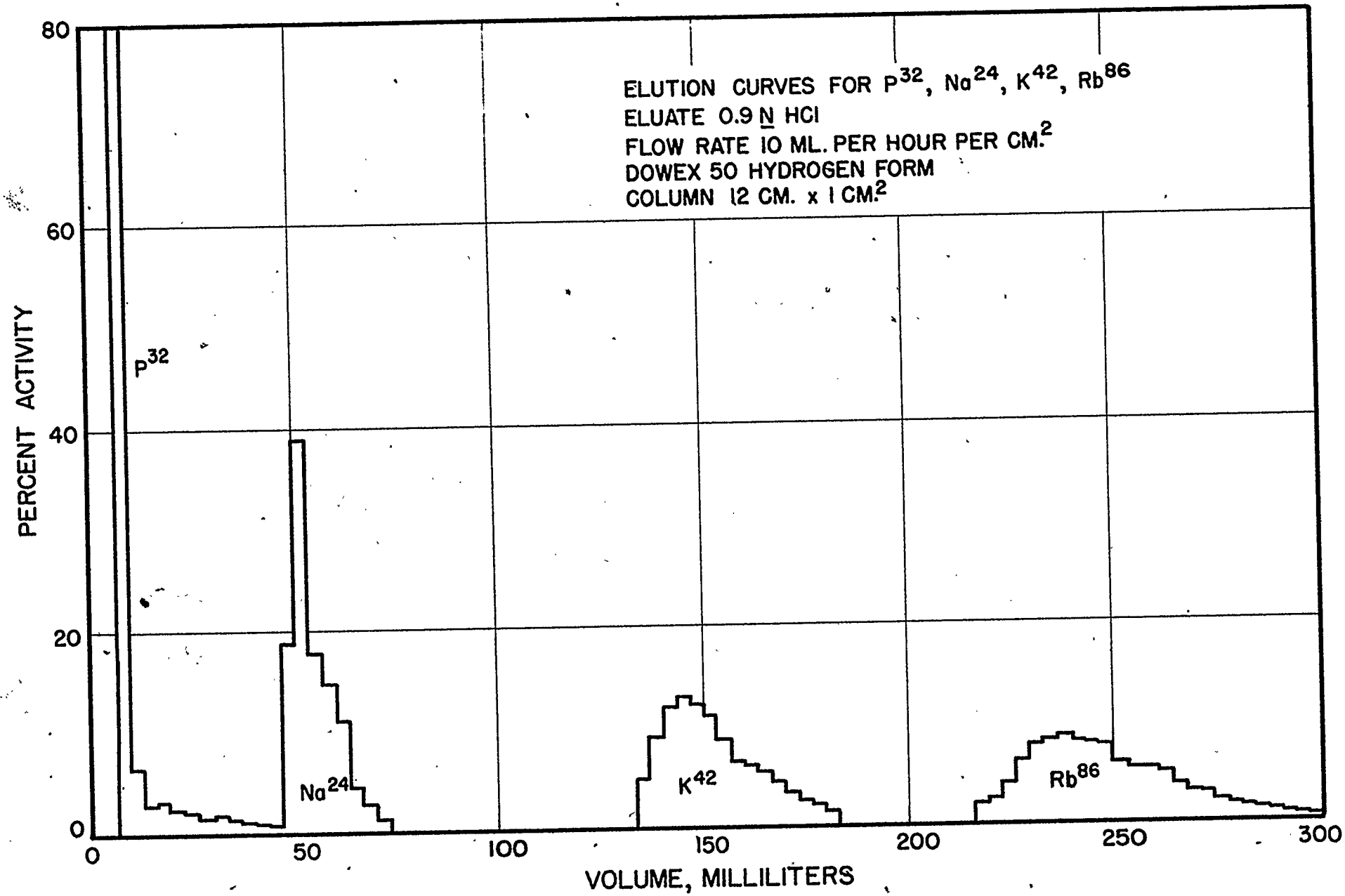
ELUTION CURVES FOR P^{32}, Na^{24}, K^{42}, Rb^{86}
ELUATE 0.9 N HCl
FLOW RATE 10 ML. PER HOUR PER CM.^{2}
DOWEX 50 HYDROGEN FORM
COLUMN 12 CM. x 1 CM.^{2}
PERCENT ACTIVITY
80
60
40
20
0
P^{32}
Na^{24}
K^{42}
Rb^{86}
0
50
100
150
200
250
300
VOLUME, MILLILITERS

FIG. 6

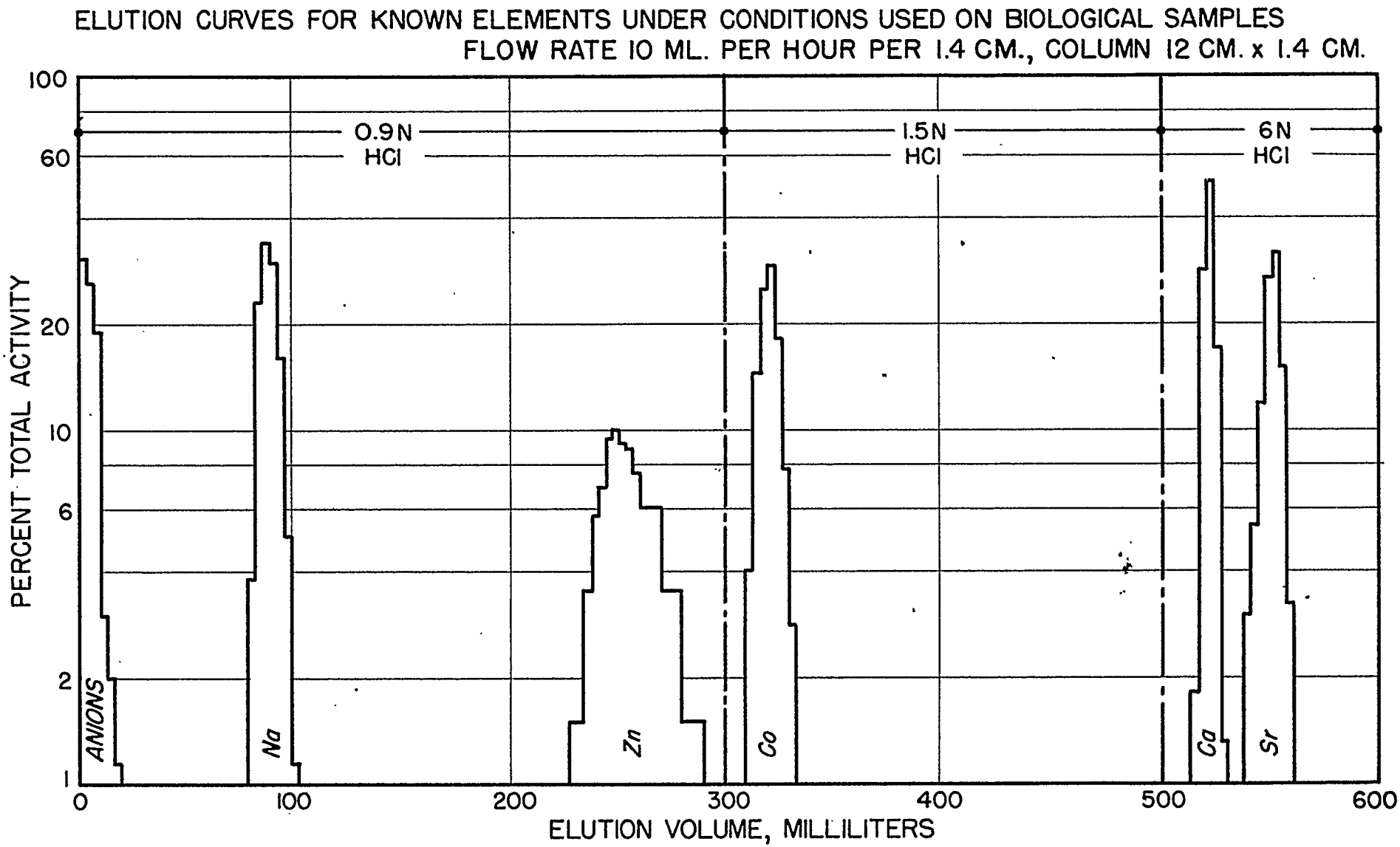
ELUTION CURVES FOR KNOWN ELEMENTS UNDER CONDITIONS USED ON BIOLOGICAL SAMPLES
FLOW RATE 10 ML. PER HOUR PER 1.4 CM., COLUMN 12 CM. x 1.4 CM.
PERCENT TOTAL ACTIVITY
100
60
20
10
6
2
1
0.9N HCl
1.5N HCl
6N HCl
ANIONS
Na
Zn
Co
Ca
Sr
0
100
200
300
400
500
600
ELUTION VOLUME, MILLILITERS

FIG. 7

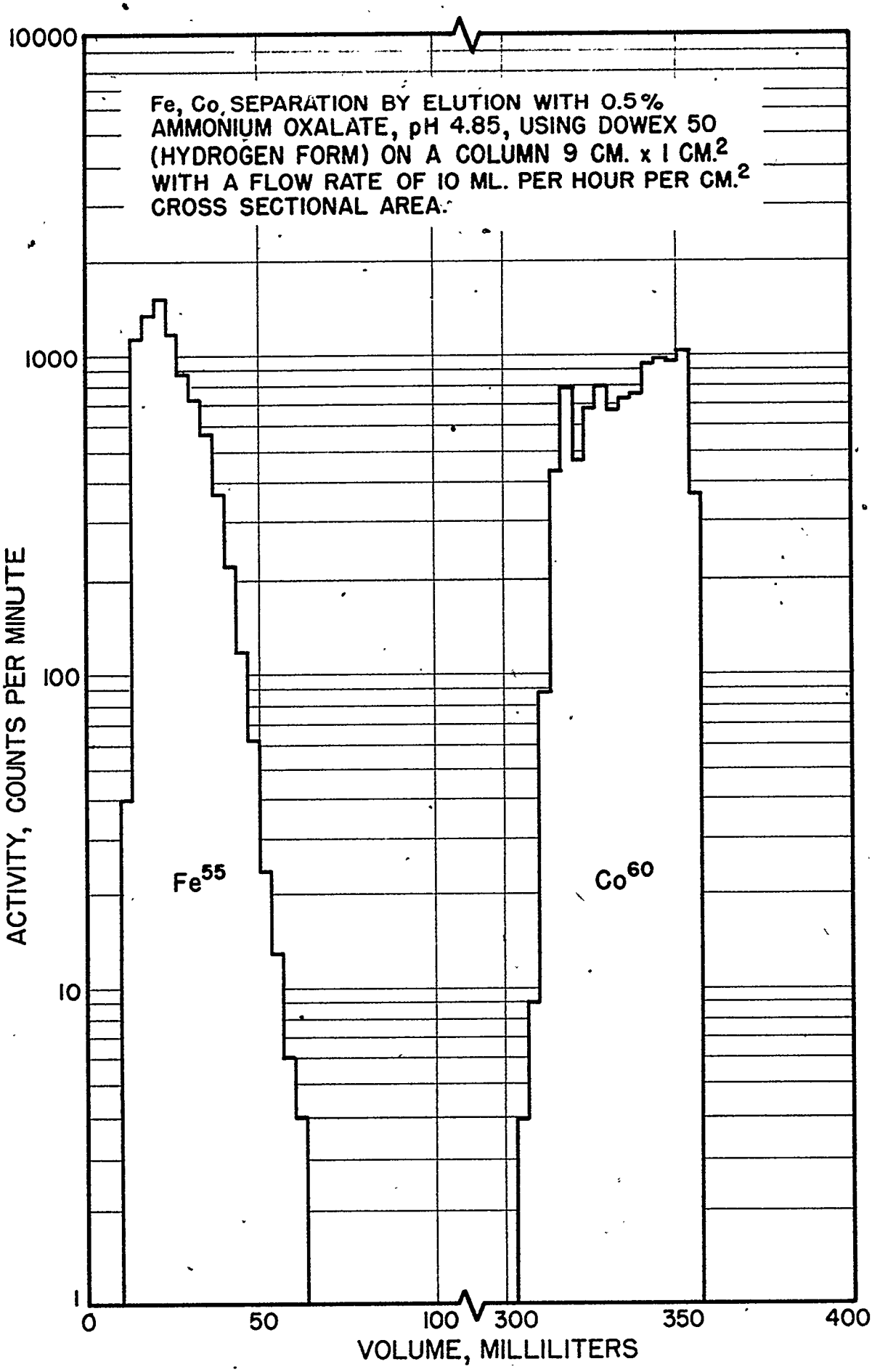
Fe, Co SEPARATION BY ELUTION WITH 0.5% AMMONIUM OXALATE, pH 4.85, USING DOWEX 50 (HYDROGEN FORM) ON A COLUMN 9 CM. x 1 CM.^{2} WITH A FLOW RATE OF 10 ML. PER HOUR PER CM.^{2} CROSS SECTIONAL AREA.
ACTIVITY, COUNTS PER MINUTE
10000
1000
100
10
1
Fe^{55}
Co^{60}
0
50
100
300
350
400
VOLUME, MILLILITERS

FIG. 8

61E49

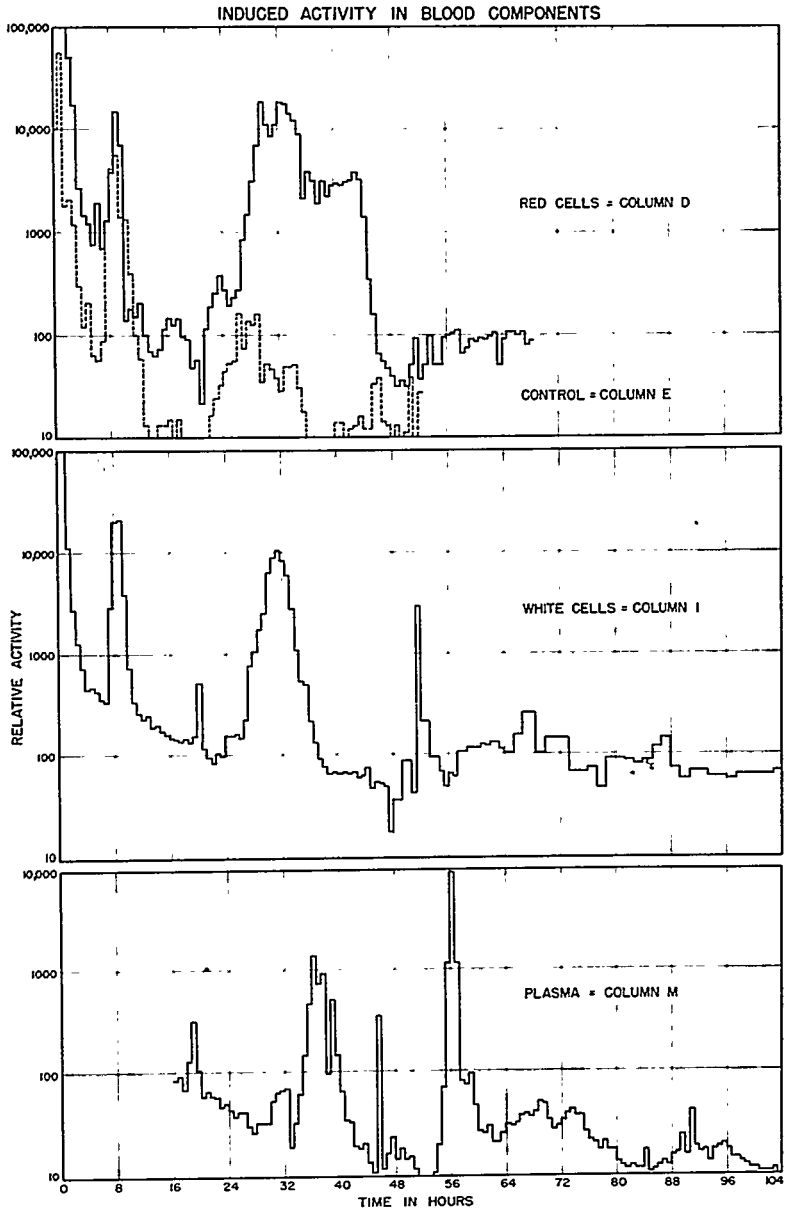
INDUCED ACTIVITY IN BLOOD COMPONENTS
RED CELLS = COLUMN D
CONTROL = COLUMN E
WHITE CELLS = COLUMN I
PLASMA = COLUMN M
RELATIVE ACTIVITY
TIME IN HOURS
100,000
10,000
1000
100
10
0
8
16
24
32
40
48
56
64
72
80
88
96
104

FIG. 9

62E49

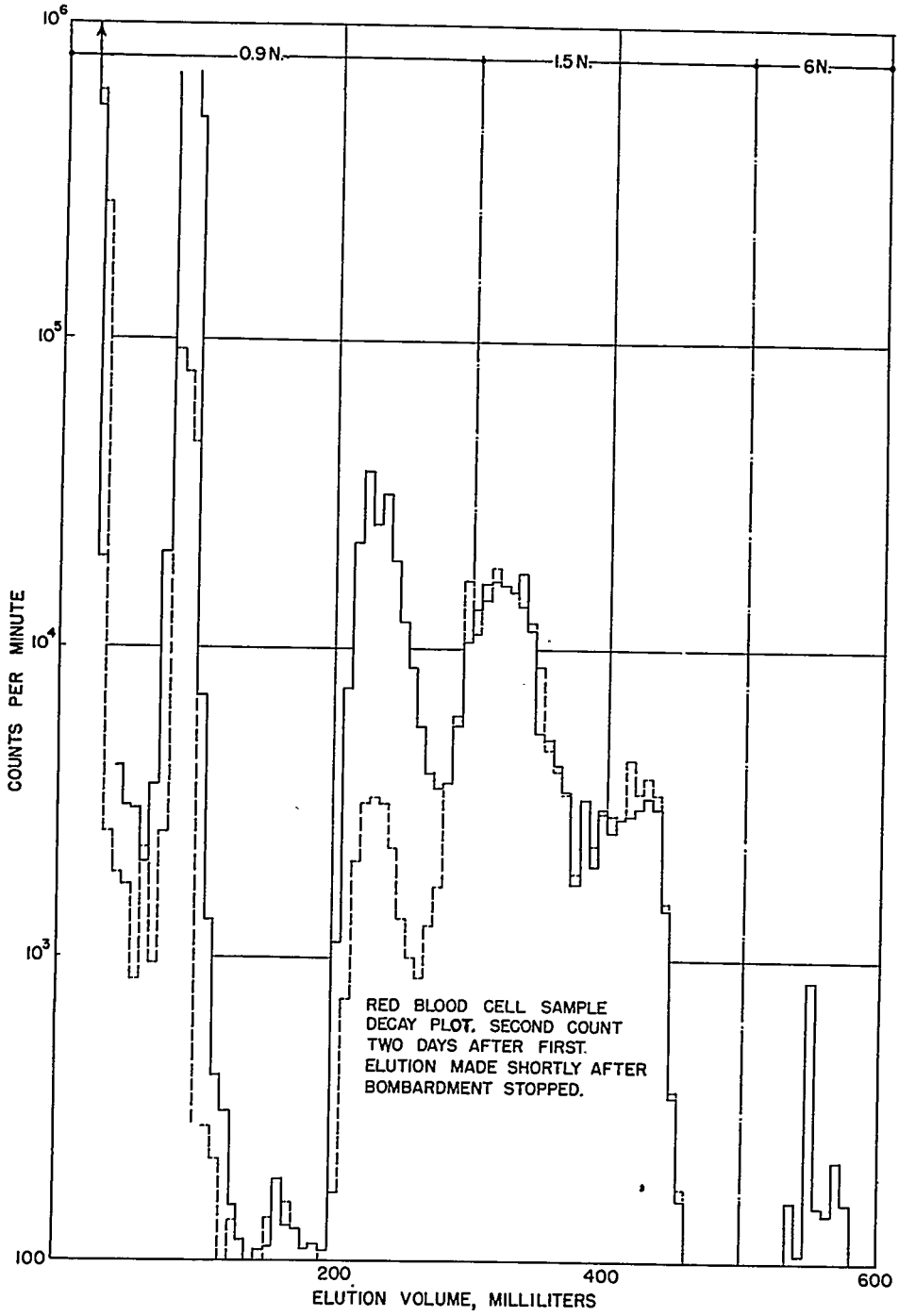
10^6
10^5
10^4
10^3
100
0.9 N.
1.5 N.
6N.
COUNTS PER MINUTE
RED BLOOD CELL SAMPLE
DECAY PLOT. SECOND COUNT
TWO DAYS AFTER FIRST.
ELUTION MADE SHORTLY AFTER
BOMBARDMENT STOPPED.
200
400
600
ELUTION VOLUME, MILLILITERS

FIG. 10

63E49

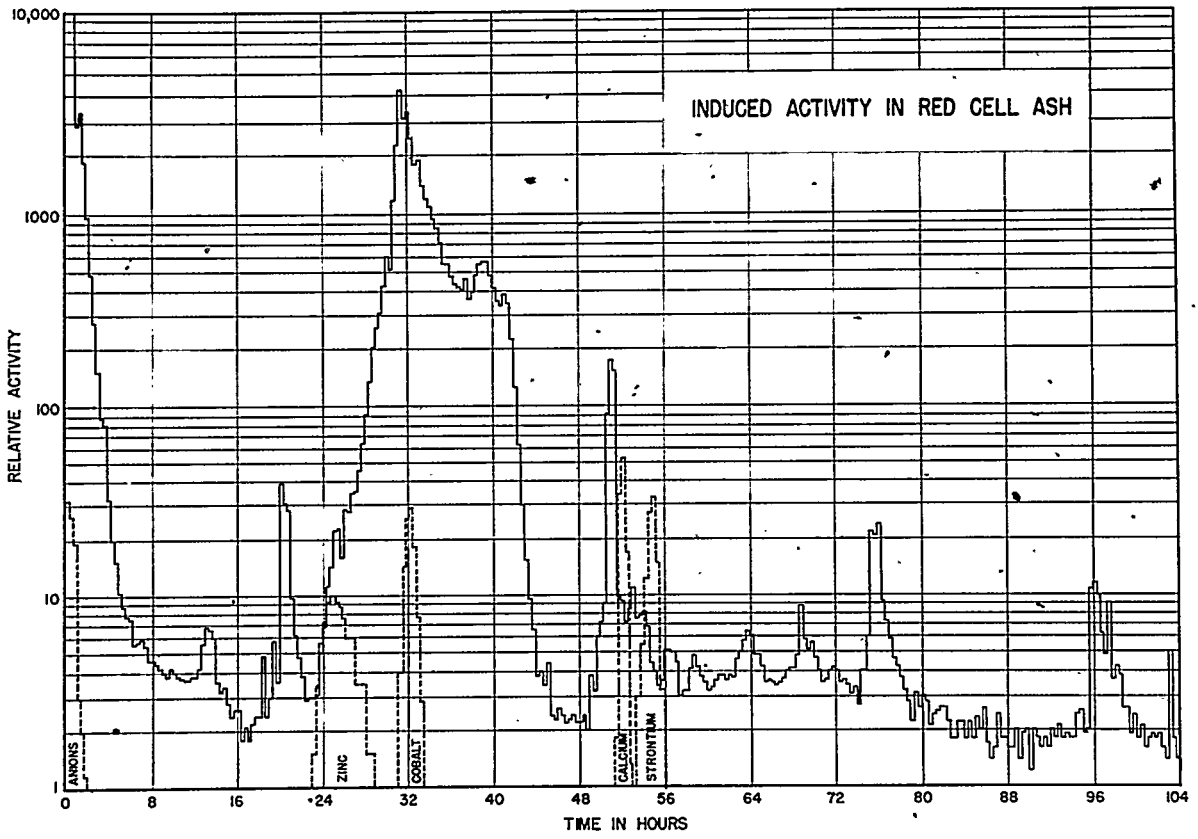
INDUCED ACTIVITY IN RED CELL ASH
RELATIVE ACTIVITY
10,000
1000
100
10
1
AMONS
ZINC
COBALT
CALCIUM
STRONTIUM
0
8
16
24
32
40
48
56
64
72
80
88
96
104
TIME IN HOURS

FIG. 11

64-49

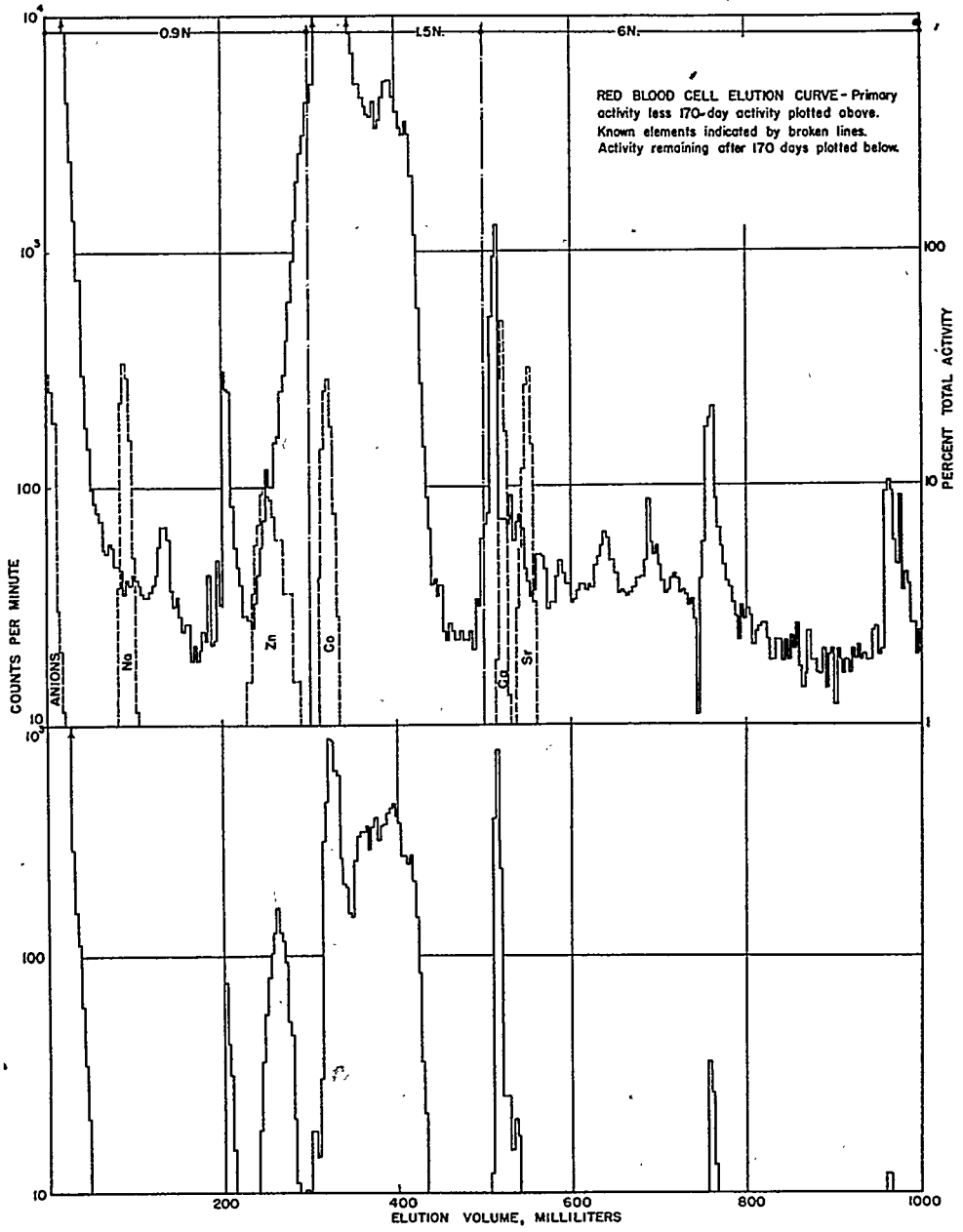
0.9N
1.5N.
6N.
RED BLOOD CELL ELUTION CURVE - Primary activity less 170-day activity plotted above.
Known elements indicated by broken lines.
Activity remaining after 170 days plotted below.
COUNTS PER MINUTE
PERCENT TOTAL ACTIVITY
ANIONS
Na
Zn
Co
Ca
Sr
200
400
600
800
1000
ELUTION VOLUME, MILLILITERS

FIG. 12

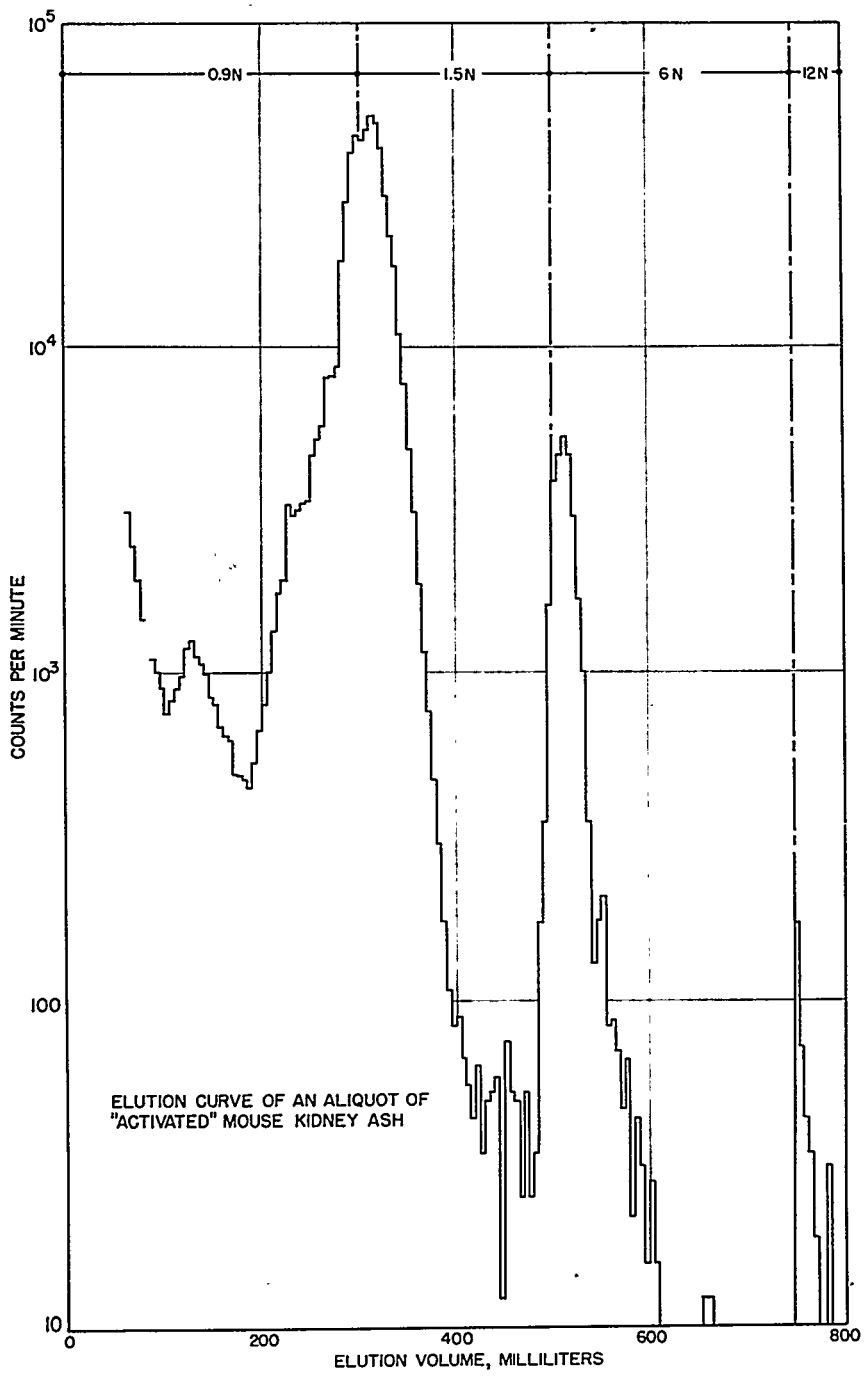
0.9N
1.5N
6N
12N
10^5
10^4
10^3
100
10
COUNTS PER MINUTE
ELUTION CURVE OF AN ALIQUOT OF
"ACTIVATED" MOUSE KIDNEY ASH
0
200
400
600
800
ELUTION VOLUME, MILLILITERS

FIG. 13

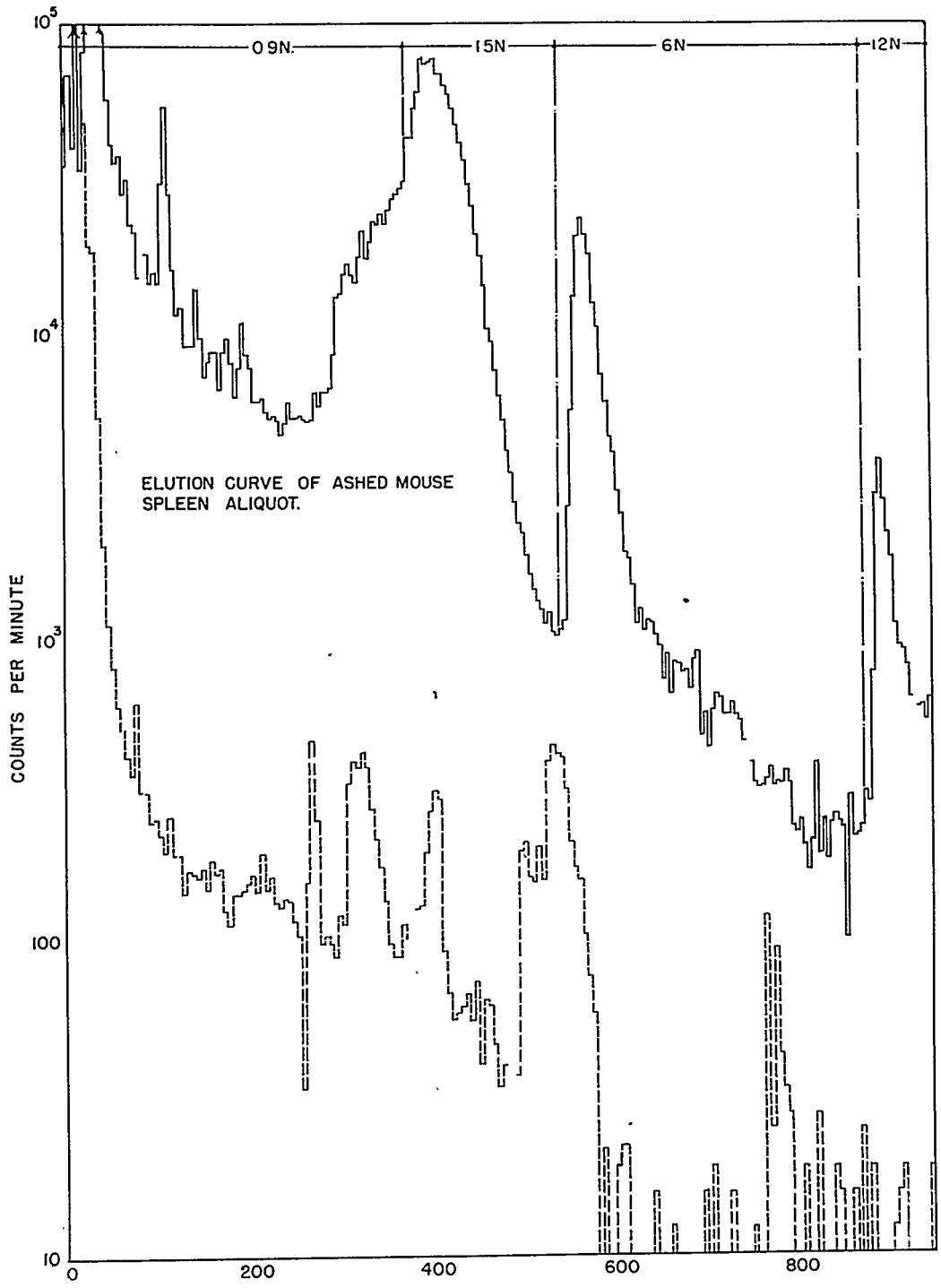
0.9N.
1.5N
6N
12N
ELUTION CURVE OF ASHED MOUSE SPLEEN ALIQUOT.
COUNTS PER MINUTE
10^5
10^4
10^3
100
10
0
200
400
600
800

FIG. 14

67E47

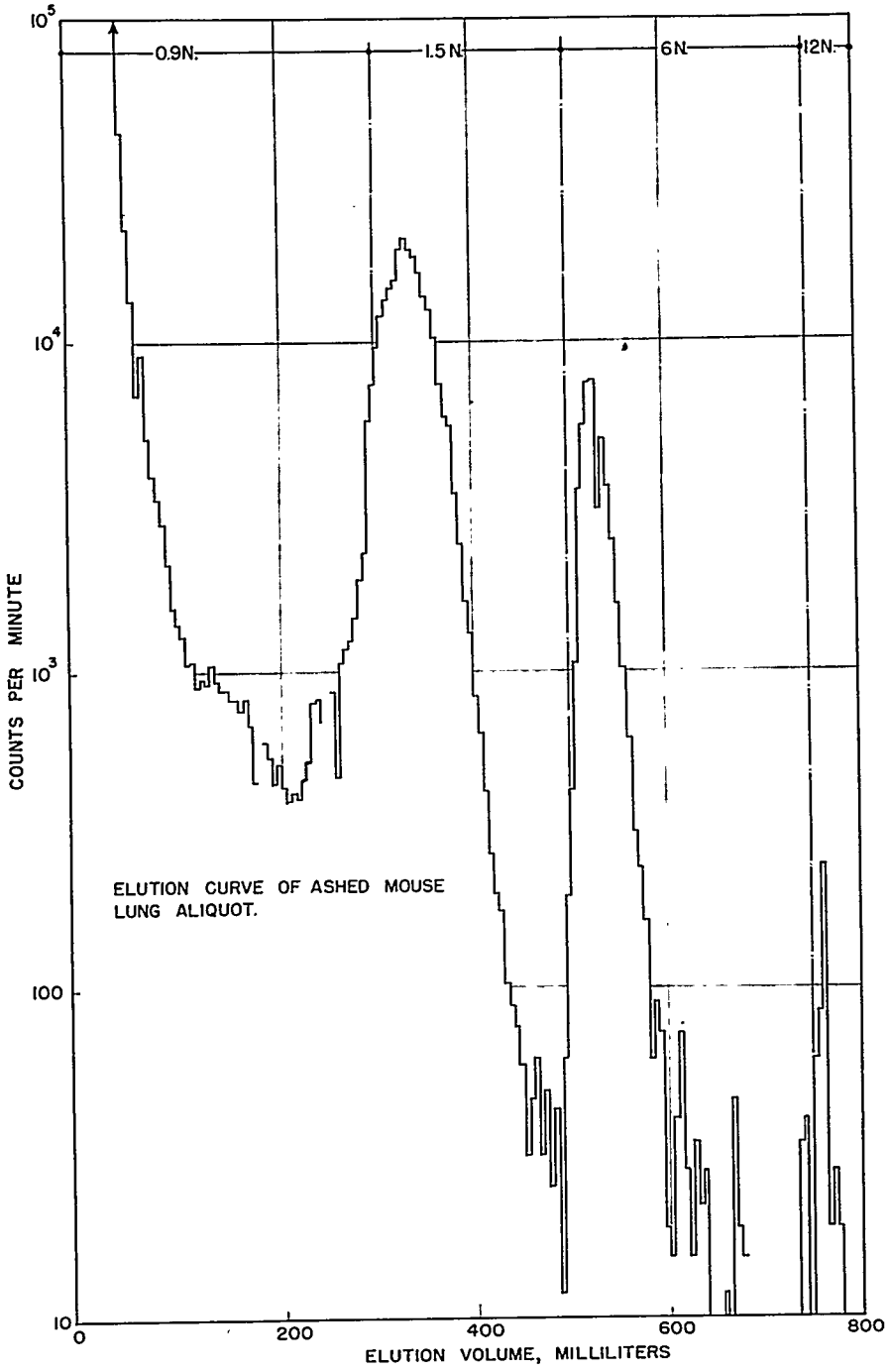
0.9N.
1.5 N.
6N.
12N.
10^5
10^4
10^3
100
10
COUNTS PER MINUTE
ELUTION CURVE OF ASHED MOUSE LUNG ALIQUOT.
0
200
400
600
800
ELUTION VOLUME, MILLILITERS

FIG. 15

68E49

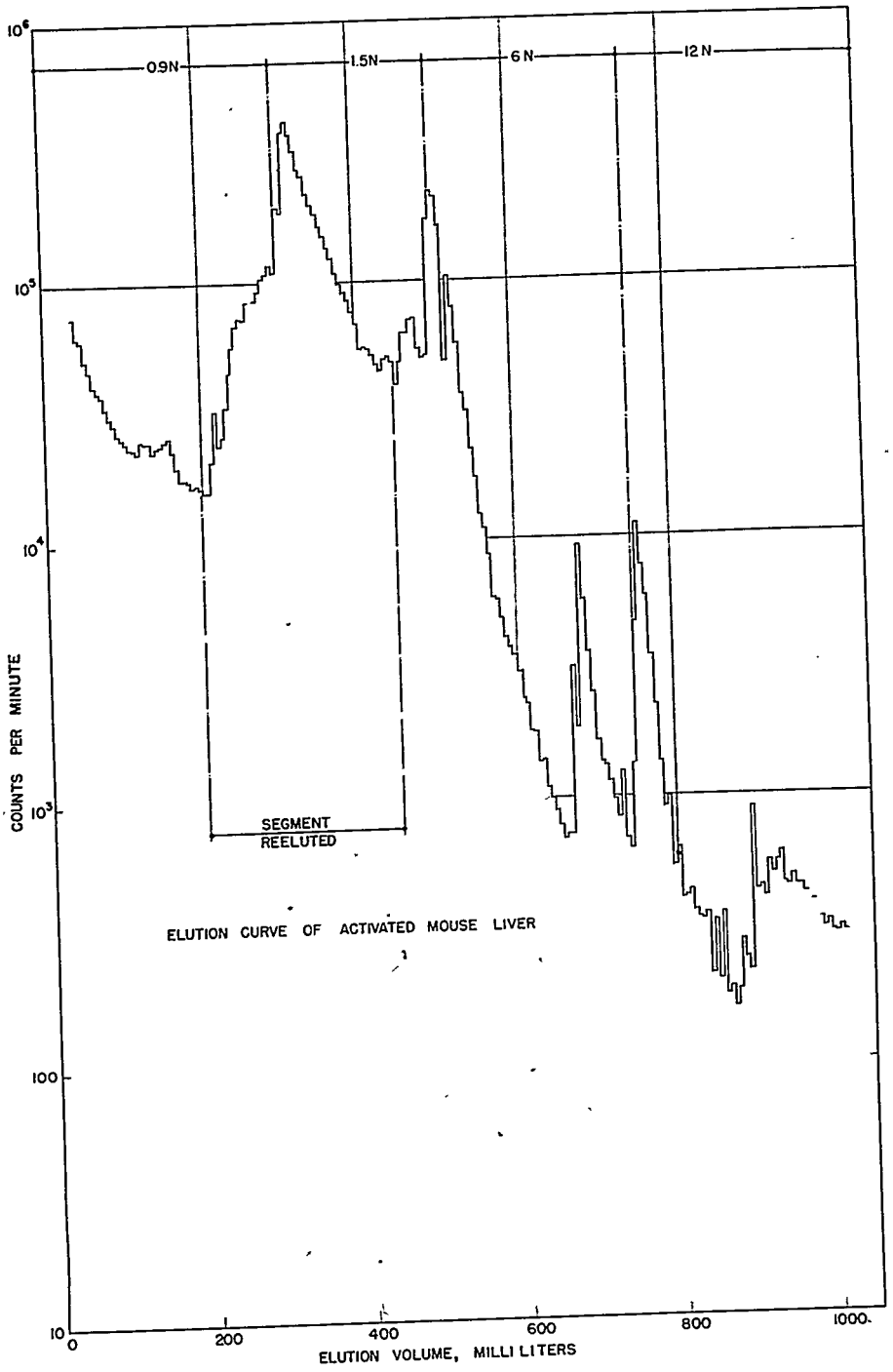

FIG. 16

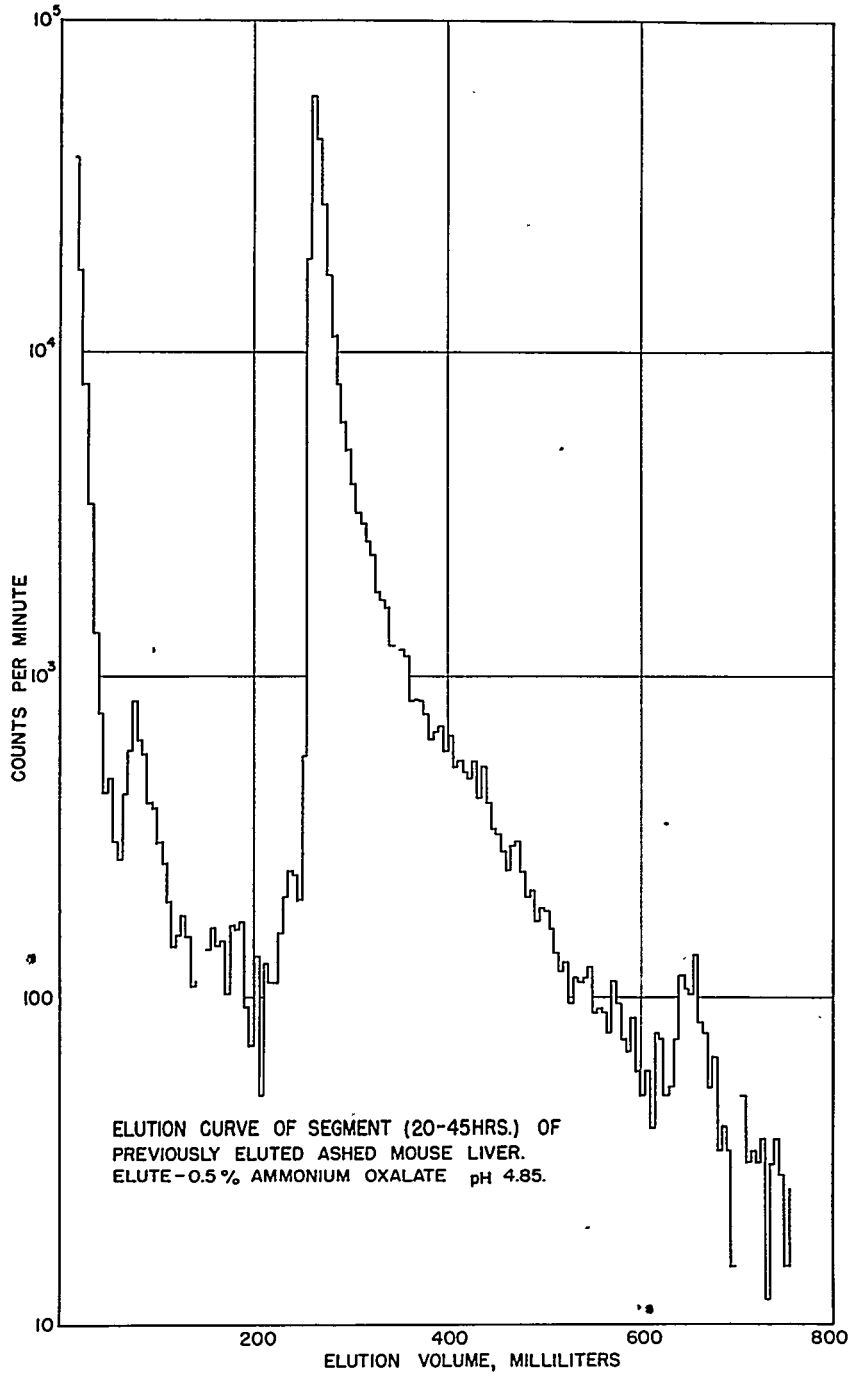
10^5
10^4
10^3
100
10
COUNTS PER MINUTE
200
400
600
800
ELUTION VOLUME, MILLILITERS
ELUTION CURVE OF SEGMENT (20-45HRS.) OF
PREVIOUSLY ELUTED ASHED MOUSE LIVER.
ELUTE - 0.5 % AMMONIUM OXALATE pH 4.85.

FIG. 17

70E49

Studies of Trace Elements with Radioactive Isotopes

The Distribution of Co^{60}, Zn^{65}, and Cu^{64} in the Cytoplasm and Nuclei

Irene Rosenfeld*, C. A. Tobias

Technical Assistance of C. Tregillus

The functions of trace elements in cellular metabolism have been emphasized by various investigators. The stimulating effect of cobalt and copper on the hematopoietic system and the functions of zinc in the enzyme systems have been greatly elucidated by the work of Keilen and Mann[1]. Radioactive isotopes of cobalt, copper and zinc have been used for tracer study by various investigators. Comar[2] recently reviewed the literature dealing with the radioisotopes of trace elements.

Some of the essential trace elements appear to exert their action as components of enzyme systems or stimulation of action of certain enzymes. Along with a study of the distribution of trace elements in the animal body** experiments are reported here which are being carried out to gain some insight into the metabolism of trace elements in animal tissues. Since, according to current principles of biochemical genetics, the action of genes and that of enzymes is closely related, it seemed reasonable to begin a study of the rate of turnover of trace elements in the cytoplasmic fraction of tissues and compare this to the rate of turnover of the same elements in the nuclear fraction. The studies reported below signify only the beginning of a wide range of experiments using radioactive tracers as indicators in the study of enzyme synthesis.

The data presented below refer to the metabolism of the trace elements zinc, cobalt and copper in white laboratory mice.

Plan of Experiments. A tracer amount of a suitable salt of the radioactive isotope with high specific activity was administered intravenously to normal white mice bearing transplanted tumors. The animals were sacrificed in regular time intervals, 3, 6, 12, 24 hours after injection. Several organs were collected and analyzed for radioactivity. A known aliquot of tissue was used for separating nuclear and cytoplasmic material. A few further tests were carried out to determine the protein fractions responsible for carrying trace elements.

Experimental Procedures. Cu^{64} and Co^{60} were obtained from the Isotope Branch, A.E.C.***. The Co^{60} was received as Co_3O_4 and converted to $CoCl_2$ in our laboratory. The Cu^{64} was obtained as metallic Cu which was dissolved in HNO_3, treated with

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* Atomic Energy Fellow, 1948-1949, on leave from the University of Wyoming, Laramie, Wyoming.

** See preceding paper.

*** The preparations of the solutions for intravenous injection were carried out by R. W. Dunn of the Donner Laboratory, and we wish to express our thanks.

successive small portions of conc. HCl and evaporated until the HNO_3 was completely replaced and the copper was converted to chloride. The $CuCl_2$ was dissolved in dilute HCl and made up to 10 ml. 0.2 ml was diluted to 10 ml. with 0.85 percent NaCl which was used for injection. In another case, isotonic acetate buffer was used for dilution; both solutions were well tolerated by the animals in the dose used.

Zn^{65} Preparation. A copper ion cone of the Berkeley 60-inch cyclotron bombarded by stray deuterons served as a source for Zn^{65}. The surface was washed with 8 N HNO_3. The solution was evaporated to expel the excess acid and converted to chloride by the addition of HCl. One mg of Zn as $ZnCl_2$ carrier was added. The acid solution was diluted with H_2O and treated with metallic aluminum until all the copper was removed. The precipitate was filtered off and the filtrate was treated with ammonium tartrate and the pH adjusted to 3.0; H_2S was bubbled through the filtrate and the precipitated ZnS was washed and dissolved in HCl. The procedure of precipitation and redissolving was repeated once more. The acid was evaporated and $ZnCl_2$ was taken up in saline solution.

The amount of radioactive element injected varied with the isotopes used; therefore, each experiment will specify the activity and the total amount of element injected.

Experimental Animals. The rradioactive elements were administered intravenously in all experiments, which is to be assumed without further repetition in the text.

Strain A mice were used as experimental animals. Tumor-bearing mice carried transplanted LCS mammary carcinoma, originally obtained by courtesy of L. C. Strong. At the time of sacrifice the tumors were 15 days old. In each experiment the tissues of 20 animals were pooled. The tissues used for separation were: tumor, liver, kidney, spleen and lung.

The method of separation of nuclei from the cytoplasm was carried out according to the method of Mirsky and Pollister[3]. The fractions containing the cytoplasm washings were collected and measured, and aliquots of the cytoplasmic fraction were used for ashing and protein fractionation. The nuclei were dried and weighed, and the total dried nuclei were used for ashing.

The cytoplasmic proteins were separated by taking aliquots from the original cytoplasmic washing and treated in the following manner:

1. "Globulin" was precipitated with one-half saturated $(NH_4)_2SO_4$. The precipitate washed with dist. H_2O; redissolved in 10 percent NaCl and reprecipitated with $(NH_4)_2SO_4$ (one-half saturation).

2. "Albumin". This fraction was precipitated with full-saturation of $(NH_4)_2SO_4$. The precipitate redissolved with dist. H_2O and reprecipitated with $(NH_4)_2SO_4$ (full saturation).

3. "Acid soluble" and "protein bound" fractions. Ten percent trichloracetic acid was used in the separation of these fractions. The precipitate obtained by this method was filtered off and called the "protein bound" fraction and the filtrate, the "acid soluble" fraction of the cytoplasm.

Assay for Radioactivity. The above fractions were used for ashing and distribution studies of the radioactive element.

The tissues were wet ashed with concentrated HNO_3 and finished by alternative additions of superoxal and HNO_3. The oxidation was complete when the ashed material slowly brought to dryness on free flame, was colorless. The residue of the ashed material was dissolved with a few drops of HCl and tranferred to volumetric flasks with distilled water.

This method of ashing was used for the determinations of Co^{60}, Cu^{64}, and Zn^{65}.

The radioactivity of cobalt and zinc was determined by electro-deposition of these elements on a copper disk. The apparatus was described by Dunn[4]. The aliquots of the sample were placed in the electro-deposition cell and the solutions described below were added.

To the aliquot of ashed material was added:

5 mg. of carrier cobalt (in 1 ml. sol. of $CoSO_4$).
4 ml. 10 percent hydroxylamine hydrochloride (freshly prepared).
20 ml. 2 percent $(NH_4)_2 SO_4$ in concentrated NH_4OH.

The cobalt is electroplated at 400 milliamperes for 3 hours.

The method for zinc plating was the following:

To the aliquot of ashed material was added:

5.0 ml (1 N NaOH + 1 percent KCN) solution.
5.0 mg. of carrier Zn as $ZnSO_4$.

The total volume was made up to 10 ml and plated for 1 hour at 200 milliamperes. The aliquots of the ashed tissues for the copper determinations were transferred to plastic cups and evaporated at 60^0 to 80^0. The samples were counted using an end-window Geiger-Muller tube.

The distribution of Co^{60} in the cytoplasm and nuclei was studied 3, 6, 12, 24, 48, and 72 hours after the administration of Co^{60} in the tissues referred to above, both in normal and tumor bearing animals. The distribution of Cu^{64} was studied 3, 12 and 24 hours after injection in tissues of normal and tumor-bearing animals. The distribution of Zn^{65} in the cytoplasm and nuclei was carried out only in tumor-bearing animals since we found that only a small amount of Zn^{65} was present in the nuclei.

Results. Gross distribution of tagged cobalt in mice was already studied by Lawrence et al.[5] The results presented below, where comparable, appear to be in agreement with their data.

Cobalt. Table I shows the results of the accumulation of cobalt in the cytoplasm of various tissues of normal and tumor-bearing animals. The optimum uptake in the carcinoma was observed at the end of 6 hours while at 3 and 12 hours the amount of cobalt in this tumor was approximately the same. There was a gradual decrease with increase in time and at the end of 72 hours only 0.81 percent of the injected dose was present. There was a differential uptake of cobalt in the liver and kidney of normal and tumor-bearing animals. The liver of tumor animals showed the highest concentration at the end of 3 hours, and the normal liver at the end of 6 hours. The kidney of normal animals during the experiment had a 50 percent higher cobalt uptake than that of the tumor bearing animals. This differential uptake in the liver and kidney cytoplasm of normal and tumor bearing animals can be seen from Fig. 1 and 2. It is difficult at the present time to explain what factor or factors are responsible for this evident difference in the rate of turnover of cobalt in the tissues of normal and tumor bearing mice. The report of Lawrence et al[5] showed a 50 percent increase of cobalt in the liver of leukemic mice at the end of 24 hours, when compared with the normal.

The cytoplasm of spleen and lung of normal and tumor bearing animals showed the same degree of uptake from 3 to 72 hours.

Table II presents the ratio of cobalt activity per gram nuclei of the various tissues of normal and tumor bearing animals as a fraction of cobalt radioactivity per gram of cytoplasm. This ratio in tumor nuclei was consistently higher than the liver nuclei of normal and tumor bearing animals. The kidney, spleen, and lung nuclei of tumor and normal animals showed about the same uptake.

Fig. 3 shows the ratio of cobalt in nuclei vs cytoplasm when plotted against time. The Co^{60} curves for the liver nuclei of tumor and normal animals run parallel to each other, the normal liver nuclei showing a higher ratio than the liver nuclei of tumor animals. Relative radioactivity of nuclei exhibit a tendency to increase with time for both liver and tumor. The relative concentration of Co^{60} in the tumor nuclei was higher at the end of 3 hours than the liver nuclei and continued to rise during the experimental period of 72 hours. Marshak[6] reported a similar increase in the uptake of P^{32} in lymphona nuclei when compared with liver nuclei.

The distribution of Co^{60} in the various protein fractions of the cytoplasm of the tissues at the end of three hours are given in Table III. The acid soluble fraction of the cytoplasm of various tissues contained the highest percentage of the cobalt and only a small percent of the injected dose was present in the protein fraction. Cobalt in the "protein bound" fraction was found in combination with the globulin. However, in the lung it was almost evenly divided between the albumin and globulin fractions.

The above experiments were repeated in most part using radioactive cobalt of different specific acitivity. There was no appreciable difference between results with 24.5 μc/mg and 2.4 mc/mg.

Carrier free cobalt also gave substantially the same result in one 4 hour experiment.

TABLE I

CONCENTRATION OF Co^{60} IN THE CYTOPLASM OF VARIOUS TISSUES*

Percent of the Administered dose recovered**

	Liver		Kidney		Spleen		Lung	
Sacrificed after inj.	Tumor	Normal*	Tumor*	Normal	Tumor	Normal	Tumor	Normal	Tumor
3 hours	4.2	8.5	15.0	1.4	0.57	0.17	0.17	0.41	0.43
6 hours	6.7	11.4	7.4	1.2	0.69	0.29	0.32	0.56	0.47
12 hours	4.4	5.5	6.2	0.74	0.62	0.13	0.18	0.34	0.31
24 hours	3.3	4.4	4.3	0.49	0.34	0.07	0.19	0.23	0.26
48 hours	0.76	3.0	2.3	0.46	0.23	0.09	0.07	0.30	0.22
72 hours	0.81	1.0	1.6	0.53	0.24	0.07	0.07	0.23	0.12

* Data, where repeat experiments were made usually agreed within 5 percent.
** Dose: 15.5×10^4 c/m/animal; contained 13.0 microgram cobalt, and had an activity level of 0.32 μc.

TABLE II

Co^{60} UPTAKE, RATIO OF SPECIFIC ACTIVITY OF NUCLEI TO SPECIFIC ACTIVITY OF CYTOPLASM*

	Liver		Kidney		Spleen		Lung	
Sacrificed after Injection	Tumor	Normal	Tumor	Normal	Tumor	Normal	Tumor	Normal	Tumor
3 hours	0.30	0.09	0.10	0.25	0.14	0.17	0.15	0.14	0.07
12 hours	0.42	0.26	0.16	0.36	0.34	0.32	0.6	0.40	0.43
24 hours	0.43	0.24	0.17	0.60	0.59	0.55	0.55	0.61	0.45
48 hours	0.52	0.23	0.17	0.62	0.48	0.42	0.46		0.50
72 hours	0.54	0.29	0.24	0.62	0.45	0.47		0.64	0.56

*Specific acitivity interpreted as disintegration rate per gram wel sample.

TABLE III

DISTRIBUTION OF Co^{60} IN THE VARIOUS CYTOPLASMIC FRACTIONS*

Percent of Administered Dose Recovered

Tissue	Acid Soluble	"Protein Bound"	Albumin Fraction**	Globulin Fraction**
Liver	6.53	1.58	0.63	
Kidney	1.56	0.49	0.09	0.33
Spleen	0.19	0.05	0.01	0.04
Lung	0.39	0.39	0.08	0.1

* Tissues of normal animals sacrificed 3 hours after injection of Co^{60}. Dose: 3.31×10^5 c/m/animal; contained 0.4 μgms Co with an activity level of 1.1 μc.
**Albumin and globulin refer to the fractions obtained with full and one-half saturation of $(NH_4)_2SO_4$.

Copper. Table IV gives the distribution of the radioactive copper in the cytoplasm of various tissues. The tumor tissue, 3 hours after injection, accumulated 11.5 percent of the injected dose. Later there was a rapid loss of the Cu^{64} so that at the end of 24 hours only 5.4 percent of the injected dose was present. The radioactive copper accumulated in higher concentration in the tumor tissue than the radioactive cobalt, in which case at the end of 6 hours only 6.7 percent of the injected dose was present in the tumor.

A differential uptake of the copper in the cytoplasm of all the tissues of tumor-bearing animals is evident from the data presented. Fig. 4 shows the concentration of Cu^{64} in the liver, kidney, and spleen of normal and tumor-bearing mice. It is evident that the cytoplasm of the liver and spleen of tumor-bearing animals contained a higher concentration of the injected dose than the same organs in normal mice, while the normal kidney accumulated more copper than the kidney of tumor-bearing animals. Greenstein and Thompson[7] observed a distinct rise in the copper content of livers and decrease in kidneys of tumor bearing animals. In a footnote of their report they stated that a decrease of copper in the spleen was obtained on semi-quantitative basis. The data presented here, however, indicate an increase of copper in the spleen and lung cytoplasm of tumor-bearing animals*. The only organ showing a decreased uptake in tumor bearing animals was the kidney as compared with that organ in normals. The changed distribution of copper is probably related to some change in the physiological function of copper in the tissues in tumor-bearing animals.

The distribution of copper in various tissue nuclei are given in Table V. Tumor nuclei shows the highest uptake at 3 hours, at 12 and 24 hours the uptake remains constant. Liver nuclei of tumor-bearing animals accumulated Cu^{64} in higher concentration than normal animals, a reversal of the cobalt uptake by these nuclei. Kidney nuclei of normal animals has measurable amounts of Cu^{64} in all time intervals but in the kidney nuclei of tumor-bearing mice, a measurable amount of Cu^{64} was present only at one time interval. The Cu^{64} in the nuclei of lung and spleen of normal animals was not consistent. The uptake was slow and the loss very rapid. Spleen and lung nuclei of tumor-bearing animals contained Cu^{64} during the experiment. As we have indicated in the table, if the counts per minute in the total nuclei were less than 20, we have assumed that there was no measurable amount of uptake of the isotope by the nuclei. The possible lack of Cu^{64} in the nuclei may be due to the small amount of uptake, the short half-life, and the low specific activities of the isotope used. The clarification of this point must wait until a longer half-life isotope of copper with a higher specific activity will be available for biological study.

A few data were collected regarding the distribution of Cu^{64} in the various other organs. These are given in Table VI. The caecum has the highest uptake during all the time intervals studied. In general the different segments of the gastro-intestinal tract have the highest concentrations at the various time intervals. All other tissues contained small amounts of Cu^{64}.

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* Mr. Welch of this Laboratory recently obtained some data with emission spectroscopy that confirmed the accumulation of copper in the spleens of tumor bearing mice.

TABLE IV

THE DISTRIBUTION OF Cu^{64} IN THE CYTOPLASM
OF VARIOUS TISSUES OF NORMAL AND TUMOR MICE

Percent of the Injected Dose Recovered

	Liver			Kidney		Spleen		Lung	
Sacrificed after Injection	Tumor	Normal*	Tumor**	Normal	Tumor	Normal	Tumor	Normal	Tumor
3 hours	11.5	29.4	36.1	1.3	1.07	0.15	0.73	0.10	0.30
12 hours	8.1	30.2	42.4	1.4	0.92	lost	0.79	0.15	0.21
24 hours	5.4	13.	35.6	0.86	0.64	0.24	0.56	0.12	0.26

* Dose 44.5 x 10^3 Cu^{64} c/m per mouse; contained 64 μgms of Cu with an activity level of approximately 3 μc at the time of injection.
**Dose 10.6 x 10^4 Cu^{64} c/m per mouse; contained 64 micrograms of Cu with an activity level of approximately 5 microcuries at the time of injection.

TABLE V

DISTRIBUTION OF Cu^{64} IN THE NUCLEI
OF VARIOUS TISSUES OF NORMAL AND TUMOR MICE

Percent of the Injected Dose Recovered

	Liver		Kidney		Spleen		Lung	
Sacrificed After Inj.	Tumor	Normal	Tumor	Normal	Tumor	Normal	Tumor	Normal	Tumor
3 hours	0.68	0.1	0.76	0.009	*	*	.03	*	0.003
12 hours	0.11	.08	0.26	0.013	0.004	0.07	.008	0.008	.004
24 hours	0.11	.04	0.10	0.002	*	0.003	.02	*	0.002

* If the total of counts was less than 20 c/m it was not included in the data.

TABLE VI

DISTRIBUTION OF CU^{64} IN TISSUES OF NORMAL ANIMALS*

Percent of the Injected Dose Recovered per gram wet weight of Tissue.

Tissue	3 hours	12 hours	24 hours
Stomach	.53	.42	0.32
Small Intestine	.38	.50	0.28
Caecum	2.18	2.66	3.40
Pancreas	.39	.33	0.28
Thymus	.13	.36	0.04
Skeletal Muscle	.23		0.007
Heart	.16	.10	0.11
Skin & Fur	.19	.08	0.05

Results are the average of 6 animals.

* Dose 44.5×10^3 c/m Cu^{64} per mouse, contained 64 micrograms of Cu with an activity level of approximately 3 microcuries at the time of injection.

Zinc. The distribution of Zn^{65} in the cytoplasm and nuclei of various tissues of tumor bearing animals are given in Table VII.

The highest concentration Zn^{65} was present in the cytoplasm of liver and tumor. The cytoplasm of other tissues accumulated smaller amounts. The uptake in the cytoplasm of the liver with zinc was higher than cobalt or copper, but little or no zinc was present in the nuclei of the various tissues studied. The liver nuclei contained at 3 and 24 hours 0.006 percent and 0.007 percent of the injected dose. The tumor nuclei at 3 hours contained no zinc and at the end of 24 hours only 0.005 percent of the injected dose was found in the nuclei. The nuclei of other tissues at the end of 3 and 24 hours contained no zinc. The trace elements studied showed great deal of variation as to the uptake of these isotopes by the nucleus of the cell. There was no correlation between the accumulation of trace elements in the cytoplasm and nuclei. Whether this selective uptake of the different elements by the nuclei is related to a specific nuclear function or whether it exists in a simple chemical combination with the nucleoprotein will be clarified only by future investigations into their role in the nuclear metabolism. Fig. 5 shows the data on hand concerning the uptake of three different isotopes in the cytoplasm of mammary carcinoma tumors.

The distribution of Zn^{65} in the various cytoplasmic fractions are given in Table VIII. Most of the zinc was present in the "acid soluble" fraction and only one percent or less of the injected dose was bound with protein. In fractionating the protein we found that only a small fraction of the "protein bound" zinc combined with the albumin and globulin.

Discussion. Although the presence of some metallic elements was previously demonstrated in all nuclei, up to now very little information was available concerning the turnover of these elements in cell nuclei. The present experiments demonstrated not only that at least copper and cobalt may be found in nuclei but also that there is a vigorous exchange of nuclear trace elements. Assuming that mouse liver nuclei contain .25 mg/g copper per cm^3 wet tissue, it would appear that about 25 percent of this amount would be exchanged per hour. It is too early to say from the available data what the form of the attachment to the nuclei and what the biochemical significance of these findings might be. The nuclear uptake has not been too clearly demonstrated in case of zinc, where the small amounts observed might be due to imperfect separation of cytoplasm and nuclei.

Significant changes were observed in the rate of turnover of copper and cobalt in the animals with mammary carcinoma as compared to normals. Some effects were observed on both the cytoplasmic and nuclear fractions. These effects may be explained in part by the general change of metabolism in organs of an animal with a growing tumor. For an explanation of the cobalt data one might assume that the overall metabolism of the liver is speeded up, since there is evidence for this from other investigations. If this were the case, one might expect a faster liver turnover, as bringing about increased uptake as well as increased rate of uptake. If in addition one assumes that the tumor cells and nuclei need cobalt in the period of their growth, one might qualitatively understand the reason for lower kidney concentration(less cobalt excretion), high tumor uptake and high tumor nucleus to cytoplasm ratio. In case of copper the picture is not complete: in this case one function of the liver seems to be storage; this is increased in the tumor animals. Increase

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TABLE VII

CONCENTRATION OF Zn^{65} IN CYTOPLASM AND NUCLEI OF TUMOR BEARING ANIMAL TISSUES

Percent of the Injected Dose Recovered.

Tissue	Tissue Fractions	Sacrificed 3 hours*	Sacrificed 24 hours**
Tumor	Cytoplasm	6.4	13.0
	Nuclei	*	0.005
Liver	Cytoplasm	35.72	38.5
	Nuclei	.006	0.007
Kidney	Cytoplasm	1.76	2.37
	Nuclei	*	*
Spleen	Cytoplasm	1.18	3.67
	Nuclei	*	*
Lung	Cytoplasm	0.43	0.92
	Nuclei	*	*

Dose: 3.82×10^4 c/m/animal; contained 3.0 microgram zinc, with an activity level of approximately 7 microcuries.

* Less than 20 c/m in the total sample.
**Dose: 4.5×10^5 c/m/animal; contained approximately 10 micrograms of Zn, with an activity level of approximately 85 microcuries.

TABLE VIII

DISTRIBUTION OF Zn^{65} IN THE VARIOUS CYTOPLASMIC FRACTIONS

Percent of the Injected Dose Recovered*

Tissue	Fractions		
	Acid Soluble	"Protein bound"	"Albumin" and "Globulin"
Tumor	11.25	0.20	.03
Liver	22.4	0.29	.03
Kidney	2.09	0.03	.01
Spleen	1.84	0.03	.02
Lung	0.43	0.01	.01

Tumor animals sacrificed 24 hours after injection of Zn^{65}.

* Dose 4.5×10^4 c/m/animal; contained 3.7 micrograms zinc.

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of copper storage in liver in human carcinoma has been reported by Sandberg, et al[8]; there must be however other functions also, as evidenced by the high spleen concentration, rapid turnover in the tumor and increases in the radioactivity of the liver and tumor nuclei.

In fractionating the cytoplasmic proteins, most of the cobalt and zinc was found in the acid soluble fraction (protein free filtrate) and only a small percent of the injected dose was bound with the protein. Since trace elements combine with the prosthetic group of the protein and these combinations are very unstable, it is possible that treatment with 10 percent trichloracetic acid dissociated these combinations and liberated the cobalt and zinc from the protein; the measurement in vitro after treatment with acid may not be an indication of the conditions existing in vivo. However, all one may state is that one percent or less of cobalt and zinc combined firmly with the protein, which combination was not broken with treatment of acid.

The separation of the cytoplasmic proteins into albumin and globulin indicates that a large percent of the "protein bound" cobalt was present in the globulin fraction, and only a small percent combined with the albumin fraction.

Summary.

1. The turnover of Co^{60}, Cu^{64}, and Zn^{65} in liver, spleen, kidney and tumor of normal and tumor bearing animals was studied as a function of time.

2. Some cobalt and copper were found in the nuclear fraction of tissues, particularly liver and tumor tissue. Zinc was also found there, but in rather small concentration. In each case cytoplasmic concentrations of the trace elements were higher than nuclear concentrations.

3. The presence of tumor altered the Co and Cu metabolism of the animals. In case of the Co there was a higher turnover in the liver, but lower concentration; a high uptake by tumor cytoplasm and nuclei; a low concentration in kidney. In case of Cu the storage function of liver and the concentration in the spleen increased; there was also rapid turnover by the tumor.

4. Separation of the cytoplasmic proteins containing the isotopes Co^{60} and Zn^{65} indicate that one percent or less of the injected dose of those isotopes were bound firmly with the proteins. Most of the cobalt and zinc was present in the acid soluble fraction.

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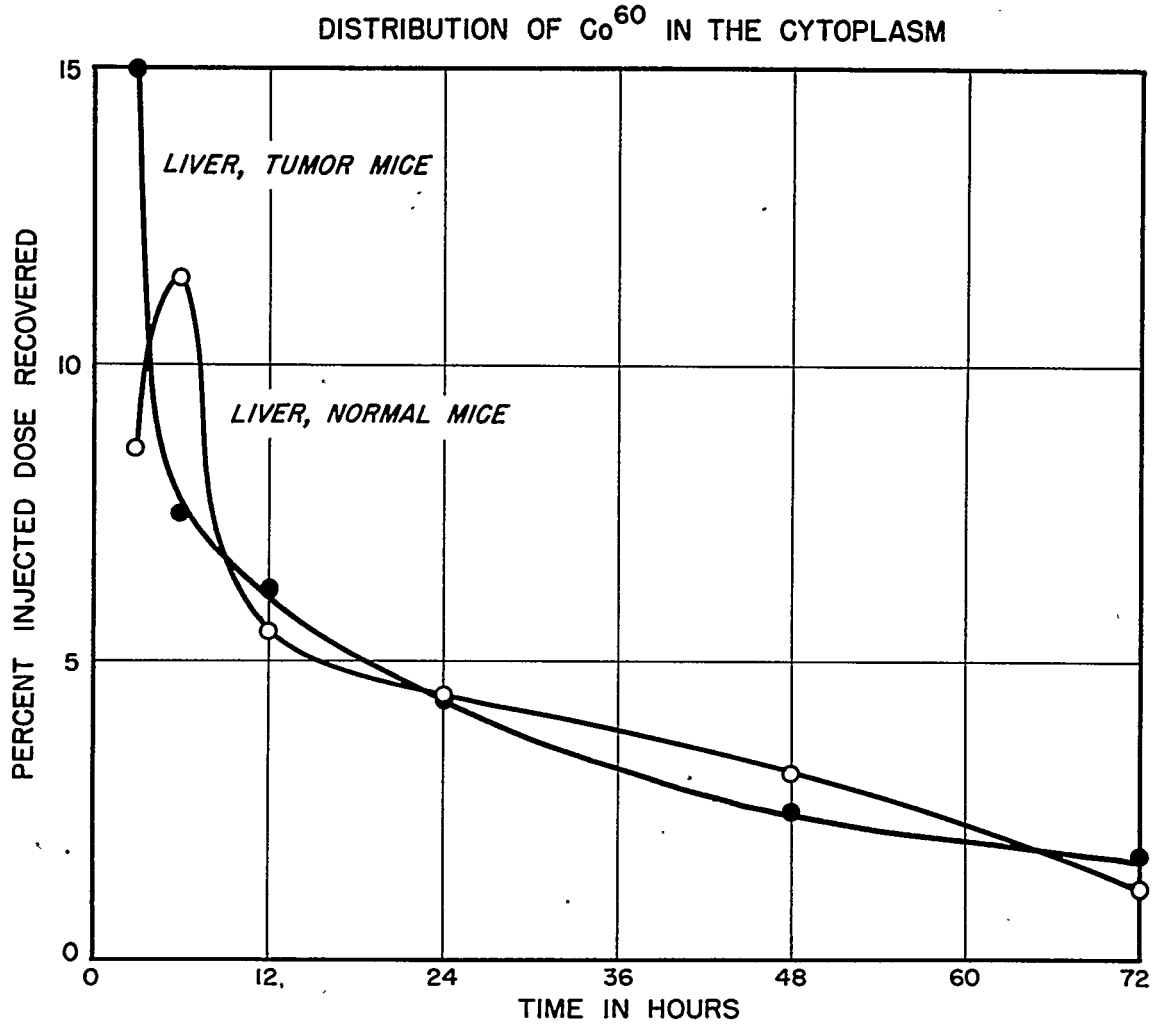
DISTRIBUTION OF Co^{60} IN THE CYTOPLASM
LIVER, TUMOR MICE
LIVER, NORMAL MICE
PERCENT INJECTED DOSE RECOVERED
15
10
5
0
0
12.
24
36
48
60
72
TIME IN HOURS

FIG. 1

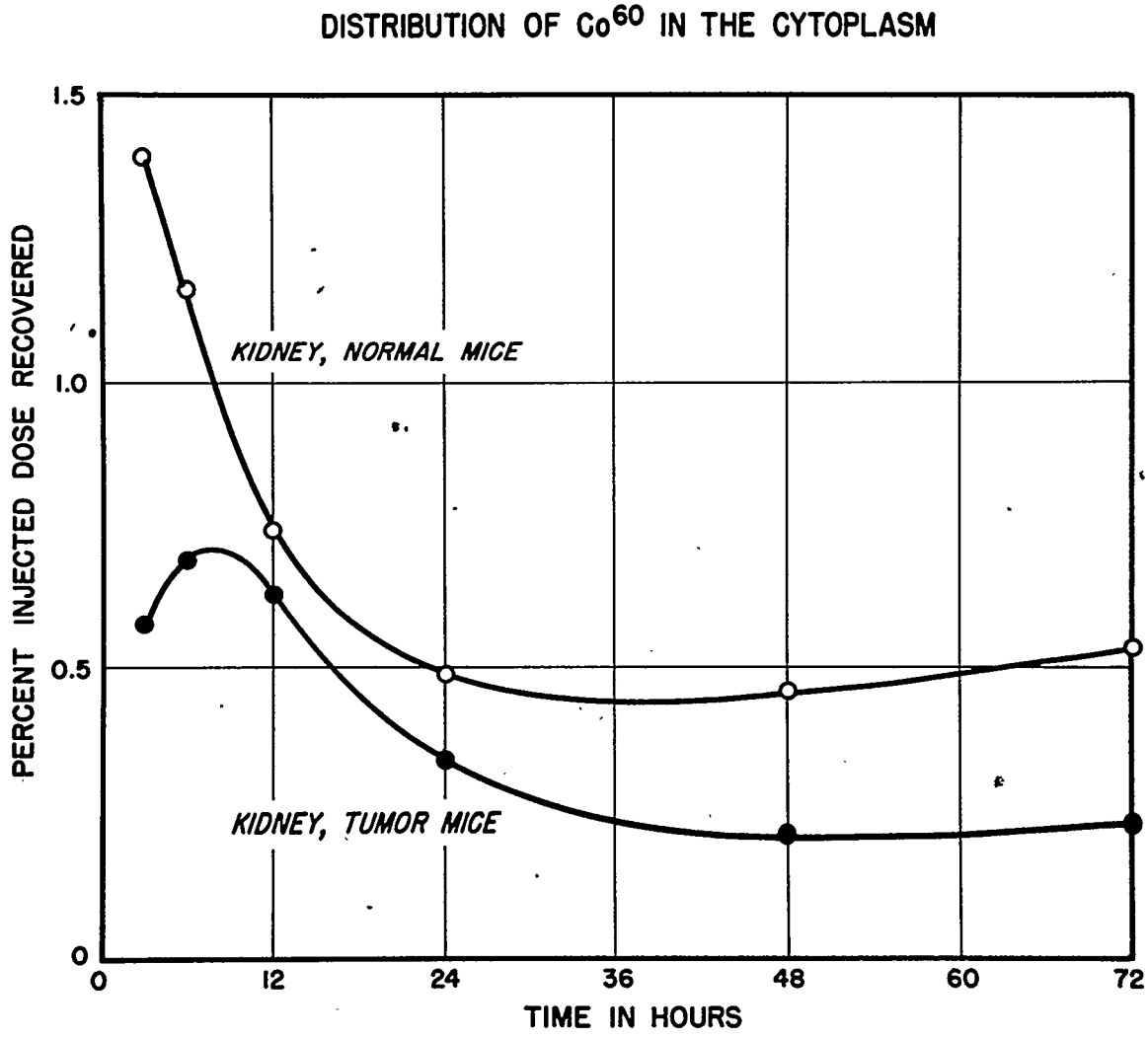
DISTRIBUTION OF Co^{60} IN THE CYTOPLASM
PERCENT INJECTED DOSE RECOVERED
1.5
1.0
0.5
0
KIDNEY, NORMAL MICE
KIDNEY, TUMOR MICE
0
12
24
36
48
60
72
TIME IN HOURS

FIG. 2

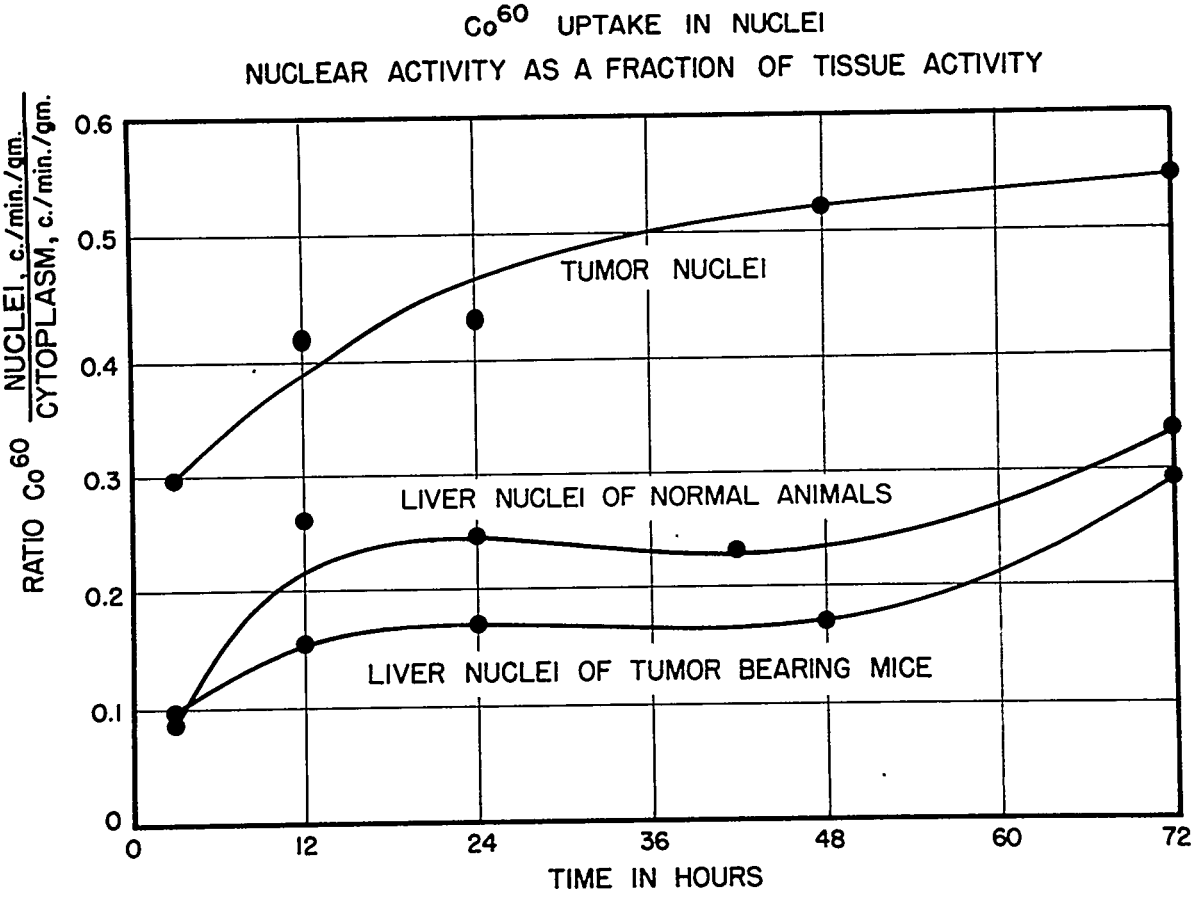
Co60 UPTAKE IN NUCLEI
NUCLEAR ACTIVITY AS A FRACTION OF TISSUE ACTIVITY
RATIO Co60 NUCLEI, c./min./gm. / CYTOPLASM, c./min./gm.
0.6
0.5
0.4
0.3
0.2
0.1
0
TUMOR NUCLEI
LIVER NUCLEI OF NORMAL ANIMALS
LIVER NUCLEI OF TUMOR BEARING MICE
0
12
24
36
48
60
72
TIME IN HOURS

FIG. 3

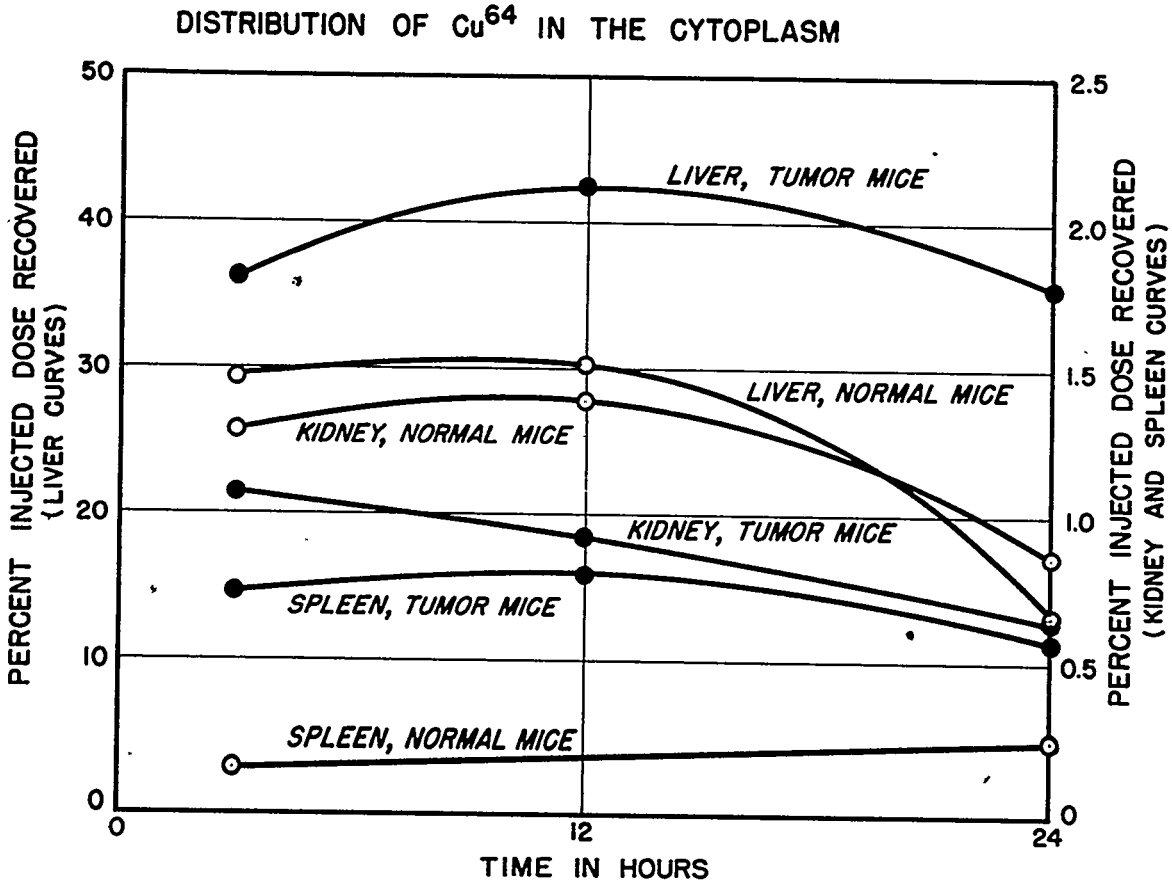
DISTRIBUTION OF Cu64 IN THE CYTOPLASM
PERCENT INJECTED DOSE RECOVERED (LIVER CURVES)
PERCENT INJECTED DOSE RECOVERED (KIDNEY AND SPLEEN CURVES)
LIVER, TUMOR MICE
LIVER, NORMAL MICE
KIDNEY, NORMAL MICE
KIDNEY, TUMOR MICE
SPLEEN, TUMOR MICE
SPLEEN, NORMAL MICE
50
40
30
20
10
0
2.5
2.0
1.5
1.0
0.5
0
0
12
24
TIME IN HOURS

FIG. 4

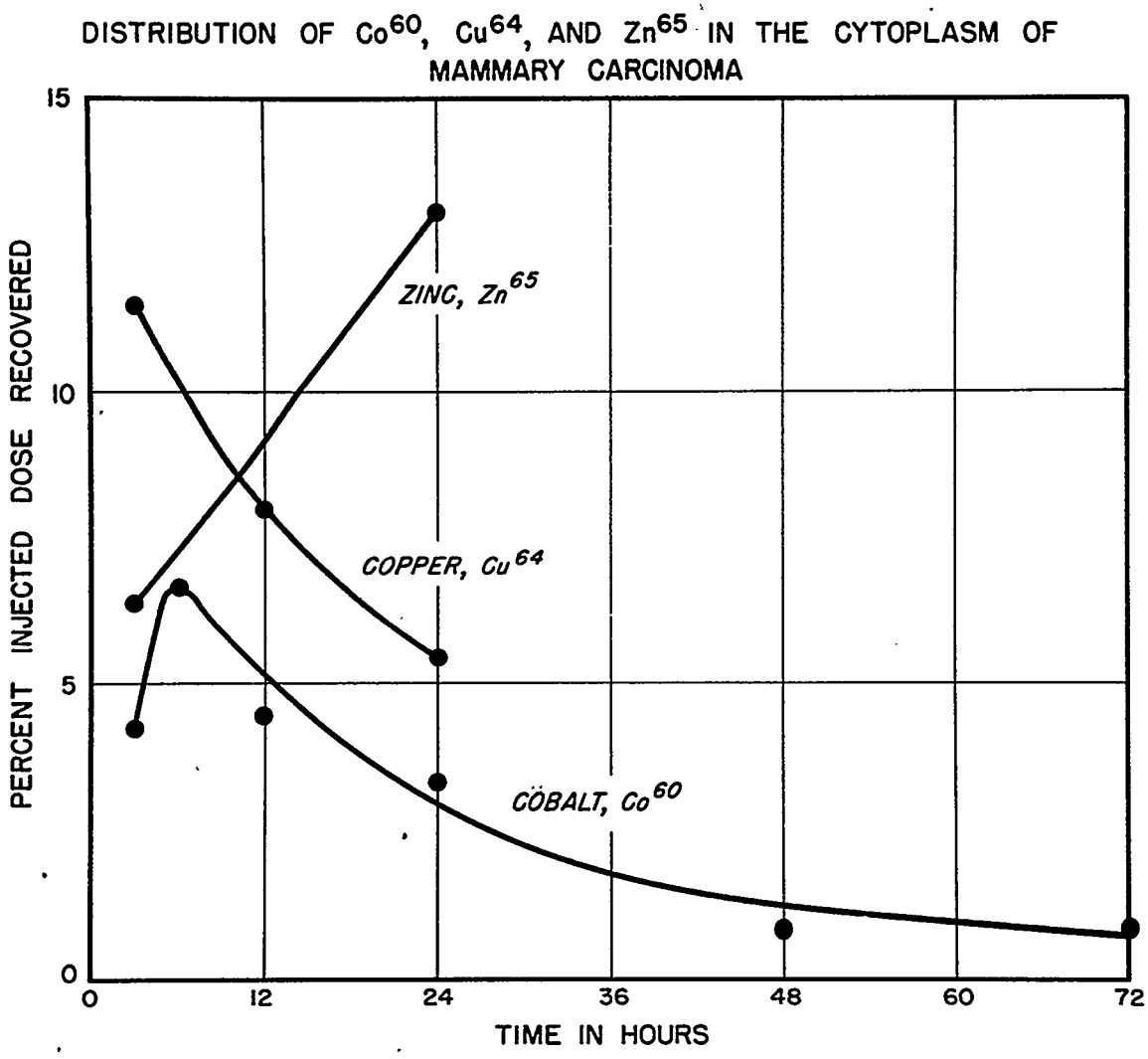
DISTRIBUTION OF Co^{60}, Cu^{64}, AND Zn^{65} IN THE CYTOPLASM OF MAMMARY CARCINOMA
PERCENT INJECTED DOSE RECOVERED
15
10
5
0
ZINC, Zn^{65}
COPPER, Cu^{64}
COBALT, Co^{60}
0
12
24
36
48
60
72
TIME IN HOURS

FIG. 5

III HEALTH CHEMISTRY AND PHYSICS

Health Chemistry

N. B. Garden

Monitoring. Organization of the monitoring group to include the new Central Research Laboratory, into which groups of chemists, physicists and biological and medical personnel will move within the coming month, has taken place, three new monitoring personnel having been hired for the over-all plan. The new personnel is undergoing training in monitoring and ascertaining chemists' wants; it is found expeditious to have new monitors engage in thorough routine laboratory checking under the supervision of a senior monitor for half the day and for the remainder of the day study the availability and uses of gadgets and other protective devices with the Research and Development group. The monitors are roughly divided into two classifications; those who will primarily check rooms routinely and monitor experiments and targets, and those who possess more of an engineering sense, whose function will also be to be the "go-between" for the chemists and development group. Both types of personnel are to know both types of functions and continuously take requests from the chemists for equipment.

Transportation, Decontamination, Disposal and Storage. The pneumatic system for transportation of certain types of targets, especially short-lived ones, is practically completed and will be tested within the next few days. A new target handling box, providing twice as much shielding and weighing twenty pounds less than previous boxes, is now successfully in use. Powder-containing targets have been packed in an improved manner from the standpoint of their unloading after bombardments. The long-awaited decontamination house is in progress of construction and the equipment therein being planned.

The waste disposal - radioactive waste embedded in cement in steel drums - continued to increase but can be adequately handled by the current system of sea disposal.

Research and Development. This group is now improving the straight-type cave equipment for remote control handling of high activity samples. Tongs for the handling of foil-type targets were constructed and put into use during this period. An air alarm counter is in the state of development, for monitoring the alpha-emitting air content, and to be used in connection with the general air filter survey in all the laboratories, which is currently being conducted. A tungsten-shielded container for isotope storage and transportation has been constructed and is awaiting test. A portable radioactive serum bottle capper was completed. Being tested is a modified glove port for use on Berkeley Boxes - modified for more safe and efficient change of gloves. The usual output of standard and modified Berkeley Boxes was issued, including a special box for use in processing radioactive chromic phosphate in the Medical Physics group.

Health Physics

B. J. Moyer

Neutron Field of Linear Accelerator. Extensive surveys of the fast and slow neutron flux densities have been made during this quarterly period. The slow neutron intensity was measured with a balanced chamber system employing argon and BF_3, and the fast neutron energy flux was measured by a polethylene-lined proportional counter.

The slow neutron component was everywhere sufficiently low, extending as high as 1500 $cm^{-2}sec^{-1}$ in the worst areas with a beam current of nearly 1/10 microampere.

The energy flux density carried by fast neutrons, in the region from 0.5-15 Mev , measured in Mev/cm^2sec, was measured repeatedly with results as shown on the attached map. The figures pertain to a proton beam current of 10^{-8} amp.

From a series of observations of the distribution and direction of the fast neutrons it was ascertained that one source was the high-energy end of the accelerator tank. Roughly half as many neutrons appear to originate in the region of B as at the target A (See Fig. 1). Modifications of the focussing grid structures are now being undertaken in an effort to eliminate or greatly reduce neutron production in the B region.

Survey Instrument for Neutrons Over 50 Mev. The presently available Bi fission chambers are too insensitive to allow satisfactory surveys to be made outside the shielding of the 184-inch cyclotron. To satisfy this need a multicellular (150 cells) fission chamber has been constructed which presents a total of 22 gm. of Bi spread over the cell surfaces with a thickness of about 2 mg/cm^2.

The method of deposition consisted of dissolving $Bi(NO_3)_3 \cdot 5H_2O$ in acetone and glacial acetic acid containing also some zapon lacquer, and spraying this on the cell walls (demountable) and subsequent baking. The baking leaves a coating of Bi_2O_3 bonded by zapon lacquer.

Tests of this instrument will begin as soon as the cyclotron resumes operation.

Statistical Summary of Monitoring Program. Survey instruments maintained:

1.	B-V Ionization chambers	28
2.	Victoreen 263 Portable Survey Meters	19
3.	I.D.L. portable survey meters	18
4.	Cutie Pies	2
5.	Recording area survey meters	11
6.	Victoreen Proteximeters	3
7.	Fast neutron proportional counters	2
8.	Slow neutron proportional counters	5
9.	Balanced chamber (Slow neutron survey instrument)	1
10.	Special tissue wall survey instrument	1

Personnel meters in use:

1.	Number of people wearing film badges	1,250
2.	Total man days coverage with pocket chambers	1,594
3.	Total man days coverage with pocket dosimeters	3,383
4.	Total man days coverage with pocket chamber, S. N.	2,190

Cases of weekly exposure above .3 r

Weekly Film Exposures Above	184" Area	60" Area	Linear Accelerator	Synchrotron	Chemistry	Total
0.3 r	5	1	9	0	33	48
0.5 r	1	0	5	0	16	22
1.0 r	1	0	0	0	4	5
1.5 r	1(5.0r)*	0	0	0	0	1

*This exposure was received while undergoing a flouroscopic examination that was in no way connected with the project.

LMB/11-29-49
Information Division

93E49

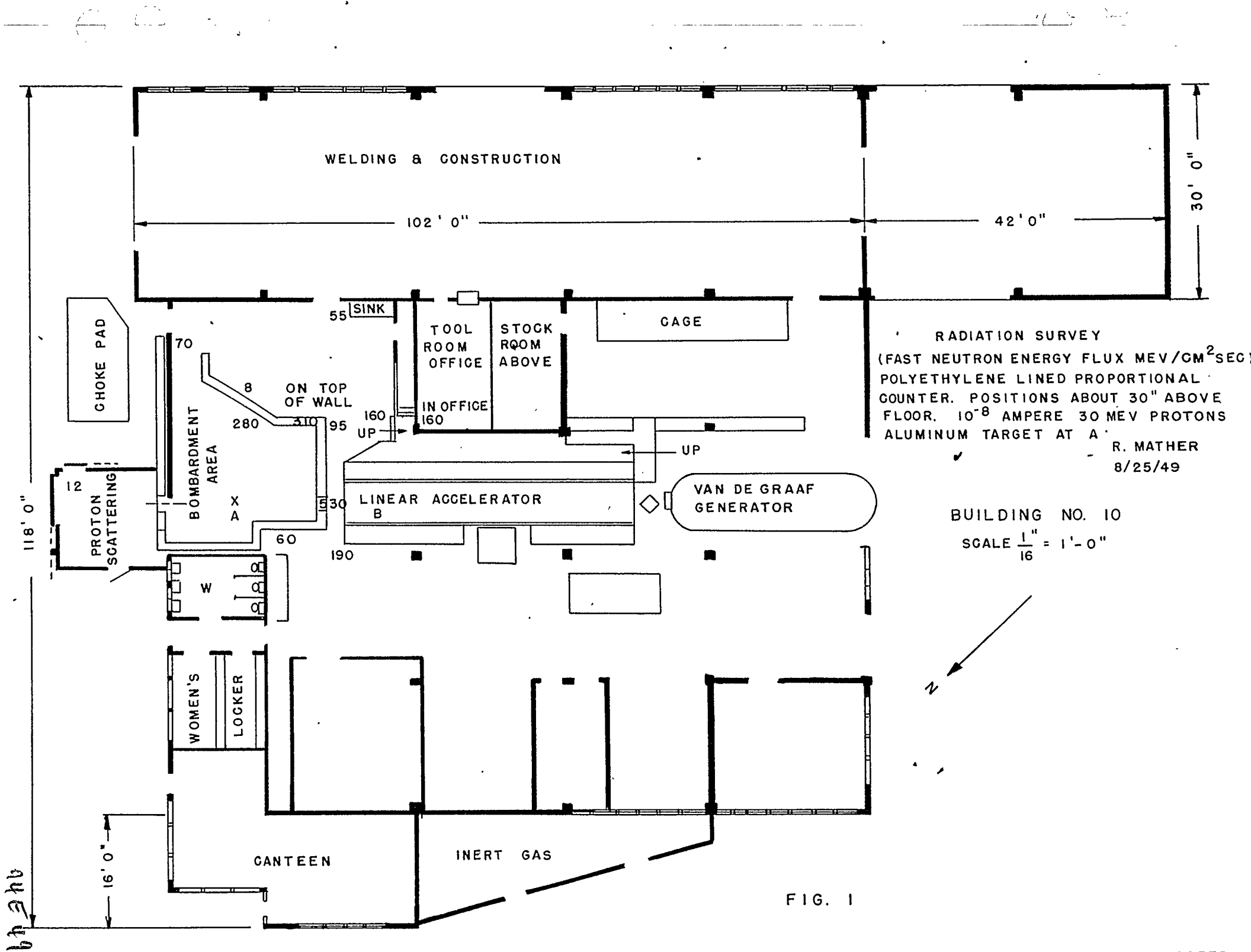
WELDING & CONSTRUCTION
102' 0"
42' 0"
30' 0"
118' 0"
CHOKE PAD
70
55
SINK
TOOL ROOM OFFICE
STOCK ROOM ABOVE
CAGE
8
ON TOP OF WALL
280
310
95
160
IN OFFICE
160
UP
UP
BOMBARDMENT AREA
X
A
12
PROTON SCATTERING
60
130
LINEAR ACCELERATOR
B
VAN DE GRAAF GENERATOR
190
W
WOMEN'S
LOCKER
CANTEEN
INERT GAS
16' 0"
N
RADIATION SURVEY
(FAST NEUTRON ENERGY FLUX MEV/CM2SEC)
POLYETHYLENE LINED PROPORTIONAL COUNTER. POSITIONS ABOUT 30" ABOVE FLOOR. 10-8 AMPERE 30 MEV PROTONS ALUMINUM TARGET AT A
R. MATHER
8/25/49
BUILDING NO. 10
SCALE 1/16" = 1'-0"

FIG. 1

14373-1