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Carbide and Carbon Chemicals Company (Y-12 Area)	19-22
Chief of Naval Research	23
Chicago Patent Group	24
Columbia University (Failla)	25
duPont Company	26-31
Iowa State College	32
Knolls Atomic Power Laboratory	33-34
Los Alamos	35-37
Mound Laboratory	38-40
Naval Medical Research Institute	41
New York Operations Office	42-44
Oak Ridge National Laboratory (X-10)	45-52
Patent Branch, Washington	53
Public Health Service	54
Savannah River Operations Office	55
UCLA Medical Research Laboratory (Warren)	56
U.S. Naval Radiological Defense Laboratory	57
University of California Radiation Laboratory	58-63
University of Chicago Toxicity Laboratory	64
University of Rochester	65-66
University of Tennessee (Comar)	67
University of Washington	68
Western Reserve University (Friedell	69-72
Wright-Patterson Air Force Base	73-74
Technical Information Service, Oak Ridge	75-89
General Electric Company, Richland	90-92
Hanford Operations Office	93
Aircraft Nuclear Propulsion Project, Oak Ridge	94-95

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TABLE OF CONTENTS

		Page
I	THE METABOLIC PROPERTIES OF VARIOUS MATERIALS	
	Radioautography	4
	Tracer Studies	8
	Chelating Experiments	19
	Radiochemistry	20
	Radiation Chemistry	23
II	BIOLOGICAL STUDIES OF RADIATION EFFECTS	
	Macrophage Migration in Experimental Atherosclerosis	31
	Radiation Hazards in High Altitude Aviation	44
	The Influence of Heparin Upon Lipoprotein Metabolism	46
III	HEALTH CHEMISTRY AND PHYSICS	
	Health Chemistry	56
	Health Physics	58

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* Previous Quarterly Reports:

Oct., Nov., Dec., 1947	UCRL-41
Jan., Feb., Mar., 1948	UCRL-98
Apr., May, June, 1948	UCRL-157
Jul., Aug., Sept., 1948	UCRL-193
Oct., Nov., Dec., 1948	UCRL-270
Jan., Feb., Mar., 1949	UCRL-332
Apr., May, June, 1949	UCRL-414
Jul., Aug., Sept., 1949	UCRL-480
Oct., Nov., Dec., 1949	UCRL-587
Jan., Feb., Mar., 1950	UCRL-683
Apr., May, June, 1950	UCRL-806
Jul., Aug., Sept., 1950	UCRL-960
Oct., Nov., Dec., 1950	UCRL-1143
Jan., Feb., Mar., 1951	UCRL-1282

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I THE METABOLIC PROPERTIES OF VARIOUS MATERIALS

J. G. Hamilton

Project 48A

Radioautography

C. W. Asling and Gretchen Thilo

The program to improve the technique of double radioautographs using astatine and radio-iodine has been continued. The radio-iodine was produced by cyclotron bombardment because interest, in the case of the monkey, was in doing in vivo simultaneous iodine-astatine uptake experiment. The radio-iodine was produced by alpha-particle bombardment of antimony from which the predominant activity is the four-day positron I^{124}. Of course, from the point of view of the radioautographic studies, this type of radio-iodine production is no different in its preparation from either the deuteron bombardment of tellurium or from the pile. In any event, the in vivo studies on the monkey were a failure due to some unforeseen difficulty with the equipment employed.

In the case of the radioautographs, it was found that by using a somewhat larger dose of radio-iodine, NTA stripping film gave quite good radioautographs of the radio-iodine deposition and, of course, far superior alpha tracks from the astatine.

Another factor is that the NTA emulsion is far less sensitive to light than the NTB and it permits much easier handling of the material because more light can be employed. Even with the decreased sensitivity of NTA to beta particles as contrast to NTB at the time intervals employed it was considered that no significant radiation damage to the thyroid would take place from the radio-iodine.

It has been discussed earlier that a significant fraction of astatine is leached from the thyroid when the tissue is fixed. Accordingly, a series of experiments were set up using guinea pigs, given both astatine and carrier-free radio-iodine and a comparison was made between the relative degree of leaching that takes place when the thyroid is fixed in either 80 percent alcohol or Bowin's fluid. It was an agreeable surprise that not only did less leaching of astatine take place with Bowin's fluid but also the histological preparations were much superior in that the Bowin fixative preserved the microscopic structure of the tissue much better than alcohol. It was interesting though, that a small amount of leaching of the radio-iodine with both alcohol and Bowin's fluid was found, even though the time interval between this administration and administration of both radiohalogens was 31 hours. In the guinea pig study, the uptake of both astatine and iodine was

only a few tenths of a percent. This it was presumed arose from the fact that these animals had been fed on a diet relatively high in iodine and were in iodine block.

The double radioautographic experiment was repeated with a young rhesus monkey; 165 microcuries of At^{211} and 35 microcuries of radio-iodine were employed. By the time this experiment was done, a large fraction of the I^{124} had decayed away and a significant fraction of the radioactivity present was due to I^{126} which is concomitantly produced by the alpha particle bombardment of antimony. Also present was a small amount of 53-day I^{125} which decays by orbital electron capture. In view of the small percentage of this radioisotope present and its characteristic of radioactive decay, it is presumed that it did not contribute any perceptible effect upon the photographic emulsion.

Fig. 1 presents a representative radioautograph obtained in this particular experiment, the monkey being sacrificed 18 hours following the administration of the two radiohalogens.

<u>C^{14} in Mouse Liver.</u> At the request of the Donner Laboratory a series of radioautographs were made for Drs. John Weaver and E. Bennett for their studies of the metabolism of stilbamidine. They have histochemical evidence to suggest that this C^{14} labeled compound was retained by the mitochondria of the liver cells. By the use of NTB stripping film an attempt was made to confirm this by the radioautographic technique. The procedures employed were those that are employed with thyroid tissue.

After development of the NTB stripping film, the sections were stained in order to make visible these cytoplasmic structures. Difficulties were encountered at first, as the emulsion tended to stain more deeply than the tissue. This was overcome by using a well-aged solution of Mallory's phosphowolframic acid hematoxylin stain instead of one freshly prepared. Also, the preparation was stained only for 30 minutes in contrast to the 24 to 48-hour interval recommended in a number of text books on histological techniques. The radioautograph indicated that the activity was concentrated in the mitochondria with relatively little in the structure of the cell nucleus.

Caution should be exercised in interpreting these results inasmuch as there initially may have been as great, or higher, a concentration of stilbamadine in other cell constituents held more loosely which may have been leached out.

<u>Plutonium Radioautographs.</u> Some time ago it was reported that radioautographs of lung tissue from rats which had previously inhaled plutonium aerosols indicated that plutonium was scattered about in the alveolar tissue. More important is the fact that even at time intervals of 64 to 256 days after exposure most of the activity appeared to arise from particles too small to be seen at a magnification of 900 times. This suggests that plutonium retained after these extended time intervals was present in particles less than one micron in diameter. This observation has been borne out by aerosol studies using other materials which have been done in laboratories both within the facilities of the AEC, notably the University of Rochester project, as well as in organizations not sponsored by the Atomic Energy Commission. In view of the increased

interest in the particle problem in the last few months we have begun to attack this problem more intensively, placing particular emphasis on a more precise localization of the plutonium particles and a careful study of the local histological changes which the preliminary studies in the past indicated were present.

A series of photomicrographs has been made for possible inclusion in a definitive report on the astatine experiments. They were black and white, or color, whichever was most appropriate for the material. Four groups of pictures were made as follows:

1. Photomicrograph at low magnification of entire sections of rat thyroid glands after varying doses of At^{211} and of radioautographic image of I^{131} fixation by these same glands.

2. Photomicrograph at high magnification of the histopathology of rat and monkey thyroid glands subjected to astatine irradiation.

3. Photomicrograph of the histological changes in the lacrimal glands, lymph nodes and spleen of rats subjected to astatine irradiation.

4. Photomicrograph of nuclear track emulsion radioautographs of iodine and astatine localization in rat and monkey thyroids after both separate and concurrent injection.

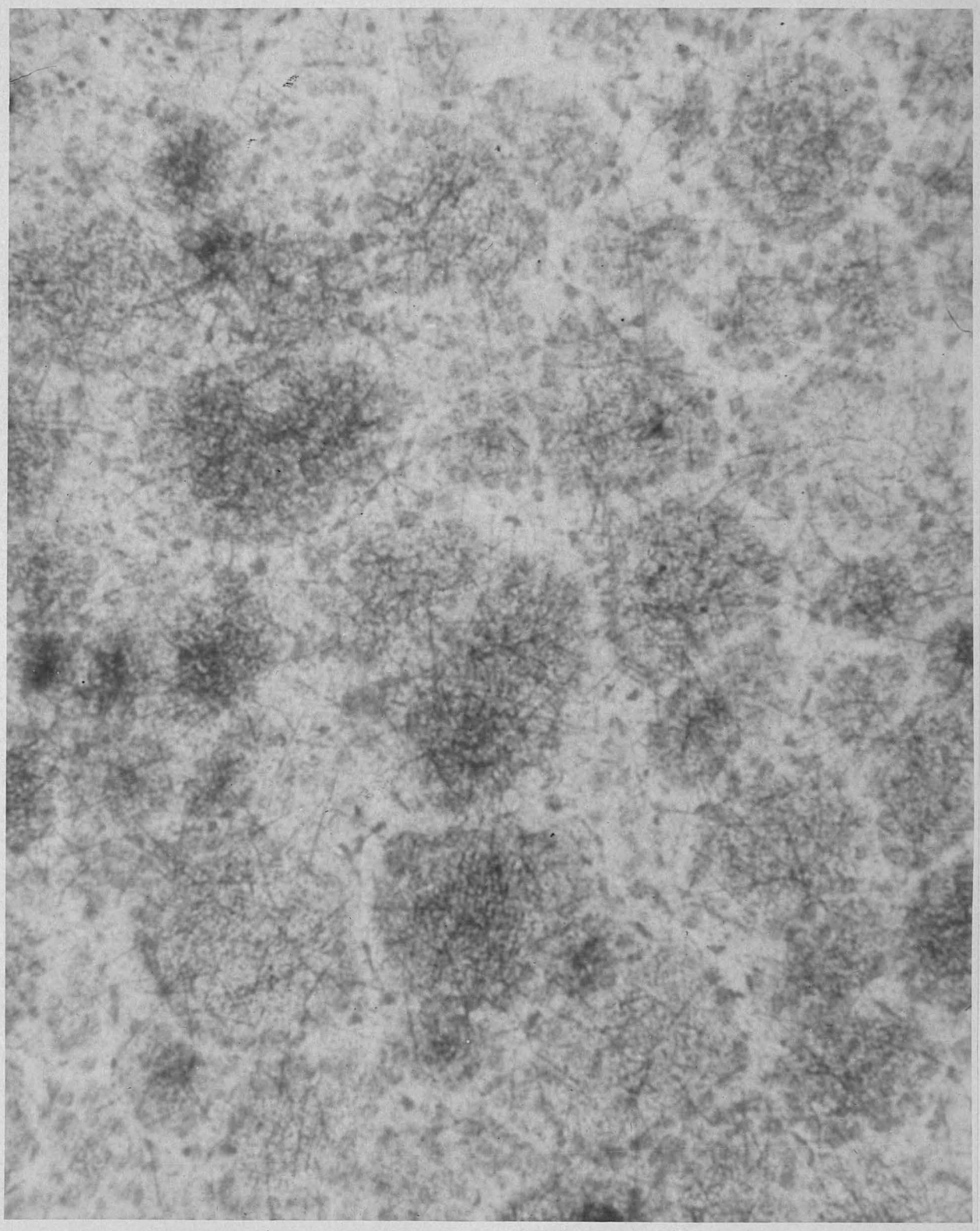

FIG. 1

Tracer Studies

K. G. Scott and J. Crowley

Platinum^{191,193}. The fate of carrier-free $Pt^{191,193}$ has been studied in the rat at various time periods up to 7 days after intravenous injection. Four hours after the parenteral administration of these isotopes, liver and kidney contained by far the largest amounts on a per gram wet weight basis. Measurable amounts were observed to be distributed in the other soft tissues and skeleton. Platinum is eliminated from the body in both urine and feces. Seven days after its administration over 70 percent was found in the excreta. The initial kidney concentration, which at 4 hours through 4 days was of the order of 5 percent of the dose per gram of kidney, continued to remain relatively high and was over 2 percent of the dose per gram of kidney 7 days after administration of the platinum. At the 7-day time period, organs having a reticulo-endothelial function, such as spleen and liver, had the next highest concentrations of platinum and when compared to kidney were 2 to 3 times higher than the other soft tissues which were measured. These data are summarized in Table I.

Similar studies were conducted with carrier-free platinum in the rat after intramuscular administration. These studies were terminated at 4 days, 7 days, 16 days and 32 days after the platinum was injected. Platinum was readily absorbed from the injection site, there remaining but 11.5, 9.5, 9.8 and 5.4 percent of the injected dose in the left leg at 4, 7, 16 and 32 days, respectively. Studies of the distribution of the platinum after its absorption from the injection site are essentially the same as those discussed in which the material was given by intravenous injection, the data being summarized in Table I. At the end of 32 days after intramuscular administration over 90 percent of the material absorbed from the injection site was excreted. However, measurable amounts of platinum were found remaining in the soft tissues such as liver, spleen and blood. These data are summarized in Table II.

Iridium^{192}. The fate of carrier-free iridium using Ir^{192} has been studied in the rat following intravenous injection and studied up to 33 days after its administration. At the early time periods iridium was primarily found in the liver, kidney and blood with measurable amounts in other soft tissues and at 2 hours after injection, 18 percent of the administered dose was found in the urine. In the succeeding time periods studied, namely, 7 and 33 days after administration, additional iridium was excreted both in the urine and feces, but the excretion rate as compared to that at 2 hours after administration was markedly reduced. Thus at 7 days and 33 days after intravenous administration the urinary and fecal excretion was 50 percent and 79 percent, respectively. Liver, kidney and spleen remain the main sites of deposition of Ir^{192} at the later time periods studied; for example, the concentration in kidney is about 80 times greater than that found in stomach and about 16 times greater than that observed in skeleton, skin, heart and lymph glands. The concentration observed in spleen was slightly higher than that observed in kidney and liver. These data are summarized in Table III. It should be noted that the metabolic behavior of iridium is similar to that of platinum.

When iridium is administered orally using carrier-free Ir^{192}, small amounts were found in the tissues 7 days after its administration. However,

TABLE I

THE DEPOSITION OF CARRIER-FREE $Pt^{191,193}$ IN THE RAT 4 HOURS 1, 4 AND 7 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE, CORRECTED FOR RECOVERY AND EXPRESSED IN PERCENT OF ADMINISTERED DOSE. EACH RAT IN THE 4 HOUR, 1, 4, AND 7 DAY GROUPS RECEIVED 0.53, 0.53, 2.2 and 6.7 MICROCURIES RESPECTIVELY.

	4 hour -.53μc		1 day-.53μc		4 day-2.2μc		7 day-6.7μc	
Organ	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.34	.33	.34	.32	.01	.02	.09	.08
Lung	1.18	.75	1.51	.87	.43	.28	.41	.27
Spleen	.66	.42	.62	.61	.37	.38	.52	.46
Blood	10.9	.66	6.61	.45	4.01	.32	2.11	.14
Liver	13.8	1.10	12.9	1.33	5.24	.62	4.44	.39
Kidney	10.9	5.18	9.85	4.93	7.26	5.64	4.95	2.24
Stomach	.34	.31	.25	.26	.11	.15	.16	.13
St. Cont.	<.01	-	<.01	-	.03	-	.02	-
Sm. Int.	2.55	.58	1.09	.37	.94	.20	.41	.10
S.I. Cont.	3.51	-	.66	-	.14	-	.10	-
Lg. Int.	1.75	.58	.80	.44	.57	.31	.35	.12
L.I. Cont.	8.30	-	1.29	-	.60	-	.10	-
Pancreas	.29	.36	.26	.27	.20	.38	.18	.10
Skeleton	8.08	.44	5.79	.34	9.36	.64	2.91	.18
Muscle	14.5	.12	11.6	.11	10.1	.13	5.28	.05
Skin	14.0	.30	14.0	.32	7.74	.28	4.81	.13
Fat	-	.31	-	.25	-	.27	-	.09
Brain	.02	.01	<.01	-	.11	.08	.03	.02
Eyes	.09	.25	-	-	.03	.11	.02	.07
Pituitary	<.01	-	-	-	<.01	-	.01	-
Gonads	.16	.06	-	-	.25	-	.08	-
Thyroid	<.01	-	-	-	.03	-	<.01	-
Adrenal	.02	<.01	-	-	<.01	-	.02	-
Lym. G.	<.01	-	-	-	-	<.01	-	.06
Urine	8.62	-	13.1	-	25.2	-	39.6	-
Feces	<.01	-	19.4	-	27.3	-	33.3	-

TABLE II

THE DEPOSITION OF CARRIER-FREE $Pt^{191,193}$ IN THE RAT 4, 7, 16 AND 32 DAYS AFTER INTRAMUSCULAR INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF ABSORBED DOSE. EACH RAT IN THE 4, 7, 16 AND 32 DAY GROUPS RECEIVED 2.2, 2.2, 6.7, AND 14 MICROCURIES RESPECTIVELY.

	4 day-2.2μc		7 day-2.2μc		16 day-6.7μc		32 day-14μc	
Organ	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	<.01	-	.12	.15	.07	.07	<.01	-
Lung	.20	.12	.31	.22	.13	.18	.18	.11
Spleen	.43	.38	.72	.68	.26	.23	.32	.27
Blood	3.29	.25	3.50	.26	1.17	.07	.59	.04
Liver	3.46	.42	2.76	.31	1.20	.10	.69	.05
Kidney	7.13	4.36	4.92	3.18	2.53	1.14	2.13	.93
Stomach	.45	.69	.21	.24	.10	.02	.05	.01
St.Cont	<.01	-	.06	-	-	-	-	-
Sm.Int.	.61	.13	.45	.15	.17	.02	.17	.01
S.I.Cont.	.51	-	.19	-	-	-	-	-
Lg.Int.	.35	.17	.34	.19	.17	.03	.08	.01
L.I.Cont.	.13	-	.45	-	-	-	-	-
Pancreas	.09	.11	.26	.20	.09	.07	-	-
Skeleton	2.67	.20	2.93	.21	2.02	.13	1.07	-
Muscle	6.52	.08	5.33	.06	2.41	.03	1.86	.02
Skin	5.73	.23	3.37	.12	2.72	.06	.99	.02
Fat	-	<.01	-	.11	-	.09	-	-
Brain	<.01	-	<.01	-	.07	.04	.10	.07
Eyes	<.01	-	.03	.01	.08	.26	-	-
Pituitary	<.01	-	<.01	-	.04	-	-	-
Gonads	<.01	-	<.01	-	.07	.04	-	-
Thyroid	<.01	-	<.01	-	.05	-	-	-
Adrenal	.12	-	.17	-	.05	-	-	-
Lym.Gl.	-	<.01	-	<.01	-	.24	-	-
Urine	39.7	-	19.8	-	55.1	-	36.2	-
Feces	28.7	-	54.1	-	31.5	-	55.5	-

TABLE III

THE DEPOSITION OF CARRIER-FREE Ir^{192} IN THE RAT 2 HOURS, 7 AND 33 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF ADMINISTERED DOSE. EACH RAT IN THE 2 HOUR, 7 AND 33 DAY GROUPS RECEIVED 1.7, 3.4 AND 5.1 MICROCURIES RESPECTIVELY.

	2 hour - 1.7μc		7 day - 3.4μc		33 day - 5.1μc	
Organ	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.33	.48	.12	.17	.04	.07
Lung	4.07	2.71	.51	.36	.32	.28
Spleen	.98	1.20	2.08	2.16	1.61	1.87
Blood	17.1	1.36	1.21	.09	.22	.01
Liver	25.6	3.41	23.3	2.91	6.52	.87
Kidney	4.16	2.97	4.40	3.12	2.22	1.66
Stomach	.23	.25	.14	.17	.08	.02
St.Cont.	.05	-	.02	-	-	-
Sm.Int.	2.41	.43	.66	.12	.31	.03
S.I.Cont.	1.77	-	.09	-	-	-
Lg.Int.	.44	.23	.36	.19	.19	.03
L.I.Cont.	.13	-	.32	-	-	-
Pancreas	.15	.22	.10	.17	.03	.04
Skeleton	6.66	.44	2.36	.14	1.53	.10
Muscle	9.74	.12	5.25	.07	3.26	.03
Skin	7.76	.26	7.45	.23	2.36	.09
Fat	-	.04	-	.06	-	.02
Brain	.07	.04	.02	.01	.01	<.01
Eyes	.02	.08	.01	.06	.01	.02
Pituitary	.01	-	<.01	-	<.01	-
Gonads	.03	.26	.04	.36	.02	.17
Thyroid	.01	<.01	<.01	-	<.01	-
Adrenal	.02	-	.02	-	.01	-
Lym.Gl.	-	.17	-	.18	-	.08
Urine	18.1	-	37.8	-	43.5	-
Feces	.20	-	13.7	-	35.7	-

appreciable amounts were found in kidney, liver, lungs and spleen. These data are summarized in Table IV. From this data the fraction absorbed from the digestive tract is estimated to be of the order of 10 percent of the administered Ir^{192}.

Mercury[196,197]. The fate of carrier-free mercury has been studied in the rat up to 8 days after intravenous administration. These studies have shown that mercury is not excreted in the urine, but that appreciable excretion in the feces occurs and was between 40 and 50 percent of the total amount of material administered 5 days and 8 days after it was given. Kidney contained the highest concentration of mercury of any tissue in the body for the time period studied, being between 10 and 200 times greater than the other tissues of the body. Of the mercury retained in the tissues 8 days after its intravenous administration approximately one-half of the radiomercury so administered was found in the kidneys. Owing to the fact that about 40 percent of the mercury administered was excreted, the balance of the tissues except for the kidneys contained approximately 10 percent of the administered dose. On a per gram wet weight basis, liver, spleen and lungs contained several times more mercury than other soft tissues such as heart, muscle, pancreas, skin, and bone. These data are summarized in Table V.

Attempts to increase the excretion of mercury using a complexing agent such as "Versene" were disappointing. The "Versene"-treated animals excreted approximately 55 percent of the mercury administered, this being only 10 percent higher than the control group. The relative distribution of mercury in the body after "Versene" administration agrees with that observed in the control group with the exception that the total amount contained in the kidneys of "Versene"-treated animals was slightly less than the control group. However, these animals had smaller kidneys than the control group, thus making the percent per organ higher in the control group and the percent per gram wet weight higher in the "Versene"-treated group. These data are summarized in Table VI.

Bismuth[206]. Studies on carrier-free bismuth after intravenous injection of the rats have been completed up to 17 days after administration of Bi^{206}. Ninety-five percent of the material so administered was excreted predominantly in the urine. Measurable amounts of bismuth remained in the liver, kidney and spleen, suggesting that this element has been taken up by the reticuloendothelial system of the body. Attention should be drawn to the fact that the skeleton is not a retention depot for this time interval. These data are summarized in Table VII.

Rhodium[105]. Deposition of carrier-free rhodium[105] has been studied in the rat up to 18 days after intravenous administration. Studies up to 6 days after intravenous administration are presented in Table VIII. These studies show that rhodium is incompletely excreted, there being only 55 percent of the rhodium administered in feces and urine at 6 days after its administration. The greatest deposition occurred in the kidney, being almost 1.5 percent of the dose per gram wet weight of the kidney at 6 days after administration. Appreciable amounts were also found in spleen and liver as well as the intestinal tract, pancreas, skin and lymph glands. With the passage of time, additonal rhodium was excreted so that the animals sacrificed

TABLE IV

THE DEPOSITION OF CARRIER-FREE Ir^{192} IN THE RAT 7 DAYS AFTER ORAL ADMINISTRATION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF ADMINISTERED DOSE. EACH RAT RECEIVED 5.0 MICROCURIES.

Organ	% per organ	% per gram
Heart	<.01	.01
Lung	.03	.03
Spleen	.03	.03
Blood	.01	.01
Liver	.17	.03
Kidney	.22	.17
Stomach	.01	<.01
Sm. Int.	.06	<.01
Lg. Int.	.07	<.01
Pancreas	<.01	-
Skeleton	.29	<.01
Muscle	.56	<.01
Skin	.58	.03
Fat	<.01	-
Brain	<.01	-
Eyes	<.01	-
Pituitary	<.01	-
Gonads	<.01	-
Thyroid	<.01	-
Adrenal	<.01	-
Lym. Gl.	<.01	-
Urine	3.61	-
Feces	94.4	-

TABLE V

THE DEPOSITION OF CARRIER-FREE $Hg^{196,197}$ IN THE RAT 4 HOURS, 1, 5 AND 8 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF ADMINISTERED DOSE. EACH RAT IN THE 4 HOUR, 1, 5 AND 8 DAY GROUPS RECEIVED 3.2, 6.4, 16.1 AND 167 MICROCURIES RESPECTIVELY.

	4 hour-3.2μc		1 day-6.4μc		5 day-16.1μc		8 day-167μc	
Organ	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.32	.33	.20	.26	.09	.16	.04	.05
Lung	1.23	.72	1.19	.70	.43	.28	.15	.10
Spleen	1.54	1.03	1.55	1.31	.66	.47	.18	.18
Blood	7.44	.53	3.62	.27	.44	.03	.32	.03
Liver	29.7	2.91	17.5	1.74	8.24	.71	2.19	.23
Kidney	24.1	13.1	35.3	22.3	26.6	14.1	47.9	26.7
Stomach	.42	.39	.48	.37	.11	.09	.04	.04
St.Cont.	.13	-	.04	-	.52	-	.01	-
Sm.Int.	2.31	.63	2.55	.56	.82	.09	.23	.06
S.I.Cont.	2.81	-	2.61	-	.52	-	.22	-
Lg.Int.	2.42	.96	2.62	.87	.45	.15	.17	.08
L.I.Cont.	2.62	-	4.95	-	.76	-	.62	-
Pancreas	.43	.29	.69	.29	.08	.11	.05	.05
Skeleton	4.37	.29	3.92	.27	1.29	.08	.58	.04
Muscle	8.47	.09	9.19	.09	3.03	.03	1.49	.01
Skin	10.7	.32	11.8	.38	4.62	.11	3.00	.08
Fat	-	.04	-	.16	-	.03	-	.03
Brain	.03	.01	.07	.04	.07	.04	.04	.03
Eyes	.02	.07	.02	.07	<.01	-	.01	.03
Pituitary	<.01	-	<.01	-	<.01	-	<.01	-
Gonads	.08	.49	.07	.46	.02	.11	<.01	-
Thyroid	<.01	-	<.01	-	.01	-	.01	-
Adrenal	.04	-	.04	-	.01	-	.01	-
Lym.Gl.	-	.38	-	.57	-	.11	-	.09
Urine	.78	-	.90	-	1.87	-	4.52	-
Feces	.05	-	.63	-	49.3	-	38.2	-

TABLE VI

THE EFFECT OF A COMPLEXING AGENT ON THE DEPOSITION OF CARRIER-FREE $Hg^{196,197}$ IN THE RAT 8 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF ADMINISTERED DOSE. EACH RAT RECEIVED 167 MICROCURIES OF $Hg^{196,197}$ AND EACH OF THREE RATS RECEIVED 0.5 MILLILITERS OF A 10 PERCENT SOLUTION OF THE CALCIUM-SODIUM SALT OF ETHYLENE DIAMINE TETRAACETIC ACID (VERSENE) ADMINISTERED INTRAMUSCULARLY, DAILY FOR 7 DAYS.

Organ	8 day + Versene % per organ	8 day + Versene % per gram	8 day - Control % per organ	8 day - Control % per gram
Heart	.03	.04	.04	.05
Lung	.13	.11	.15	.10
Spleen	.20	.22	.18	.18
Blood	.26	.02	.32	.03
Liver	3.03	.35	2.19	.23
Kidney	34.2	32.6	47.9	26.7
Stomach	.04	.05	.04	.04
Stomach Cont.	.14	-	.01	-
Sm. Int.	.27	.07	.23	.06
S. I. Cont.	.24	-	.22	-
Lg. Int.	.15	.10	.17	.08
L. In. Cont.	.61	-	.62	-
Pancreas	.03	.07	.05	.05
Skeleton	.62	.05	.58	.04
Muscle	1.73	.02	1.49	.01
Skin	4.01	.13	3.00	.08
Fat	-	.02	-	.03
Brain	.04	.02	.04	.03
Eyes	<.01	-	.01	.03
Pituitary	<.01	-	<.01	-
Gonads	<.01	-	<.01	-
Thyroid	<.01	-	.01	-
Adrenal	<.01	-	.01	-
Lym. Gl.	-	.08	-	.09
Urine	10.9	-	4.52	-
Feces	43.3	-	38.2	-

TABLE VII

THE DEPOSITION OF CARRIER-FREE Bi^{206} IN THE RAT 17 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF ADMINISTERED DOSE. EACH RAT RECEIVED 10 MICROCURIES.

Organ	% per organ	% per gram
Heart	0	-
Lung	.18	.02
Spleen	.15	.16
Liver	2.40	2.24
Kidney	.59	.30
Stomach	<.01	-
St.Cont.	-	-
Sm.Int.	.02	<.01
S. I. Cont.	-	-
Lg. Int.	.11	.04
L. In. Cont.	.03	-
Pancreas	.02	.01
Skeleton	.82	-
Muscle	.25	<.01
Skin	.39	<.01
Fat	-	-
Brain	.01	<.01
Eyes	<.01	.02
Gonads	<.01	-
Thyroid	<.01	-
Adrenal	<.01	-
Lymph Gland	-	.03
Urine	-	73.1
Feces	-	21.9

TABLE VIII

THE DEPOSITION OF CARRIER-FREE Rh^{105} IN THE RAT 2 HOURS, 1, 2 AND 6 DAYS AFTER INTRAVENOUS INJECTION. VALUES ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED IN PERCENT OF THE ADMINISTERED DOSE. EACH RAT IN THE 2 HOUR, 1, 2, AND 6 DAY GROUPS RECEIVED 2, 2, 4, AND 6.5 MICROCURIES OF Rh^{105} RESPECTIVELY.

	2 hour-2μc		1 day - 2μc		2 day - 4μc		6 day - 6.5μc	
Organ	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Heart	.30	.30	.24	.27	.22	.22	.15	.15
Lung	1.51	.56	1.16	.52	.74	.41	.66	.32
Spleen	.39	.18	.34	.32	.51	.40	.64	.55
Blood	11.4	.61	6.47	.45	5.01	.31	1.25	.08
Liver	5.41	.34	3.92	.40	4.34	.35	2.65	.27
Kidney	3.03	.87	2.89	1.61	3.41	1.37	3.36	1.42
Stomach	.48	.30	.44	.25	.31	.24	.22	.20
Stom.Cont.	.03	-	.02	-	.12	-	<.01	-
Sm.Int.	1.33	.25	1.13	.25	1.10	.15	.82	.16
Sm.Int.Cont.	1.23	-	.62	-	.41	-	.10	-
Lg.Int.	1.24	.32	2.57	.39	.90	.27	.89	.27
Lg.Int.Cont.	.25	-	.66	-	.69	-	.28	-
Pancreas	.67	.28	.35	.30	.23	.22	.14	.22
Skeleton	8.76	.39	4.07	.24	5.29	.28	3.03	.15
Muscle	18.6	.16	13.4	.14	15.1	.13	11.7	.11
Skin	21.4	.43	14.4	.52	21.6	.47	17.5	.40
Fat	-	.23	-	.15	-	.08	-	.14
Brain	.05	.03	.03	.02	.03	.01	<.01	<.01
Eyes	.04	.12	.05	.14	.04	.15	.02	.08
Pituitary	<.01	-	<.01	-	<.01	-	<.01	-
Gonads	.49	.14	.55	.23	.46	.17	.47	.19
Thyroid	<.01	-	<.01	-	<.01	-	<.01	-
Adrenal	.04	-	.02	-	<.01	-	.02	-
Lym. Gl.	-	.50	-	.61	-	.37	-	.37
Urine	23.3	-	35.2	-	29.1	-	35.5	-
Feces	.03	-	11.4	-	10.4	-	20.6	-

at 18 days after its administration excreted approximately 50 percent in the urine and 30 percent in the feces. At this time period, kidney, spleen, lymph gland and skin contained the highest amounts of rhodium still present in the body, the concentration in kidney being much higher than the other soft tissues observed. When administered by stomach tube, the animals being sacrificed 4 days later, rhodium did not accumulate to appreciable degree in any of the soft tissues which were measured with the exception of kidney which contained 0.04 percent of the administered dose. Thus concentration is approximately 30 times below that observed when the rhodium is administered intravenously.

Chelating Experiments

Harry Foreman

Chronic Toxicity Studies. Studies to determine the results of prolonged feeding of CaEDTA and Fe-3* have been terminated. Animals fed on a stock diet containing 5 percent CaEDTA (calculated on the basis of the EDTA content) were sacrificed after 177 days. The animals gained on the average of 15 to 20 grams in this time period as compared to a weight gain of 20 to 25 grams for the control animals fed only a stock diet throughout the time period. Diarrhea occurred during the first month but after this time the animals had soft, semi-formed stools until the termination of the experiment. There were no gross unusual findings either during their lifetime or at autopsy. They showed approximately the same levels of activity as the control animals. At autopsy the lower bowel was filled with watery stool, but there was no induration, thinning, or inflammation of the wall. Liver, kidney, spleen, duodenum and cecum were taken for histological examination.

Animals fed on a stock diet containing 10 percent Fe-3 were sacrificed at 125 days. As indicated in previous reports, the animals began to manifest spasmodic tremors about 3 weeks prior to sacrifice. The tremors became more and more pronounced until shortly before sacrifice the animals exhibited irregular convulsions. The profuse diarrhea persisted throughout the entire experiment. By the end of the experiment the animals had lost on the average of 10 grams as compared to an approximate weight gain in the control animals of 20 grams for the 125-day period. It is interesting to point out that the experimental animals showed a red count shortly before death of an average of 6.5 as compared to a level of 11.8 in the control animals. Their hemoglobin average was 44 as compared to 82 in the control animals. Hematocrit for the experimental animals averaged 22 as compared to 40 for the control animals. The animals on autopsy showed no unusual gross findings. Liver, spleen, kidney, duodenum, and cecum were taken for histological study. The brain appeared normal.

Animals fed 5 percent Fe-3 in a stock diet were sacrificed after 128 days. The animals all showed no change in weight in this time period. Diarrhea persisted throughout the entire feeding experiment. The animals showed no unusual findings during their lifetime. At autopsy one of the animals showed a mediastinal tumor weighing approximately 0.5 grams, which was filled with a fatty material. A portion of the wall was taken for histological analysis. In all likelihood there is no relationship between the occurrence of the tumor and feeding of Fe-3. There were no other gross unusual findings. Liver, spleen, kidney, cecum, and duodenum were also taken for histological analysis.

Plutonium^{238}. The experiment in which animals injected with Pu^{238} are fed daily doses of CaEDTA and Fe-3 is now in approximately the third month. The preliminary analyses show that the administration of CaEDTA in this manner results in a seven- to tenfold increase in urinary excretion of the Pu, even after the feeding has continued for 50 days. The detailed and final data will be presented in the next Quarterly Report.

*A commercial detergent whose formula is held secret by the manufacturer.

Radiochemistry

W. M. Garrison, H. R. Haymond, J. D. Gile and D. C. Morrison

Carrier-Free Fe^{59} from Co. The radionuclide, Fe^{59}, produced by the nuclear reaction $Co^{59}(d,2p)$ has been isolated without the use of added isotopic carrier by a separation procedure based on the observation that Fe^{59} forms radiocolloidal aggregates which may be removed by filtration. The radio-iron was produced by bombarding a cobalt metal target with 20 Mev deuterons in the 60-inch cyclotron at the Crocker Laboratory. The only other transmutation products formed concurrently by deuteron bombardment of cobalt include 2.6 hour Mn^{56} (from n,α reaction) which was allowed to decay out prior to chemical separation and approximately 10^5 year Ni^{59} (from d,2n reaction) which is too long-lived to be produced in detectable amounts by the bombardments reported here.

The target was 10 mil thick foil of "iron-free" cobalt[1] silver-soldered to a water-cooled copper probe. Approximately 100 milligrams of the bombarded surface was removed by dissolution in 12 N HCl. After centrifugation, the solution was diluted with an equal volume of water and neutralized to pH 9 with 15 N NH_4OH to give a clear "solution" which was slowly drawn through a Whatman No. 50 filter paper. The carrier-free Fe^{59} was quantitatively retained on the filter as adsorbed radiocolloid. The filter was washed first with dilute NH_4OH until Co could not be detected in the filtrate and then washed with distilled water. Over 98 percent of the Fe^{59} remained on the filter paper. The activity was then removed with 6 N HCl solution which was evaporated to incipient dryness and diluted to 0.1 N at which pH the Fe^{59} remained in true solution.

The Fe^{59} was identified by the assigned 46-day half-life and by the 0.46 Mev and the 0.26 Mev beta particles which have been reported. The gamma radiation had a half-thickness in lead of 11 gm/cm^2 which agrees with the previous observations. Chemical separation of an aliquot amount of the activity in the Fe fraction after the addition of Ni, Co, Fe and Mn carriers further identified the activity as Fe^{59}.

Carrier-Free $Tl^{201,202}$ from Hg. A carrier-free procedure used in isolating radio-thallium from a mercury target which had been bombarded with 19 Mev deuterons in the 60-inch cyclotron at Crocker Laboratory is described below. At this energy, deuteron bombardment of mercury produces $Tl^{201,202}$ by the nuclear reactions, $Hg^{200}(d,n)Tl^{201}$, $Hg^{201}(d,2n)Tl^{201}$, $Hg^{201}(d,n)Tl^{202}$ and $Hg^{202}(d,2n)Tl^{202}$. Other possible shorter-lived radioisotopes of thallium were allowed to decay out prior to chemical separation.

The target chamber was made from a 100 ml pyrex flask and had a concave glass window approximately 4 cm in diameter and 0.15 mm in thickness (with a total volume of 30 ml). The target flask was connected to a small reflux condenser which was cooled by a stream during bombardment. The target was filled with 30 ml liquid mercury[2] and bombarded with 19 Mev deuterons for

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1 The cobalt target was prepared by Mr. R. W. Dunn of the Donner Laboratory. Spectographic analysis showed the iron content to be less than 20 p.p.m.

2 The mercury was C.P.-triple distilled-thallium free.

a total of 30 μa-hr at an average beam intensity of 3.5 μa. After removal of the mercury from the target, it was found that the radio-thallium had deposited quantitatively on the walls of the glass flask. The mercury was discarded and the flask was washed twice with 16 N HNO_3. The HNO_3 solution which contained the radio-thallium and any mercury which had adhered to the glass surface was evaporated to 2 ml, treated with excess 12 N HCl to destroy the HNO_3 and diluted to approximately 3 N HCl. This solution was then extracted with ethyl ether which had been previously equilibrated with 3 N HCl. Over 95 percent of the carrier-free radio-thallium was removed from the aqueous phase. The ether fraction was washed with water to remove HCl and the activity was quantitatively retained in the organic phase. Fifty milligrams of NaCl was added to the ether solution and the mixture evaporated to dryness on a steam bath. The carrier-free $Tl^{201,202}$ redissolved quantitatively in 5 ml of distilled water to adjust to pH 6 to give an isotonic saline solution for biological investigation.

The radio-thallium was identified by half-life determinations, absorption measurements and by chemical separation with added carriers. The decay curve was followed for 60-days and showed two periods: 72-hour Tl^{201} and 11.8-day Tl^{202}. Eleven days after bombardment the activity showed only the 11.8-day period. The aluminum absorption measurement four days after bombardment had an end-point of approximately 130 mg/cm^2. Lead absorption measurements showed the 0.4 Mev gamma ray previously assigned to Tl^{202}. Over 98 percent of a trace amount of activity which had been added to a solution containing milligram amounts of Au, Hg and Tl was recovered chemically in the thallium fraction.

Carrier-Free $Pt^{191,193}$ from Os. Radioactive platinum produced by bombardment of osmium with 38 Mev alpha particles, has been isolated without addition of isotopic carrier. Two radioisotopes of platinum are produced by (α,n), (α,2n) and (α,3n) reactions with alpha particles of this energy: 3-day Pt^{191} and 4.3 day Pt^{193}. The carrier-free radio-platinum was separated from the target element and from the possible radioisotopes of Ir (from n,p reaction) by a solvent-extraction procedure based on the solubility of chloroplatinous acid - ethyl ether.

A 2 mm layer of C.P. osmium[3] powder supported on a grooved water-cooled aluminum target plate by a 0.25 mil tantalum foil was bombarded for 40 μa-hr at an average beam intensity of 7 μa in the 60-inch cyclotron at Crocker Laboratory.

The bombarded osmium was dissolved in aqua regia. Additional 16 N HNO_3 was added and the osmium was volatilized as the tetraoxide. After the osmium had been volatilized, the HNO_3 was destroyed with excess of 12 N HCl. The acid solution containing the active $Pt^{191,193}$ was diluted with distilled water to make the solution 3 N in HCl. One ml of 10 percent $SnCl_2$ was added, and the chloroplatinous acid was extracted with ethyl ether which had previously been saturated with 3 N HCl. Under these conditions over 95 percent of the radio-platinum is recovered in the organic phase. The ether layer was washed with 6 N HCl and the activity was quantitatively retained in the organic layer. Twenty milligrams of NaCl were added to the ether phase and the mixture was evaporated to dryness on a steam bath. The carrier-free $Pt^{191,193}$ was redissolved quantitatively in 2 ml of distilled water at pH 6 to give an isotonic

[3] The osmium was obtained from Johnson Mathey and Co. Pt was not detected by spectrographic analysis.

saline solution for biological experiments.

The radio-platinum was identified by half-life determinations, absorption measurements and by chemical separation with carrier. The decay was followed for 40 days and showed the 3.0 day Pt^{191} and the 4.3 day Pt^{193}. Seven days after bombardment the activity showed only the 4.3 day period. Aluminum and lead absorption measurements 2 days after bombardment showed the approximately 0.5 Mev conversion electron and the 0.57 Mev gamma ray previously reported for Pt^{191}. A trace amount of activity added to a solution containing carrier amounts of Os, Ir, Au and Pt was quantitatively recovered in the Pt fraction following chemical separation.

Carrier-Free Hg^{197} from Au. The Hg^{197} was prepared by the reaction Au^{197} (d,2n). The Au target was dissolved in excess aqua regia and the solution was taken to dryness and redissolved in HCl. Insoluble material was removed by centrifugation. The acid solution was made 2.7 N in HCl and extracted twice with equal volumes of ether which had been previously equilibrated with 2.7 N HCl. The first extraction removed over 95 percent of the Au from the aqueous phase. A second extraction was required to remove Au below a concentration level equivalent to approximately 10 micrograms of Au. The radioactivity in the aqueous phase remained constant with continued extractions indicating that the Hg^{197} is not extracted under these conditions. The solution of carrier-free Hg^{197} was then evaporated to dryness on 3 ml of isotonic saline solution and redissolved quantitatively on the addition of water. The activity followed Hg quantitatively in the chemical separation of a mixture of Hg, Au and Pt in milligram amounts containing a trace amount of the activity. The decay and radiation characteristics agreed with published values.

Other Radiochemistry. The following carrier-free radioactivities were separated from cyclotron targets using previously reported procedures: F^{18}, W^{181}, $Rh^{101,102}$, A^{37}, At^{211} and $I^{124,125,126}$.

Radiation Chemistry

W. M. Garrison, H. R. Haymond, and D. C. Morrison

The detailed mechanism of the indirect action of high energy radiation on dilute aqueous solutions is of fundamental importance in the interpretation of many radiation effects in biological systems. Although a considerable amount of data on radiation induced reactions in dilute solutions has been adequately described in terms of generally accepted free radical mechanisms involving a primary dissolution of solute molecules, detailed determination of the nature and relative amounts of reaction products, particularly for organic substances, has been limited to a relatively few compounds under limited experimental conditions. Most of the available information on the radiation chemistry of dilute solutions of organic substances has been obtained using x- or γ-radiation and, except for a few notable instances, has involved the identification and determination of one or perhaps two of the non-volatile reaction products. To contribute to the basic mechanism of the biological effect of high energy radiation, a program has been initiated at this laboratory with the object of studying the detailed chemical changes produced by heavy particle irradiation of dilute solutions of the simpler organic compounds having functional groups of biological importance. It is proposed to make quantitative assessment of the amount and nature of the chemical changes as a function of the type and energy of the radiation. Identification and measurement of reaction products will be facilitated by the use of C^{14}, S^{35} and H^{3} labeled compounds. The reactions which are being investigated will be separated into three general categories:

1. Decomposition of fatty acids, alcohols, amino acids, sugars, etc., and the identification and yield determinations of volatile and non-volatile products.

2. The synthesis in aqueous solution of polyatomic organic molecules from carbon dioxide, hydrogen, nitrogen, etc.

3. Oxidation-reduction reactions of inorganic solutes for actinometric purposes.

The radiation source for these studies is the 60-inch cyclotron at the Crocker Laboratory. Because of the relatively large energy input which is available and because the determination and measurement of radiation products, particularly those formed in the low yield, can be made more conveniently when large volumes of solution are irradiated, an all-glass circulating target assembly has been developed which permits the irradiation of essentially liter amounts of solution under controlled conditions. A cross section of the circular target pump is shown in Fig. 2. The arrangement of the target and the complete target assembly is schematically presented in Fig. 3. Figs. 4, 5, and 6 are photographs of the assembly which is currently in use.

In a typical run, the solution to be irradiated is placed in the flask (7A) which is connected through a ground glass joint to a centrifugal pump arrangement (7B). The shaft of the pump is connected to an air motor (8).

As the shaft is rotated, the solution is drawn up to fill the pump chamber (7B) and is returned to the storage vessel through (7C). The chamber (7B) has a platinum window (6b) which is retained by a gasket (6a) and a metal bracket (6) which in turn is connected to the upright supporting the air motor and is an integral part of the portable target platform (9). The reservoir (7A) is equipped with inlet and outlet ports so that the solution during irradiation can be saturated with a desired gas. The temperature is measured by a thermometer placed directly in the storage vessel through a standard taper ground glass joint, and cooling is obtained by means of the bath (7D). The beam is delimited by the aperture (2) and the exposure time is controlled by the shutter arrangement (5). The entire apparatus up to the aluminum window is insulated from the cyclotron and is so designed that the ionization produced in the column of air between the aluminum window and the platinum window of the target assembly does not result in loss of beam current. The beam current to the aqeuous solution is monitored through the platinum window which is in direct contact with the conducting solution. With this apparatus it is possible to follow the course of a radiation induced reaction over a prolonged period of time and samples of the solution may be removed at intervals. This can be accomplished by pipetting the solution from the reservoir while the liquid is being circulated, or by passing the circulating solution through the stopcock in (7C). The mercury seal (7E) permits the solution to be irradiated while saturated with hydrogen, oxygen, inert gas, etc.

Preliminary data using this target assembly have been obtained for acid solutions of ferrous ammonium sulphate saturated with oxygen. In a typical experiment, 500 ml of a solution 0.25 M in $Fe(NH_4)_2(SO_4)_2$ and 1 N in H_2SO_4 was bombarded in the circulating target with 35.5 Mev helium ions at a beam current of 0.5 μa. Samples were removed and titrated potentiometrically with standard 0.01 N $KMnO_4$. A composite curve showing the ferrous ion oxidation as a function of bombardment is shown in Fig. 7. The curve which is given shows only the linear relationship between the amount of ferrous ion oxidized and the energy absorbed over the concentration range studied and the precision of the experimental techniques. The ion-pair yield, i.e., the slope of the curve in Fig. 7 cannot be reported in terms of absolute value inasmuch as the alignment of the defining slit and the target window have not been completely established. This work is in progress and these preliminary data will be extended to include the effect of particle energy for alpha particles and for protons and deuterons. Other experiments in progress include a continuation of work previously reported on the radiation induced reduction of CO_2 to formic acid and formaldehyde in aqueous solution.

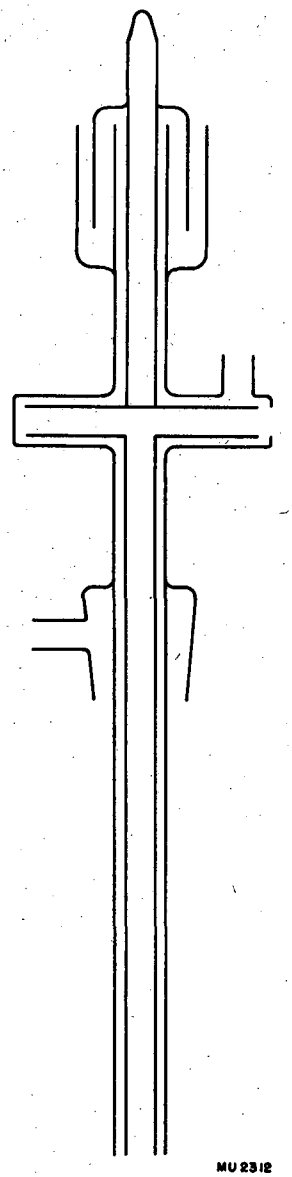

Fig. 2

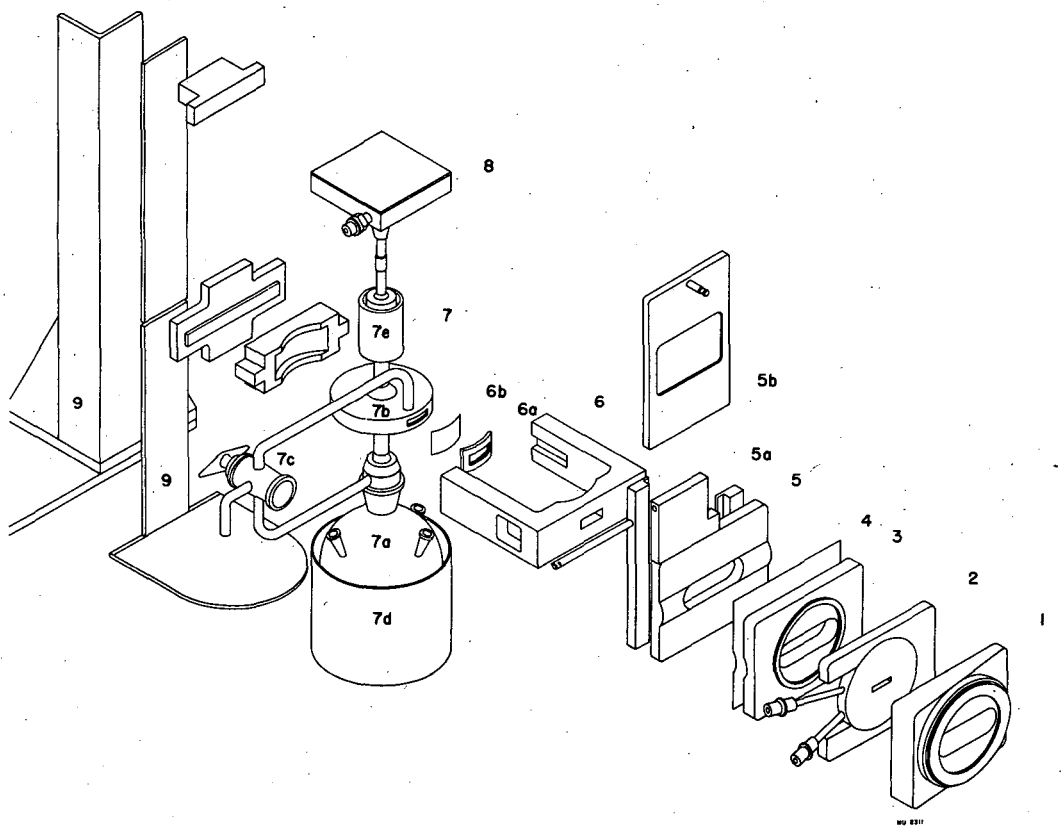

Fig. 3

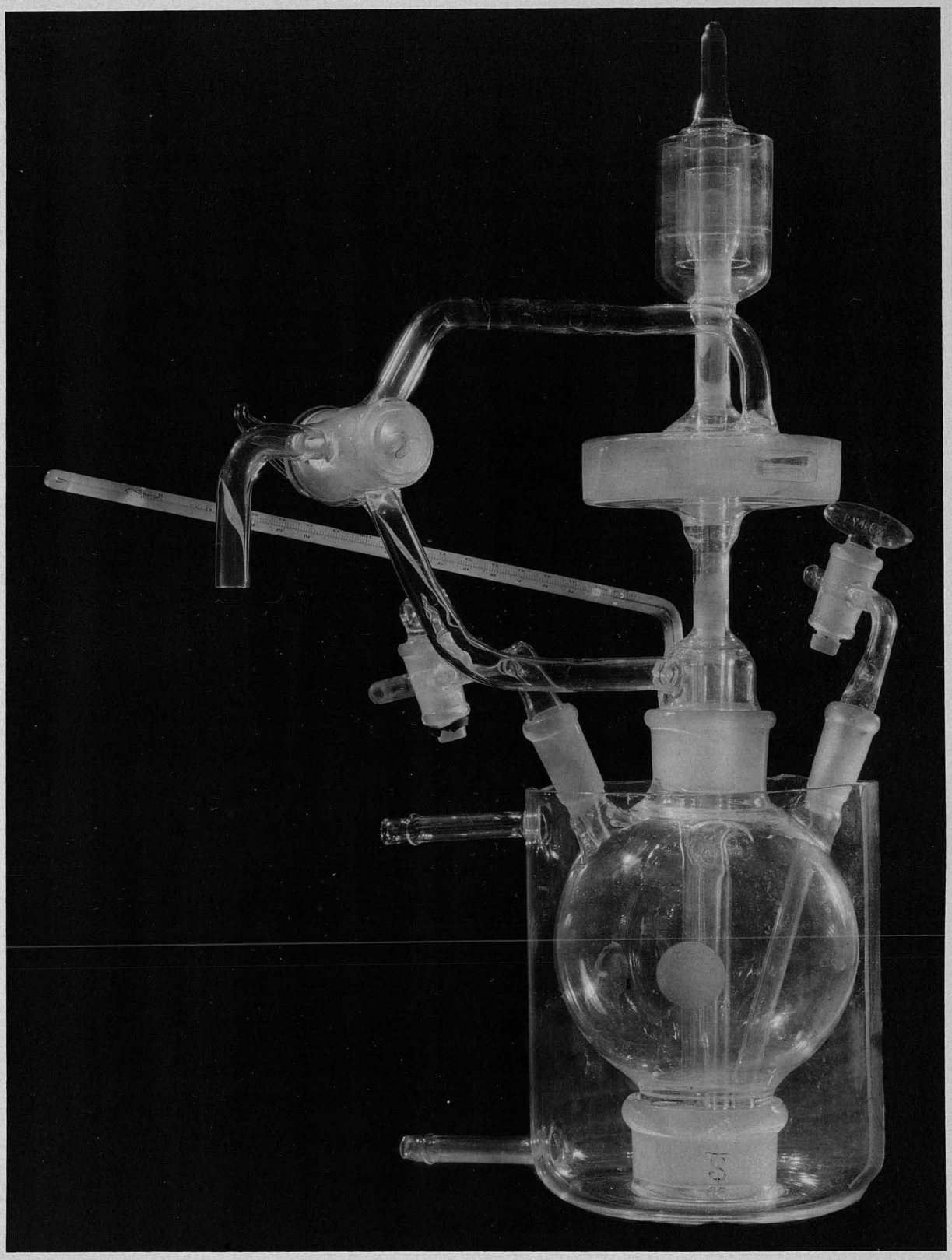

FIG. 4

OZ 1149

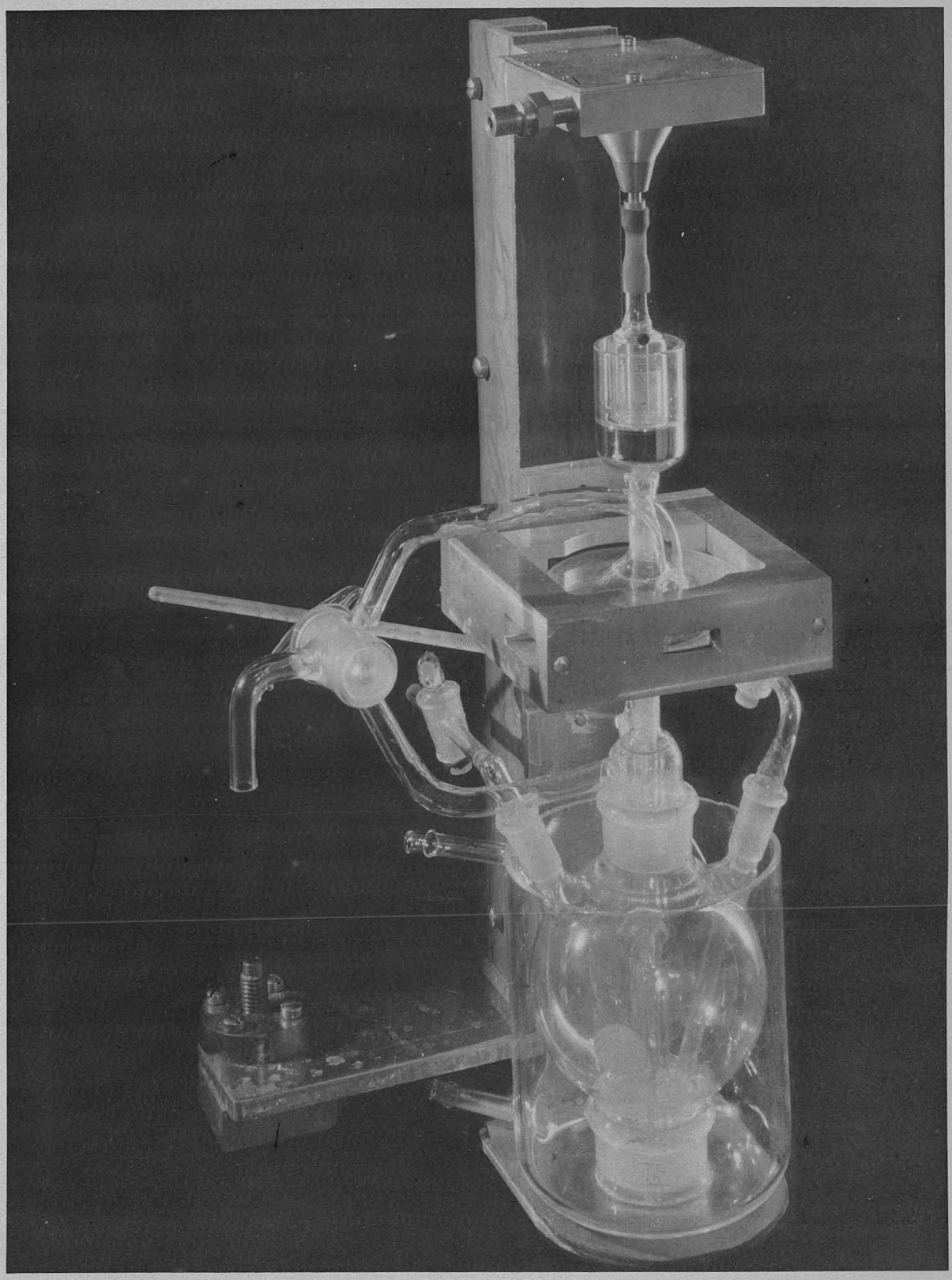

FIG. 5

OZ 1150

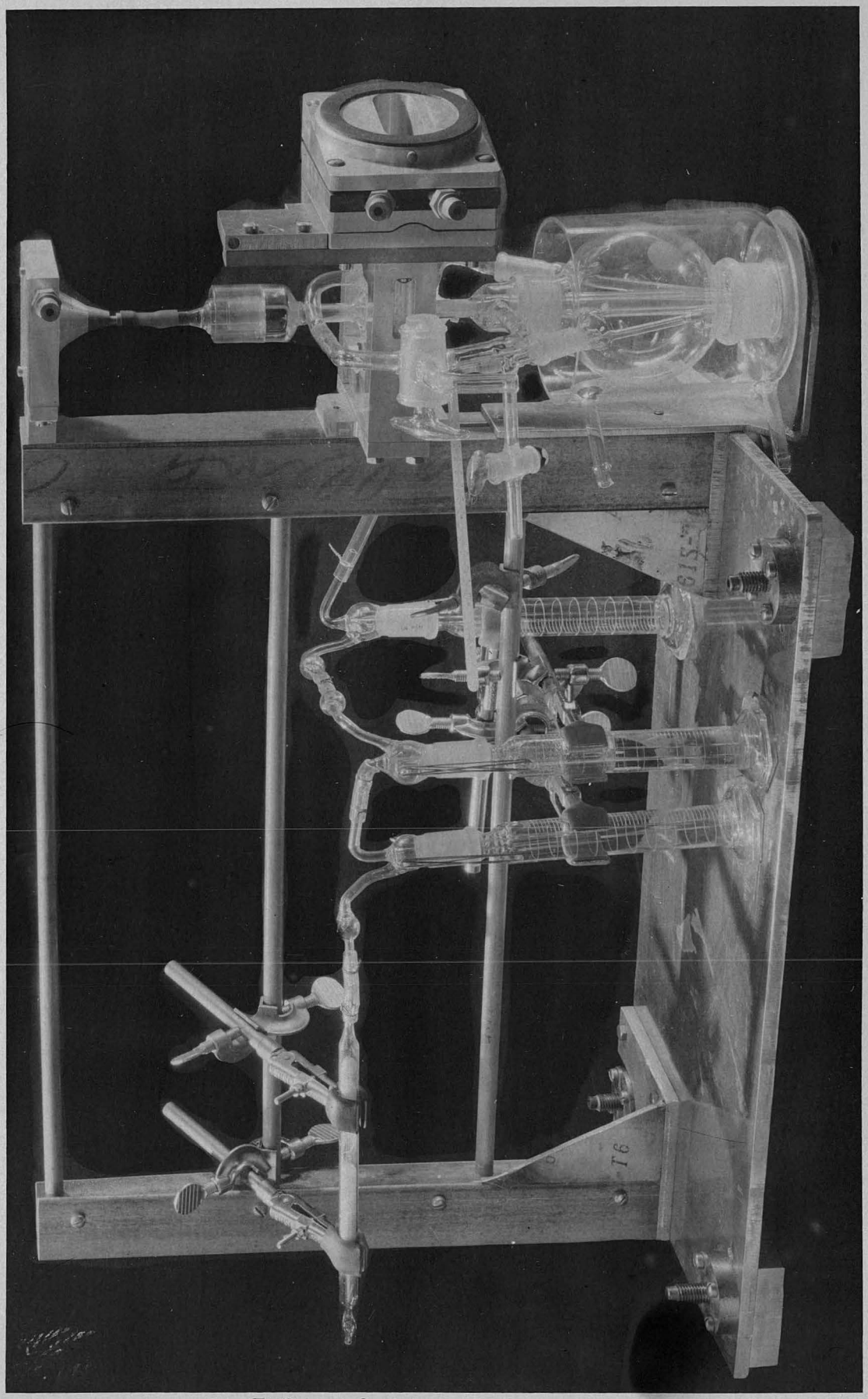

FIG. 6

OZ 1148

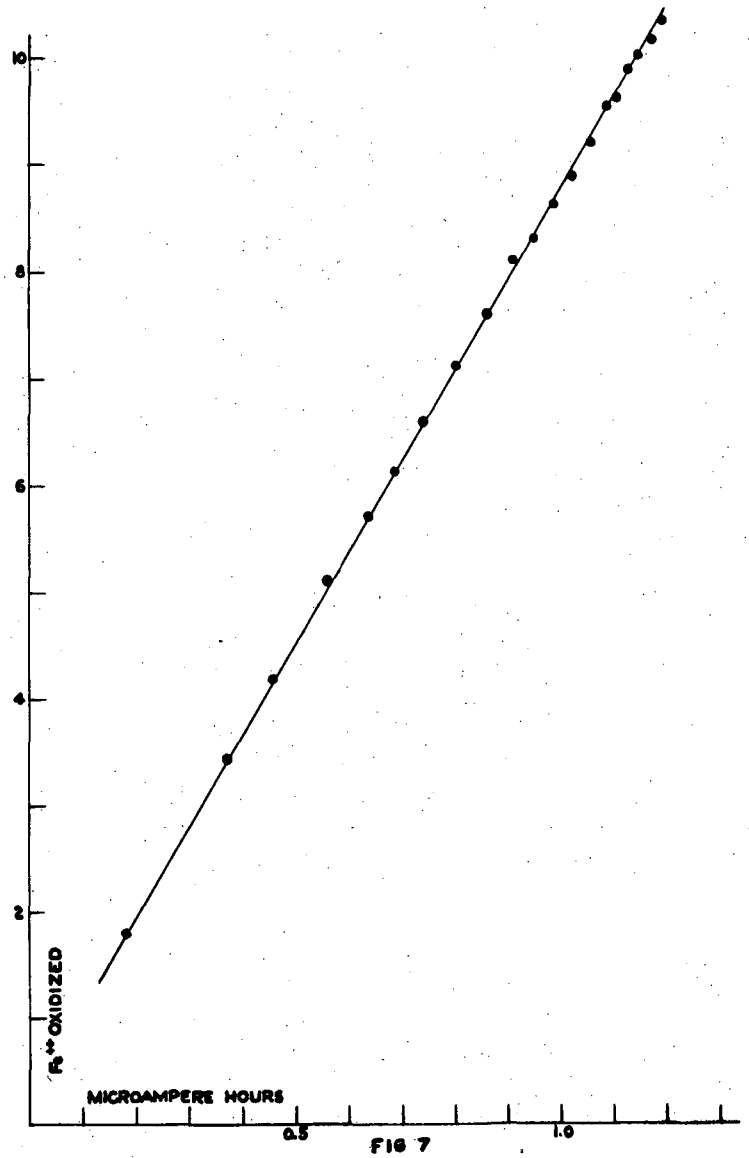

FIG 7

MU 2284

II BIOLOGICAL STUDIES OF RADIATION EFFECTS

J. H. Lawrence - in charge

Project 48A-1

Macrophage Migration in Experimental Atherosclerosis

John H. Simonton and John W. Gofman*

The source of the lipid material occurring in atheromatous lesions has been attributed to: (a) local fatty changes; (b) movement of lipid-bearing blood macromolecules into the arterial intima; (c) movement into the intima of lipid-filled cells which have originated elsewhere in the body.[1]

The present study represents an attempt to evaluate the last hypothesis, specifically with reference to the migration of Kupffer cells from the liver through the circulation to the aorta.

Means of concentrating radioisotopes in liver, spleen and bone marrow were available to the authors in this laboratory.[2] It was strongly suspected that the colloidal vehicle employed was taken up by the cells of the reticuloendothelial system existing in these tissues. That this is, in fact, the case has been shown in the present work. If a representative number of liver macrophages were thus labeled, and if they migrated to the aorta during the course of cholesterol feeding to rabbits, then a relatively great increase in radioactivity would be expected to appear in the aortic plaques. Conversely, if the activity in the diseased aortae of cholesterol-fed rabbits failed to exceed that appearing in control animals, it was felt that strong evidence against macrophage migration, as a mechanism in this disease, would have been adduced.

Methods. The production and morbid anatomy of cholesterol-induced rabbit atherosclerosis has been so thoroughly described in the literature as to require little repetition here.

Young white rabbits ranging in weight from 2035 to 3460 grams were divided into two groups, both of which received the radioactive isotopes, while only one was fed cholesterol.

Albers Rabbit Family Ration was ground, mixed with crystalline cholesterol, and the resulting powder made into tablets. Calcium stearate and powdered sugar were added in small amounts to improve the cohesiveness of the tablets. This special diet was adminstered on alternate days so that each animal received an estimated three grams of cholesterol per week. After a one or two day period of sluggish eating, the rabbits were found to consume the tablets readily. The special diet was supplemented with the stock chow, carrots, greens, water and salt ad lib. All the animals were found to gain weight on this regime. No external signs of cholesterol toxicity

* This work was supported in part by the Atomic Energy Commission and the U. S. Public Health Service.

were observed over the experimental period.

The control animals received an identical diet with the exception of the Albers-cholesterol mixture.

Thorium 230 (Ionium*) was used as the tracing isotope. It was injected intravenously in a colloidal citrate-hydroxy vehicle[2] previously employed in localizing certain other radioisotopes in liver, spleen and marrow.[3]

Five of the animals received their colloid in divided doses in order to label a maximum number of macrophages.

The distribution of the radioactivity was determined by autographic methods and checked by radioassay of the critical organs. The animals were sacrificed at intervals determined by the length and amount of cholesterol feeding and by the state of the serum.

Samples of liver, spleen, marrow, lung, adrenal, aorta, and when present, atheroma, were taken from each animal and imprint-preparations made upon nuclear track plates.** These plates were thus exposed to the alpha radiation present in the tissue and developed at successive intervals of time. (The number of tracks present will increase with time following the basic radioactive decay law.) After development, rinsing, fixing, and washing of the plates, the tissue adhering to the emulsion surface was stained either with Jenner-Giemsa or with Oil Red O and Harris Hematoxylin, and the plate examined microscopically.

The alpha tracks were manifested as short, linear rows of exposed silver grains occurring as occasional tracks or dense bursts. The rays are emitted from the parent atoms through a 4π solid angle and for a reasonably complete count it is necessary to observe several focal planes through the emulsion.

This technic has the advantage of a minimum of steps during which radioactivity might be lost, and the all-important advantage that many cells will be sufficiently removed from their neighbors so that precise localization of the alpha-bursts can be made. It has the disadvantages of some loss of tissue in the development process as well as some deterioration of tissue during the weeks of exposure prior to fixation and staining. Clumping of tissue occurs also, so that dense, unresolvable masses of cells and tracks are found. However, it was felt for the purposes of this experiment that the advantages of the method outweighed its deficiencies.

Ordinary tissue radio-autographs made by exposing histologic sections to the emulsion were tried but it was found that cells were too closely packed for definite matching with emitted alpha tracks.

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* Ionium: a radioactive isotope of half-life 8.3×10^4 years, emitting alpha particles of 4.66 Mev, and having a range in air of 2.85 cm (in the NTA-plate emulsion, of about 20 microns).

** Kodak-NTA: emulsion thickness - 25 microns; surface area - 1 x 3 inches; recording alpha-particles, protons and deuterons.

Tissues surviving best through photographic development were the densely cellular ones: liver, spleen and marrow. The normal architecture of lung alveoli and aortic intima was not preserved in the imprint preparations. Attempts were made to obtain isolated foam cells but a reproducible technic was not found.

Fatty material was expressed through razor cuts made into fresh aortic plaques and deposited upon the emulsion surfaces of NTA plates. This material subsequent to the development of the plate was stained with oil Red O and Harris Hematoxylin, and studied for associated alpha tracks.

Formalin-fixed aortae were cut on the freezing microtome into strips five to seven microns thick. These were floated upon the NTA plates which were then allowed to expose. The formalin in which the tissue was fixed and the water in which the strips were floated were tested and found to contain a negligible amount of radioactivity. These "strip" preparations took a fat stain and retained sufficient structure to permit identification of plaque areas and to serve as a check on the direct imprint preparations.

Routine counts were made of the tracks occurring on liver and aorta plates. Typical results are recorded in Tables II and III.

Samples of six organs were removed from each animal, weighed, wet-ashed, reduced to volume, and an aliquot mounted on platinum discs and assayed for alpha radioactivity. Because of the limited amount of diseased aorta it was not practicable to assay pure plaques; therefore, the figures reported for aorta represent parts rich in (60 to 80 percent of the intimal surface) but not exclusively made up of atheromas.

Each animal received between one and two cc of India ink within the last twenty-four hours of survival. This facilitated the identification of macrophages and incidentally demonstrated that phogocytic function of the macrophages had not been impaired by the presence of radioactivity throughout the feeding period.

An occasional liver-imprinted NTA plate was fat-stained. While detail was poor, sufficient structure remained so that cord and Kupffer cells could be distinguished and cytoplasmic sudanophilism of both cell types observed. Fat-stained histologic sections of livers from each of the atheromatous animals were made and showed fatty cellular changes and the presence of fat and India Ink within the same Kupffer cells. Almost every Kupffer cell in a given field appeared to be fat and ink-laden.

Discussion. We have found no evidence in microscopic preparations of aortic atheromatous tissue of the presence of "labeled" Kupffer cells, or of other labeled macrophages from spleen or bone marrow. The "label" consisted of the presence of bursts of three or more alpha tracks radiating from a central intracellular area. Radio-autographs of splenic phagocytes showed an activity of the order of that associated with liver macrophages, while those of marrow macrophages exhibited considerably more.

The scattered single alpha tracks observed in the aorta preparations, as well as the occasional bursts of three, four or five tracks, were found

to be strikingly fewer than those found in liver imprint preparations. If plaque tissue arises entirely or largely from migrated macrophages, we would be justified in expecting an activity greater, per gram of tissue, than that of the liver. It seems more probable that what little radioactivity is observed microscopically in aortic preparations and assayed in samples of aortic tissue represents phagocytosis of the colloidal vehicle directly from the blood stream by aortic cells.

The possibility cannot be ruled out that "labeled" Kupffer cells may have undergone necrosis during the course of the experiment and their contents been scavenged by other Kupffer cells. However, if these macrophages are stimulated by a continuous excess of liver cholesterol to release themselves into the blood stream and to migrate to the arterial intima, the presence of such "late"-labeled Kupffer cells might reasonably be expected in plaque areas.

Total liver radiation which the animals received over the four to six month experimental period, ranged from 0.25 reps to 0.5 reps.* Because of their concentration of the isotope, liver macrophages would, on the average, receive a dose of the order of 10 reps for the experimental period. However, macrophages showing far more than ten tracks were still capable of engulfing India Ink at the end of the experimental period indicating that their phagocytic function at least was not impaired by the radiation itself.

Kuntz and Sulkin[4], injecting hypercholesterolemic rabbits with India Ink and lithium carmine, found the dyes in visceral phagocytes but none in the foam cells of the atherosclerotic lesions. However, we felt that it was of prime importance to introduce a macrophage-labeling agent prior to the inception of cholesterol feeding in order to expose these cells to the processes of cholesterol metabolism. Thus, a foam cell already imbedded in the atheromatous lesion might be a phagocyte of extra-arterial origin but yet be unable to get access to the circulating dye once within the arterial intima.

Summary. In order to test the macrophage-transport hypothesis of atheromatosis, rabbits were injected with a radioisotope which was demonstrated to concentrate in visceral macrophages. Cholesterol feeding was then instituted. At the end of the feeding period, the animals were sacrificed and the distribution of radioactivity between various organs and the atheromatous plaques studied. It was found that the amount of radioactivity in the plaques was relatively very low. It is felt that this indicates macrophage migration is not a significant source of the foam cells in rabbit atherosclerotic lesions.

Acknowledgment. The authors wish to express their gratitude to Miss Anita Syrek for her valuable assistance in preparing tissues.

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* Roentgen Equivalent Physical

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TABLE I

GENERAL DATA

No.	Weights (gm) Initial	Weights (gm) Terminal	Est'd. Cholesterol Intake	Duration Feeding Period	Serum Total Cholesterol Levels Initial	Serum Total Cholesterol Levels Terminal	Dose of Io	Severity of Lesions
50	3385	4985	88½ gms	210 days	19 mg%	230 mg%	1/4 cc	Minimal
51	3460	4550	73 "	190 "	63 "	720 "	1/2 "	Moderate
52	3950	4725	111 "	235 "	23.5 "	--	1/2 "	Minimal
53	2900	4365	69 "	149 "	22 "	467 "	1/4 "	Moderate
55	2620	4200	65 "	150 "	57 "	770 "	1/4 "	Severe
78	2815	4670	61 "	147 "	--	368 "	1/4 "	Moderate
54	2660	4790	87½ "	208 "	22 "	109 "	1/2 "	None
56	3120	5485	0 "	214 "	--	--	1/4 "	Control
57	2035	3925	0 "	122 "	--	--	1/4 "	Control

TABLE II

NTA Plates:

Liver - Imprint Preparations

No.	Kupffer cells with burst of 3 or more 'a' tracks	Mean Number of Tracks/Cell	No. of Cells Counted	Exposure Time
51	79%	10 (range: 0-43)	226	53 days
52	80%	14 (range: 0-60)	151	31 days
53	87%	10 (range: 0-38)	158	53 days
55	84%	12 (range: 0-60)	135	74 days
78	71%	5 (range: 0-50)	125	25 days
56	81%	10 (range: 0-48)	70	51 days
57	84%	12 (range: 0-70)	113	36 days

Explanation: (1) 56 and 57 are controls; they received the radio-isotope but no colesterol.

TABLE III

NTA Plates: Aortic Imprint Preparations

No.	Tissue Areas*	Maximum Burst No. of Tracks	-	Occurrence	Exposure Time
51	Alpha tract density within Bg** limits	5		once	59 days
53	Alpha track density within Bg limits	1		four times	53 days
55	"	3		once	67 days
78	"	4		once	34 days
56	"***	4		once	51 days
57	"	3		twice	36 days

*These areas were stained with Oil Red O and Harris Hematoxylin. Cellular elements were frequently observed but definition was not considered satisfactory.

**Background limits: the number of alpha tracks observed on oil-power scan of blank slides ranged from 24 to 49.

***56 and 57 received the radio-isotope only.

TABLE IV

NTA Plates: Aortic "Strip" Preparations

No.	Plaque Area*	Alpha Tracks Total	Alpha Tracks Bursts	Exposure Time
51	a	1	none	22 days
	b	7	none	22 "
	c	15	3-once	41 "
	d	6	none	46 "
	e	19	2-twice	46 "
	f	10	none	53 "
	g	16	2-twice	53 "
52	a	44	2-three times	36 "
	b	32	2-once	36 "
78	a	10	2-once	36 "
	b	2	none	36 "
	c	6	none	28 "
	d	9	none	28 "
	e	7	2-once	57 "
	f	4	none	57 "
	g	6	none	57 "

* These plaques varied in length from 1/4 to 1/64 inches. Thus a plaque with longer exposure time might show fewer total tracks than a plaque with a shorter exposure but greater area. These plaques were studied under oil-immersion magnification. The track density in the subjacent media was of the order of that of the intima.

TABLE V

Specific Activity (% of injected dose per gram of tissue)

No.	Aorta	Liver	Lung	Adrenal	Marrow	Spleen
50	0.012 ± .003	0.10 ± .01	0.016 ± .006	0.008 ± .002	0.21 ± .03	0.12 ± .02
51	Bg*	0.42 ± .01	0.007 ± .002	0.005 ± .002	0.11 ± .03	0.085± .005
53	0.009 ± .002	0.12 ± .02	0.029 ± .003	0.014 ± .003	0.33 ± .10	0.21 ± .01
54	0.013 ± .001	0.13 ± .01	0.010 ± .002	0.013 ± .002	0.59 ± .04	0.32 ± .03
56	Bg*	0.09 ± .01	0.007 ± .002	0.016 ± .004	0.19 ± .03	0.11 ± .01
57	0.022 ± .007	0.35 ± .02	0.11 ± .01	0.089 ± .015	0.90 ± .18	0.52 ± .06

* Background: in these two instances, the radioactivity of the samples was so low as not to differ significantly from "background" radiation.

Explanations:
1) Nos. 50, 51, 53, 54 were fed cholesterol.
2) Nos. 50, 51, and 53 developed gross lesions. 54 failed to develop gross lesions.
3) Nos. 56, 57 received the radio-isotope only.

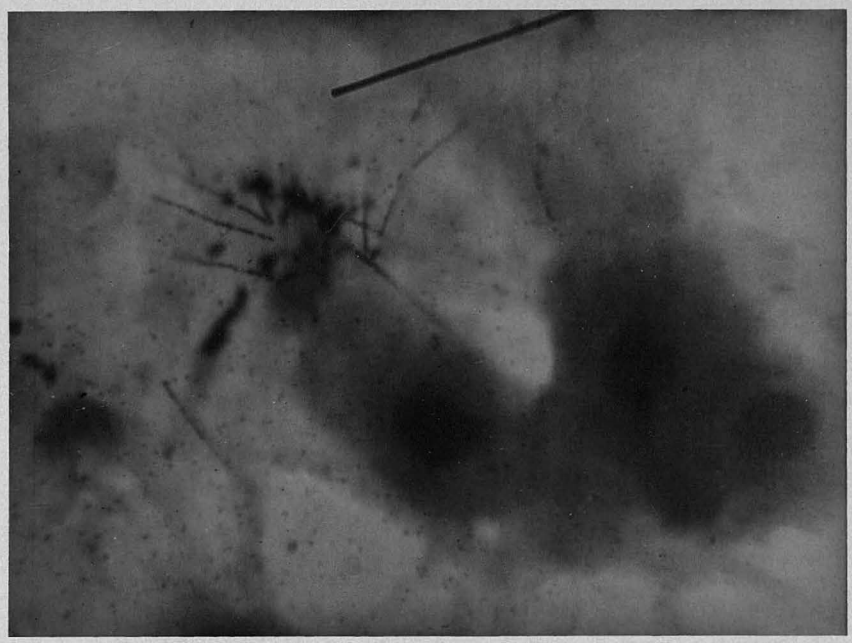

FIG. 1 - PHOTOMICROGRAPH MADE FROM A LIVER-IMPRINT UPON AN NTA PLATE. NOTE HEPATIC CORD CELLS AND ADJACENT KUPFFER CELL WITH ITS CONCENTRATION OF ALPHA TRACKS. THIS PLATE WAS EXPOSED SEVEN DAYS; STAINED WITH JENNER-GIEMSA. OIL EMERSION. RABBIT. 58

FIG. 2 - THREE CORD CELLS ARE SHOWN WITH A MACROPHAGE. NOTE "BEADS" OF INDIA INK BESIDE THE NUCLEUS OF THE MACROPHAGE. EXPOSED FOR 46 DAYS; STAINED WITH JENNER - GIEMSA. RABBIT 51

OZ 1099

FIG. 3 - THE SAME LIVER CELLS SHOWN IN FIG. 2, BUT PHOTOGRAPHED IN A DEEPER FOCAL PLANE. NOTE α TRACKS.

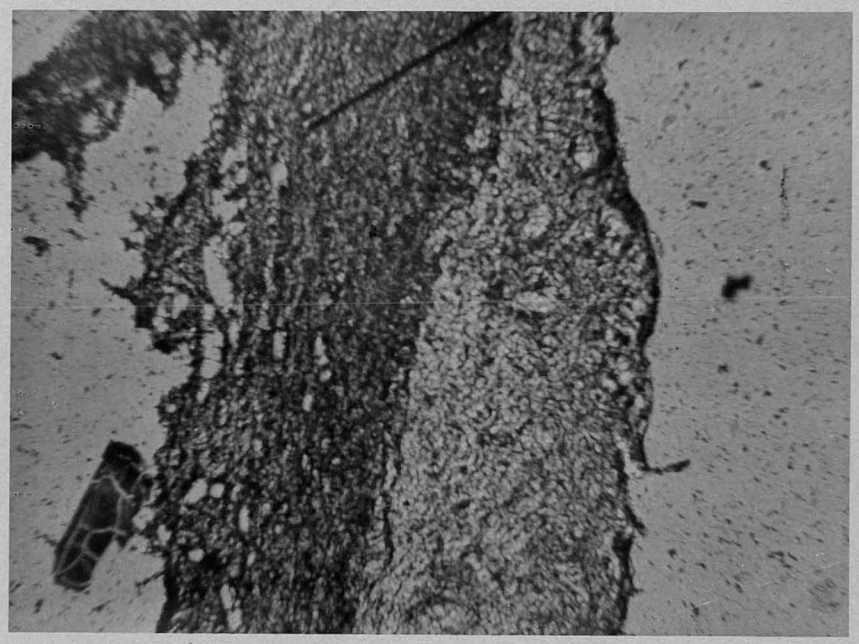

FIG. 4- A "STRIP" (SEE TEXT) PREPARATION MOUNTED ON AN NTA PLATE. PLAQUE TISSUE IS VISIBLE ABOVE THE MEDIA. OIL RED O AND HARRIS HEMATOXYLIN. 27 DAYS EXPOSURE. LOW POWER, RABBIT 78.

0Z1200

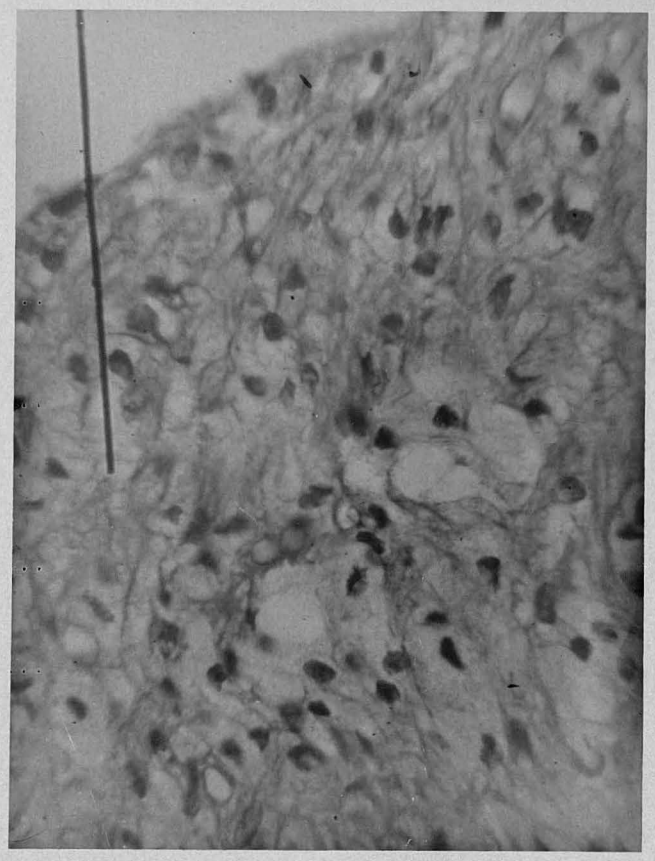

FIG. 5- HISTOLOGIC SECTION MADE THROUGH PLAQUE TISSUE CONTIGUOUS TO THAT USED FOR THE STRIP PREPARATION SHOWN IN FIG. 4. NOTE FOAM CELLS. HEMATOXYLIN AND TRIOSIN. HIGH POWER. RABBIT 78.

OZ 1201

Radiation Hazards in High Altitude Aviation

C. A. Tobias

An assay has been made of the daily dose in cosmic radiation near the top of the atmosphere. Even though a great part of the radiation is in the form of nuclei, heavier than helium, due to their high velocity the contribution to biological effects is expected to be small. Table VI gives conservative estimates of dose per 24 hours at medium geomagnetic latitudes, at the top of the atmosphere.

Somewhat less than one half of the dose is due to the "heavy" particles; the rest of it, about 6 milliergs per day is chiefly due to protons and helium ions which are near their minimum ionization.

By assuming factors in each region of rate of energy loss for the "relative" biological effectiveness, one can arrive at a tentative figure of cosmic ray dosage near the top of the atmosphere in REM units as shown in Table VII.

It would appear that the overall daily dose is in the neighborhood of the accepted "safe" dose level. In spite of this reassuring finding, it is hard to predict the exact biological effects of the cosmic ray nuclei, particularly when certain specific effects are considered. Among the unknown factors are: possible carcinogenic effects, possible induction of leukemia, effect of radiation on nerve tissue (e.g. vision), radiation cataracts. It seems unlikely, however, that any such effects would result.

TABLE VI

CONTRIBUTION TO DAILY DOSE IN COSMIC RADIATION

Atomic Number Z > 6 Only

Rate of Energy Loss in Time $erg^{1}cm^{2}$		Fraction of Energy Spent	Contribution to Daily Dose Millirep/day	Nature of Biological Effects
smaller than	larger than			
10^8	$1.7 \cdot 10^6$	18%	0.8	Similar to those of X-rays.
10^9	10^8	65%	3	Similar to fast neutron effects from pile.
10^{10}	10^9	13%	0.6	Similar to low energy alphas and carbon nuclei.
	10^{10}	4%	0.2	

TABLE VII

24 HOUR DOSE IN COSMIC RAYS NEAR TOP OF THE ATMOSPHERE

(all rays included)

REM units

Rate of energy loss $erg^{-1}cm^2$		Dose Millirep/day	Range of Relative Biological Effectiveness Experimental	Relative Biological Effectiveness Assumed	Dose Millirem per day
smaller than	larger than				
10^8	$1.7 \cdot 10^6$	6.2	0.5-3	1	6
10^9	10^8	3	0.5-14	10	30
10^{10}	10^9	0.6	0.3-25	25	15
	10^{10}	0.2	? 25 ?	100	20
Total		10			71

The Influence of Heparin Upon Lipoprotein Metabolism

Dean Graham, John W. Gofman, and Hardin Jones

The demonstration of the existence of certain special classes of lipoprotein molecules associated with atherosclerosis in several experimental animal species and in the human developing or manifesting atherosclerosis has been previously reported.

Further study of these lipoprotein molecules associated with atherosclerosis as well as several other lipoprotein species has revealed that essentially all of the blood lipids (including fat, cholesterol, cholesterol esters, and phospholipids) exist in the blood only in the form of structural entities within several of these lipoprotein molecular classes. Current work is directed toward an understanding of the factors involved in the defect which results in the abnormal elevation of such molecules as the S_f10-20 lipoproteins in certain individuals. Each lipoprotein, which can be identified in its native state by the ultracentrifugal analysis of serum, presumably has a different functional role in lipid metabolism. Its concentration in the blood is the resultant balance between formation and utilization at any particular steady state of physiological activity. Physiologic factors already reported to influence serum lipoprotein levels include (a) dietary intake of fats and cholesterol, (b) thyroid function, (c) experimental adrenal cortical hyperactivity (rabbit only), (d) age, (e) sex, (f) pregnancy. Several errors in lipid metabolism have now been classified by the ultracentrifugal analysis of serum lipoproteins. Such are manifest in (a) atherosclerotic state (b) nephrosis (c) xanthoma tuberosum (d) biliary obstruction (e) acute hepatitis (f) certain cases of diabetes (g) hypothyroidism (h) hypercholesterolemia (i) xanthelasma. In addition, there are certain bizarre, but characteristic ultracentrifugal lipoprotein patterns which are seen in presumably normal individuals, but are as yet unclassified.

All individuals show some of the lipoprotein species in the serum. The individual variations encountered are derived from two variables, (a) the number of different lipoprotein species present, (b) the relative abundance of the different lipoproteins in a single serum. A variety of evidence suggests that the typical "normal" of human beings shows one or more lipoprotein species in the range up to 6 S_f units (including the S_f 2, S_f4, S_f 6 lipoproteins). An analogous situation exists in the rabbit, chicken and dog, with minor differences in the S_f rate of the normally occurring lipoproteins. In atherosclerosis and certain other lipid metabolic derangements there exist in addition varying concentrations of lipoproteins in the classes S_f 8, S_f 10, S_f 13, S_f 17, S_f 17-20, S_f 20-40, S_f 40-40,000. In the range S_f 8-S_f 40, the higher members of the group of lipoproteins make their appearance in serum only when appreciable levels of all lower members are present. However, the relative abundances of these various lipoproteins are variable from one disease category to another. In the range S_f 40-40,000 there are also characteristic patterns associated with disease states, but lipoproteins of the S_f 40-40,000 class also represent part of the alimentary "lipemia" of normals. Development of atherosclerosis in the human as well as other species is nearly always assocated with elevation of the S_f 10-30 range of lipoproteins. However, at least certain of the higher members may also be present in this disease,

but as the variability in concentration in a given individual approaches the variability of groups, they have not been used as a correlative guide.

As an extension of studies of the factors involved in the maintenance of the levels of the various serum lipoproteins, it has now been found that heparin can produce a profound alteration in the lipoprotein pattern in a manner which may prove of value in the further understanding of atherosclerosis. Heparin, of course, has long been used clinically in the therapy of thromboembolic disease, but not for any possible influence it might have upon metabolic factors leading to atherosclerosis.

Hahn reported that heparin abolished alimentary lipemia in vivo, but not in vitro. Weld showed that the phenomenon is not restricted to any particular vascular bed by perfusing various body regions with heparinized lipemic plasma. Recently, Anderson has shown that the turbidity of lipemic plasma may be cleared in vitro by mixing lipemic plasma drawn before heparinization with that drawn five minutes after heparinization. He hypothesized an "anti-chylomicronemic" factor which results from heparin injection and discussed the possibility of a heparin-phospholipid complex being a surface active agent responsible for the clearing of lipemic plasma. Waldron reported that other anticoagulants (sulfonated polysaccharides and protamine fast pink B.L.) produced effects similar to heparin, and furthermore, that heparin enhances the absorption of fats from the intestine as evidenced by lipemia production.

Experimental Results. A single injection of sodium heparin intravenously in the cholesterol-fed rabbit produces marked changes in the lipoprotein spectrum, characterized in general by a decrease in concentration of molecules of the high S_f classes, with a concomitant increase in concentration in those of the lower S_f classes.

The association of these two changes suggests a progressive conversion of the higher S_f lipoproteins into those of lower S_f rates. An immediate response is observed within 5 minutes in the lipoproteins of the highest S_f classes, with successive changes in those of the lower S_f classes, with maximum effect being observed at about three hours. Subsequently the pattern "reverts" to its initial state in approximately 24 hours.

To obtain some evaluation of chronic effects of heparin administration, a series of eight rabbits were fed one gram of cholesterol dissolved in Wesson Oil in their Albers food and divided into two groups. One group of four animals received daily I.V. injections of fifteen mg of heparin, the other group received no heparin. The four animals receiving heparin showed levels of the S_f 10-50 class of lipoproteins three to six times lower than did three of the four control animals. One of the control animals was of the "refractory" type, developing only low levels of the S_f 10-50 class in spite of cholesterol feeding. The heparinized and control animals were paired and autopsied between 2-½ and 4 weeks. The four heparinized animals failed to develop gross atherosclerosis, whereas three of the four non-heparinized animals showed moderate atherosclerosis. The one non-heparinized animal which failed to develop atherosclerosis had also failed to develop appreciable level of the S_f 10-50 class of lipoproteins. Thus it appears that

the effect of heparin in depressing the development of the S_f 10-50 lipoproteins is associated with a suppression of atherosclerosis.

Rabbits refractory to development of S_f 10-50 lipoproteins and atherosclerosis under comparable experimental conditions, are seen in our laboratory experience with a frequency no greater than 1 in 20. Thus it appears that the chance of seeing four such rabbits in this single group to which heparin had been administered is beyond reasonable expectation of chance ($p = < 0.01$). This effect is being studied further varying heparin dosage and fat and cholesterol feeding.

In the human being, even more striking effects are seen following heparin administration. In Fig. 6 is shown the progressive changes in the spectrum of lipoproteins following intravenous injection of 100 mg of sodium heparin. This patient, a myocardial infarction survivor, showed initially high levels of lipoproteins in the S_f 10-20 class, as well as in the S_f 20-100 class. Within 15 minutes there has been essentially a "wipe-out" of lipoproteins from S_f 20-S_f 100, associated with an <u>increase</u> in the concentration of the S_f 10-20 class. Then during the next 6 hours there has been an overall shift within the S_f 10-20 class, such that the S_f 12-20 lipoproteins are markedly reduced in concentration below their original level, while the S_f 10-12 and S_f 6-10 lipoproteins have increased in concentration over the initial level. Progressively over a period of 24 hours the entire pattern reverted toward the initial pattern, although there was still a lower S_f 12-20 level than initially. This same general effect has been observed in each of thirty subjects (normals and myocardial infarctions) studied in this manner, the only variations observed being in the degree of response and in the time relationships of the sequence of events which occur. Dosages ranging from 15 mg - 100 mg of sodium heparin I.V. have proven definitely effective, although graded dosage in a single patient requires further evaluation.

<u>In vitro</u> attempts to alter the lipoprotein pattern by heparin addition, have failed in all of numerous trials. However, samples of plasma from heparinized patients have been found effective in altering the lipoprotein spectrum of serum drawn from the same individuals before heparin administration or of serum of other individuals. Thus it appears that the injection of heparin results in the <u>in vivo</u> production of an "active principle" capable of producing these conversions of lipoproteins <u>in vitro</u>. This result is similar to the studies of the "anti-chylomicronemic" factor of Anderson. In an effort to elucidate the mechanism of the effect on lipoproteins, we have found that the "active principle" appears to reside in the ultracentrifugal globulin region. The serum albumin fraction and the low density lipoprotein group both appear inactive. Globulin fractions from post-heparin plasma when incubated with earlier post-heparin plasma appear to cause further changes in the lipoprotein distribution in such plasma <u>in vitro</u>.

As yet no "active principle" in serum has been detected from individuals who have not received heparin, although it is conceivable such a factor may circulate at low concentration.

In an effort to determine the effect of a maintained heparin level on the serum lipoprotein pattern some studies have been made of individuals receiving repository heparin (R)*. Fig. 7 shows the effect of heparin repository

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* R - Lederle Repository Heparin.

in the same patient as studied in Fig. 6 with I.V. heparin. It is noted that in 4 hours with repository heparin, this patient has lost essentially all of her lipoproteins above S_f 10 units, representing in essence a reversion to a "normal" lipoprotein pattern. After 24 hours there has been a partial return of S_f 10-20 molecules (50 percent), but a much lower fractional reappearance in the S_f 20-100 class of molecules. (Even after 2 and 4 days there is a significant depression of the S_f 10-20 level.)

A series of twenty patients have received intravenous heparin at intervals varying from 2-14 days. There appeared to be no reduction of ability of patients to show the response described in Fig. 6, even after numerous heparin injections. A small proportion of the patients showed depression in S_f 10-20 levels which persisted for the full 3 to 14 day intervals between I.V. injections of heparin, but in general the levels observed 3-14 days after injection showed no average trend toward reduction. In this particular series samples were drawn throughout just prior to each new heparin injection. From what has been observed in the 24 hour period following such injections, we may anticipate that averaged over the entire period between injections, the S_f 10-20 level may have been appreciably lower than its pre-heparin value. In one patient studied over a one month period, who had shown a maintained S_f 10-20 depression for the 3 day period between heparin injections, showed a slow progressive rise approaching his original S_f 10-20 levels over a one month period after cessation of heparin injections.

An incidental and unexpected clinical observation made by Lyon and Yankley in the patients receiving repeated heparin injections was that 22 patients with moderate or severe angina pectoris reported marked relief from this symptom with a drastic decrease in nitroglycerin requirement. Reports on the effect of heparin in relieving pain of myocardial infarction and as a coronary vasodilator have appeared in the literature. However, the presently reported responses in angina have been observed with small single doses of heparin (20-100 mg) given at intervals of several days. All of seven patients whose severe angina had been relieved by the heparin injections complained of return of angina when saline placeboes were injected instead of heparin. Most of the patients in this series were on a partially fat-restricted diet before and during the heparin administration period. However, the immediate and dramatic effect of heparin was in contrast to the slower and less marked reduction in angina accompanying the dieting itself. However six of the patients who responded well to heparin had not been on a restricted diet.

We are cognizant of the many difficulties in evaluating objectively efficacy of drug relief of angina. However, the striking character of the response in patients with relatively fixed anginal patterns and nitroglycerin requirements, plus the loss of response when saline placeboes were used would appear to militate against the response being in any way psychogenically determined. Extended studies of this response are in progress now. We are not able at this time to provide any suggestion of a possible relation of the heparin effect on blood lipoproteins to that in relieving angina pectoris, although such may be the case.

Discussion. Heparin appears to act profoundly and rapidly in altering the blood lipoprotein spectrum. Shifts among the lipoproteins are observed both

in the human and the rabbit from molecules of the higher S_f classes to those of successively lower classes in times of the order of minutes to hours following a single heparin injection. The decrease in concentration of a particular S_f range of molecules, accompanied by an increase in the concentration of molecules in the next lower S_f classes suggests that the former molecules may be actually transformed into the latter by the influence of heparin. This appears to occur in several successive stages over a period of hours. This represents one of the first clues on the possible interrelationships of the various classes of lipoproteins of serum. Inasmuch as the lipoproteins of the S_f 17-100 classes represent in the human the major glyceryl ester (fat) bearers of serum, the observed interconversion accentuated by heparin, may represent steps in the normal pathway of transport and metabolism of fat. It is appropriate to consider the possibility that heparin itself, or some substance of similar properties, may normally be involved in the physiological interconversion of lipoproteins. Thus, in individuals who usually show high levels of S_f 10-20 and S_f 20-100 lipoproteins there may be a blockage in the utilization pathways of such molecules (possibly due to a deficiency of heparin-like substance), such that a piling up in concentration of such molecules occurs in the blood. In supposedly normal individuals (especially young adults and children of both sexes) all these molecules, if present, are in very low concentration, which would be expected if utilization pathways were greatly facilitated in these individuals.

As reported by Anderson for the in vitro clearing of alimentary lipemia by in vivo heparinized plasma, the present work indicates that heparin in free form does not directly induce any alteration in lipoprotein spectrum. However, plasma from heparinized patients contains a factor that is effective. This factor resides in the ultracentrifugally determined globulin fraction and induces changes in vitro which simulate, at least in part, the changes in lipoproteins which follow in vivo administration of heparin. Further search for such an "active factor" in the sera of "normal" individuals who show low levels of S_f 10-100 lipoproteins appears warranted. If present, such a factor might be anticipated at very low concentration, from the negative results of our searches for it to date. Again, such a factor might not normally reside in appreciable concentration in plasma, but might be called forth in response to lipid loading.

Heparin administered to rabbits apparently protects the animals from development of atherosclerosis under circumstances which otherwise induce atherosclerosis. Since the protective effect of heparin is accompanied by a suppression of development of high levels of the S_f 10-50 lipoproteins, the observation strengthens the previously reported evidence linking these molecules with atherosclerosis, and further suggests the value of maintaining low levels of these molecules in prevention of experimental atherosclerosis. The effect of lowering the similar classes of molecules in the human on progression of the clinical manifestations of atherosclerosis is being evaluated.

The dramatic relief of angina pectoris by intermittant heparin administration parallels the profound effect of this substance upon lipoprotein metabolism. However, at this time we have been unable to demonstrate

that these two simultaneous effects are actually related. It may be that the relief heparin provides in angina pectoris due to its vasodilator or to its anti-thrombotic activity. The effect on angina pectoris is seen with small doses of heparin and persists much longer than the anti-coagulant effect of the doses used. Currently under study are the effects of heparin in several diseases which are marked by extreme elevation of lipoproteins of the S_f 10-100 classes (e.g., nephrotic syndrome, hypothyroidism and xanthoma tuberosum).

Summary. 1. Heparin administered to rabbits and human beings causes profound reorientation in the distribution of low-density lipoproteins, characterized by a shift of lipoproteins of high S_f rates to those of successively lower S_f rates.

2. Heparin administered to cholesterol-fed rabbits prevents the development of high levels of S_f 10-50 lipoproteins and retards the development of atherosclerosis in such animals.

3. Following heparin administration the plasma contains an "active principle" associated with the ultracentrifugal globulins, which produces similar re-orientation of the lipoprotein spectrum in vitro.

4. Heparin added directly to serum is ineffective in vitro.

5. Intermittent heparin administration to patients with severe angina pectoris results in relief from symptom for periods of several days beyond a single injection.

LEGEND for Figure 6 a and b

The Effect of a Single Intravenous Injection of Heparin on Human Low-Density Lipoproteins

The accompanying ultracentrifugal flotation patterns show the progressive changes in the lipoproteins of a 63 year old female patient with coronary artery disease treated with a single 100 mg dose of heparin given intravenously.

In all the figures successive frames are taken at 0, 6, 12, 22, 30 and 38 minutes after full rotor speed of 52640 RPM has been reached. Frames 2 and 5 in Fig. 6 (b), lower pattern, for the calculation of the S_f rates of any peak appearing in these frames, respectively. These rulings can be used to calculate S_f rates in the corresponding frames of the other figures.

All stippled areas represent the measure of the S_f 20-100 class of lipoproteins; all cross-hatched areas represent the measure of the S_f 12-20 class of lipoproteins.

Figure	Time after heparin	S_f 12-20 (mg%)	S_f 20-100 (mg%)
6(a) upper pattern	Pre-heparin	167 mg%	530 mg%
6(a) lower pattern	20 minutes	239	50 (see note below)
6(b) upper pattern	6 hours	128	52
6(b) lower pattern	26 hours	143	344

Note: The sample recorded in 6(a) lower was ultracentrifugally concentrated only to 3/4 the level in the other samples. Hence the measured area from the diagram was corrected for this in preparing the tabulation of results.

It is seen that while the 6 hour post heparin sample shows an S_f 12-20 level not over 25 percent lower than the preheparin sample, there is a marked shift in distribution of the lipoproteins of the S_f 12-20 class toward the lower ranges of this class.

FIG. 6

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LEGEND for Figure 7 a through g

The Effect of Heparin Repository on Human Low-Density Lipoproteins

The accompanying ultracentrifugal flotation patterns show the progressive changes in the lipoproteins of a 63 year old female patient with coronary artery disease treated with a single 200 mg dose of repository heparin.

In all the figures successive frames are taken at 0, 6, 12, 22, 30 and 38 minutes after full rotor speed of 52,640 RPM has been reached. Frames 2 and 5 have been ruled in Fig. 7(d) for calculation of the S_f rates of any peak appearing in that frame. The ruling of Frame 2 in Fig. 7(d) may be used to calculate S_f rates in Frame 2 of any of the other figures. Similarly the ruling of Frame 5 in Fig. 7(d) may be used to calculate S_f rates in Frame 5 of any other figures.

All patterns shown represent two different sera run simultaneously in the ultracentrifuge. In each figure only the upper pattern is involved in this study. The lower pattern is from another individual and is to be completely disregarded.

All stippled areas represent the measure of the S_f 20-100 class of lipoproteins; all cross-hatched areas represent the measure of the S_f 12-20 class of lipoproteins.

Figure	Time after heparin	S_f 12-20 (mg%)	S_f 20-100 (mg%)
7(a)	Pre-heparin	197 mg%	357 mg%
7(b)	70 minutes	147	18
7(c)	3 hours	102	14
7(d)	4 hours	21	0
7(e)	6 hours	83	18
7(f)	24 hours	81	160
7(g)	72 hours	107	164

In addition the figures reveal a great increase in the concentration of lipoproteins of the S_f 6-10 and S_f10-12 classes accompanying the decrease in concentration of lipoproteins in the classes above S_f 12. The change in concentration of all lipoproteins between S_f 12 and S_f 100 in four hours after heparin administration is 533 mg percent. Assuming a plasma volume of approximately 2500 cc, this represents a minimum of 13 grams of lipoprotein cleared by the action of 200 mg of heparin in the repository form.

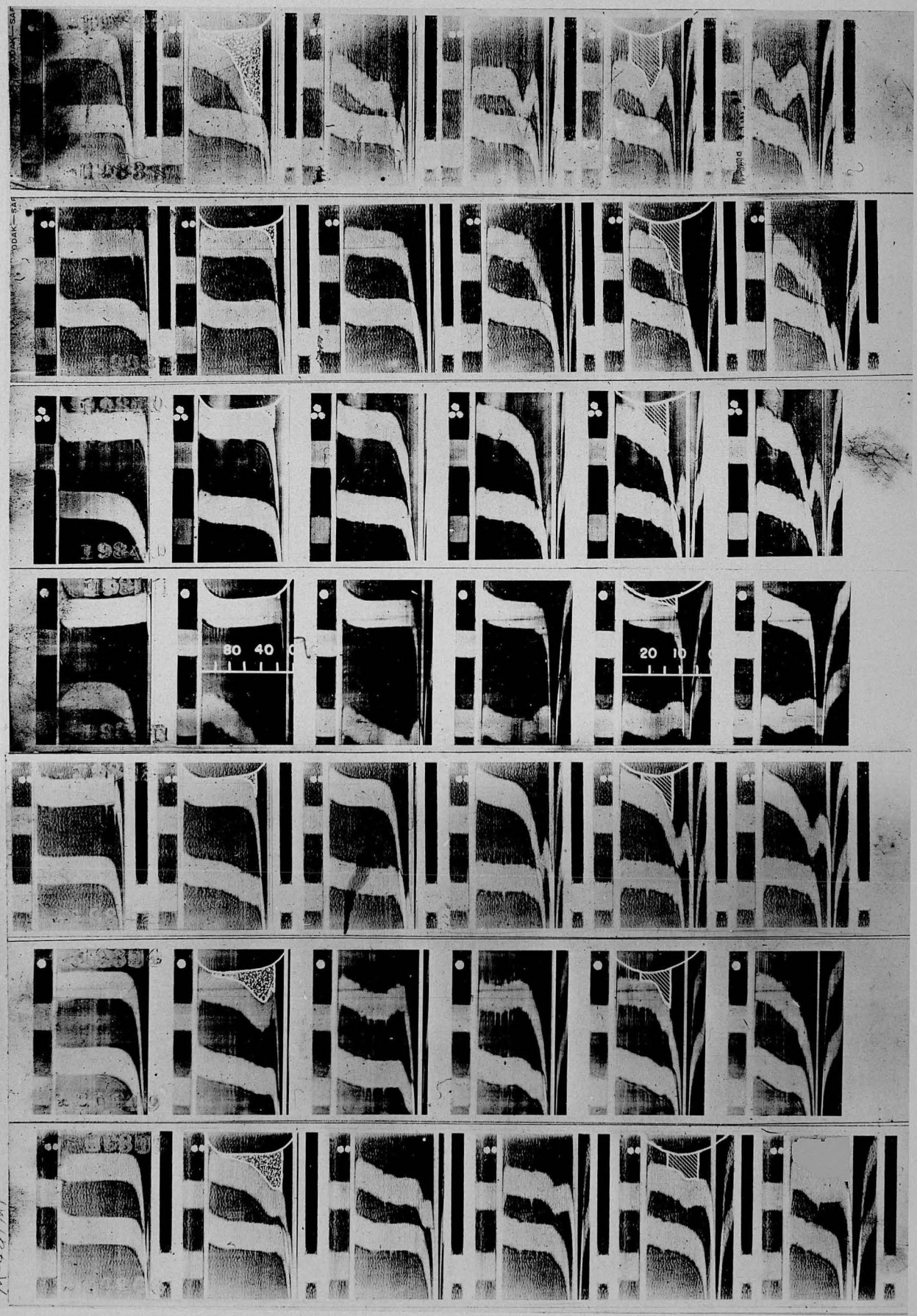

FIG. 7

III HEALTH CHEMISTRY AND PHYSICS

Health Chemistry

N. Garden

Research and Development Group. In addition to supplying gloved boxes and auxiliary equipment to chemists working with radioactive materials, the Research and Development group of Health Chemistry has adapted standard remote control equipment to a number of unusual situations in experimental work. This included the study of dissolving products of a bombarded thorium target in a liquid sodium-potassium alloy. It required a closed thermal syphon for recycling the liquid alloy. The setup had to be in an inert argon atmosphere behind two inches of lead shielding for personnel protection and required shielding between parts of the apparatus in order to provide a low background in the counting chamber, an integral part of the setup. Also created was equipment for analysis of pile-bombarded animal tissue, requiring a multiple resin ion exchange column system, with units at the same temperature (now in design stage); this is remotely controlled. In addition a shielded continuous solvent extractor, remotely controlled, to be used in bombarded iron-cobalt experiments has been made as well as liquid control valves and pumps made of teflon to combat corrosion in HF lines.

Activity Handling Group. The barge, towed by a navy tug boat, used for dumping cement and waste-filled drums at sea, has been improved by increasing its capacity. A shack for the crew has been placed on the barge to eliminate the transfer of personnel from tug boat to barge at the dumping grounds. The storage area for drums of waste and stock of empty drums has been enlarged. A new type of tong for transferring the cyclotron-bombarded targets from the target-carrying box to the chemist's working space has been built. It is a simple device with no moving parts and offers a full visibility to the target when it is being moved. It is now being used routinely with success. A new, simplified target-carrying box for transportation of all three types of targets bombarded on the 184-inch cyclotron has been built and is being used with success. Redesign of the increasingly popular "pistol-grip" target holder, used for bombardments on the 60-inch cyclotron, is complete; six have been ordered from the shops. A new water circulation system for cooling 60-inch targets containing large quantities of alpha emitters, greatly increases safety of bombardment of such targets. It also facilitates their handling. A new system whereby the entire target assembly from a 60-inch cyclotron bombardment is brought to the chemist's laboratory for disassembly has been put into effect. This removes the hazard of contamination to the cyclotron area should rupture or other malfunction take place during bombardment. The modified decontamination chamber, designed after more than a year of trial of the model chamber, is completely designed and will be under construction shortly.

Airborne Activity Control Group. Investigations made by the Airborne Activity Control Group include routine air samplings and filter changes in the laboratories as well as increasingly frequent mercury vapor surveys. A new detection device is being designed for this service. Studies are being made on the ammonia fume control in the drafting print room and a special air sampling study is

being done for the Atomic Energy Commission. A report has been made to Proceedings of the Harvard Air Cleaning Seminar. The 600 CFM emergency air cleaning unit has been used in a planned experiment for flame spraying hot targets with aluminum. The group has also procured CWS No.6 filters for use with protective masks for personnel. Vanes are now being used on all current gloved boxes for indication of ventilation air flow. Preliminary tests are being made on AAA "Fibreglas" Navy filter paper, which presents difficulties for routine use because of delicacy of the material. Testing is being continued.

During this period many members contributed their services to a session of the Radiological Civil Defense School, held at Pleasanton, California. Instruction in practical problems was provided to the State-wide student group attending.

Health Physics

B. J. Moyer

Fast Neutron Monitoring with Nuclear Plates. Ilford, C-2, 50 μ emulsion nuclear plates are being used as a supplementary method of measuring fast neutron fluxes around the 184-inch cyclotron. The recoil proton tracks formed when fast neutrons strike hydrogen atoms in the emulsion are observed with a microscope, the number of proton tracks per field of view being proportional to the number of fast neutrons passing though the emulsion. Twenty-five fields of view were observed on each plate, each field being 350 μ in diameter. The average number of recoil protons per field varied from 7.56 to 1.08. Corresponding percent probable errors are ± 24 percent and ± 67 percent. Standard plates were exposed to 10 weekly tolerance doses of fast neutrons* from a Po-Be source. To these standard plates were compared the monitor plates. An exposure to less than 2 or 3 weekly tolerances is indistinguishable from the background due to changes in humidity, sensitivity and statistical fluctuation; but exposures above this level agree fairly well with each other and with data taken with a proton recoil proportional counter.

Two examples are given in Table I.

TABLE I

Weekly tolerances compared with Po-Be**	Weekly tolerances measured by proton recoil counter	Location
8	10	South wall of deflected beam cave
6	8	Same, but with additional shielding.

Because the proton recoil counter is a rate measuring device while the Ilford plates integrate the exposure, comparisons are difficult.

The work is being continued and the possibility of using the nitrogen content of the emulsion to measure thermal neutrons by means of the $N^{14}(n,p)C^{14}$ reaction is being investigated.

The Brass BF_3 Filling System. The new brass BF_3 filling system and the simplified method of filling have proved satisfactory. Counters filled six months ago have retained their threshold and plateau characteristics. Using a standard 10,000 gain amplifier and a standard scaler with the discriminator set 5 volts above noise, the threshold is between 1300 and 1400 volts for a group of five counters. The counters, with the same operating conditions as above have a 200 volt plateau at 1600 volts.

* 86 Mev/cm^2/sec for 40 hours is 1 weekly tolerance dose.

** Based on approximately 40 hours deflected beam operation.

Besides being more compact and much easier to operate, the brass system has other advantages over the glass system. The most important advantage is that no impurities are introduced by the brass system such as were experienced using the glass system. A mass spectrograph analysis of the residual gas during the purification process was made with the following results: 50 percent BF_3, 40 percent air, traces of H_2O and CO and < 10 percent unidentified material (principally peaks at 65 and 85). From the standpoint of satisfactory counter operation the most objectionable impurities are absent.

Fast Neutron Scintillation Counter. Work is continuing on a fast neutron scintillation counter. At present a hollow sphere of polyethylene coated on the inside with Zn S-A (RCA phosphor 33-Z-20A) and viewed at with a 5819 photomultiplier looks promising. Preliminary tests show it to be inferior to proportional counters in efficiency, but it is expected to be more stable, requiring less electronic gain and not subject to gas variations.

Statistical Summary of Monitoring Program.

Survey instruments maintained:

1.	B-γ Ionization chamber.	38
2.	Victoreen 263 meters	19
3.	I.D.L. portable survey meters	20
4.	Cutie pies	3
5.	Recording γ- intensity meters	16
6.	Victoreen proteximeters	3
7.	Fast neutron proportional counters	5
8.	Slow neutron proportional counters	15
9.	Balanced chamber (slow neutron survey instrument)	2
10.	Balanced chamber (fast neutron survey instrument)	1
11.	Special tissue wall survey instrument	1
12.	Fast neutron proportional counter (portable)	1

Personnel meters in use:

1.	Total people covered with film badges	1902
2.	Total man days coverage with pocket chambers	1670
3.	Total man days coverage with pocket dosimeters	3555
4.	Total man days coverage with pocket chambers (SN)	3570

Cases of weekly exposures above 0.3r:

Weekly film exposure above	184" Area	60" Area	Linear Accelerator	Bldg. 52 Area	Chemistry	Total
0.3r	2	10	35	5	16	68
0.5r	1	4	6	5	7	23
1.0r	0	0	0	0	3	3
1.5r	0	0	0	0	0	0

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