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Health and Biology

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QUARTERLY TECHNICAL REPORT

January 1, 1952 thru March 31, 1952

It should be noted that the Quarterly Technical Reports of The University of Rochester Atomic Energy Project do not attempt to describe progress in all of the research programs but only in those in which some significant results have been achieved but which are not sufficiently complete to be written up as final reports.

Submitted by: Henry A. Blair
Director

Date of Report: 5/14/52

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INTERIM REPORT ON TWO-YEAR EXPERIMENTAL INHALATION STUDIES OF $BeSO_4$ AT A LEVEL OF 4 μg Be/m³

By
Robert Root, C. J. Spiegl, H. E. Stokinger et al.

Abstract

The first year (50 weeks) of the proposed two year $BeSO_4 \cdot 6H_2O$ mist exposure, at a desired concentration of 4 μg Be/m³ has been completed. The overall, spectrographically determined, concentration for the first year was 3.8 μg Be/m³, with an extreme range of 2.1 - 8.8 μg Be/m³. The animal data do not indicate any positive results with the possible exception of the rat groups. The mortality of exposed rat group (86%) was slightly higher than that of the control rat group (64%).

Although data are incomplete in the two-year study of the effects of a low level of $BeSO_4$ in the atmosphere, it seems advisable to report the trends apparent after one year's (50 weeks) exposure.

Concentration and Methods of Sampling: During the first 50 weeks of this experiment, a concentration of $BeSO_4$ mist was satisfactorily maintained at an atmospheric level of 3.8 μg Be/m³ with an extreme range of from 2.1 to 8.8. Our goal of 4 μg Be/m³ average for the period of 2 years thus appears to be easily attainable.

The primary standard for concentration is spectrographic analysis. Two 3-hour samples are collected on filter paper during each 6-hour day at an air-sampling rate of 14 liter-minute and are analyzed for total Be content.

A very useful secondary method has been developed to obtain approximate concentrations rapidly and thus to permit adjustment of the aerosol generating unit for hour-to-hour control. Samples are collected during 30 minutes of each hour by impingement of chamber atmosphere (2.1 cubic

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feet/min) at sonic velocity on a metal plate coated with a water soluble resin. The sample is then eluted with distilled water and analyzed colorimetrically for Be by a method utilizing the formation of a Be naphthazarin or alkanin lake (1). The mean of the colorimetric determinations was 2.0 μg Be/m^3 with a range of 1.0 to 2.9. Compared to spectrographic methods, the efficiency for this method was only about 56%, but nevertheless the recovery was sufficiently constant for the purpose of rapid chamber control.

Animal Data: Clinical criteria of toxicity thus far are either negative or inconclusive. Only mortality statistics for the rat appear to show a difference between experimentals and controls; but even in this case the difference is questionable because it must be remembered that this species approaches the termination of its average life-span in about 18 to 20 months. In a group of 64 rats exposed to $BeSO_4$, 52 died (81%), whereas 26 of 40 control rats died (65%). One dog of 5 died during exposure, no control dogs; and 3 of 16 rabbits as compared with 4 of 12 control rabbits. In no case could death be unequivocably attributed to the effects of Be. There is no sign thus far of typical acute Be poisoning.

Clinical biochemistry and hematology are negative. Histologic examinations of tissues arenot yet complete.

Discussion: Experimental difficulties in producing and controlling an atmosphere containing the extremely small concentration of 4 μg Be/m^3 in the form of $BeSO_4$ mist are being adequately handled and should present no further problems during the rest of the test.

The animal results of the past year, completion of the data in process, and the use of a suitable animal replacement schedule during the next year will, it is believed, give answers to the questions propounded at the start of this 2-year experiment.

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1) Can 4 μg Be/m^3 of a soluble Be compound be considered as a "minimal effect" level for inhalation?

2) Do any chronic lesions develop in animals during a period of inhalation of Be that represents a considerable part of the life span?

3) What is the pattern of tissue deposition, and bodily retention; does a dynamic equilibrium of intake and excretion become established when the intake is constant but not acutely overwhelming?

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EXCRETION OF RADIUM BY DOGS AFTER A SINGLE INTRAVENOUS INJECTION

by
John B. Hursh

Abstract

Two adult dogs received 50 μc radium as an intravenous injection. Daily urine and fecal samples were collected and analyzed. It was found that dogs excreted respectively 45.7 and 46.5 per cent of the dose during the first 10 days.

Background: In the course of preparing two dogs with radium burdens for use in other experiments it was decided to collect and analyze daily urine and feces samples. The measurement of the radium content of the samples is reported below.

Method: Two adult beagle dogs each received 50 μc radium chloride solution as a single injection in the jugular vein. Immediately before use the injection solution was adjusted to pH 6.0. After the first injection an amount equal to that injected was taken up into the syringe and discharged into a bottle containing 20 ml of 5 per cent HCl. The contents of the bottle were designated as the dummy injection. The value of 50 μc accepted as the dose is based on analysis of this solution and is equal to 97 per cent of the value calculated from the activity of the radium stock solution.

Each dog was placed in a metabolism cage and daily urine and feces samples were collected for a period of 12 consecutive days. Subsequently spot samples were collected as tabulated in the results. The urine drained into a glass bottle containing 25 ml of 3.0 N HCl. The feces were scraped from the stainless steel bottle tray and transferred to tared pyrex beakers containing 200 ml 3.0 N HCl.

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The total urine volume was measured and a suitable aliquot was digested with 10-25 ml concentrated HNO_3. The clear digested sample was transferred to a deemanation flask and analyzed for radium by the deemanation method (1).

The fecal sample stood in acid for 24 hours and then was homogenized by mechanical stirring, weighed, and an aliquot (ca 25 gm) was transferred to a tared Kjeldahl flask. The weight of the sample was determined by reweighing the remainder in the beaker and checked by weighing the Kjeldahl flask plus sample. The sample was digested with the concentrated nitric and perchloric acid technique (1). An aliquot of the digested sample was measured for radium according to the same techniques used for the digested urine samples.

Results: The results are presented in table 1 (page 8) and Figures 1 and 2 (pages 9, 10). The data agree with those obtained by Norris and Kisieleski (2) using rats in the sense that the experimental points fall along a straight line on a log-log plot as shown in Figures 1 and 2. The rat excretion was about 37 per cent of the dose at 10 days as compared with 45.7 per cent dose and 46.5 per cent dose for dogs No. 151 and 153 respectively.

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TABLE 1

RADIUM CONTENT OF DAILY EXCRETA

Days After Injection	Dog No. 151 Daily Fecal Excretion		Dog No. 151 Daily Urine Excretion		Dog No. 153 Daily Fecal Excretion		Dog No. 153 Daily Urine Excretion	
	μg Ra excreted	Per cent dose	μg Ra excreted	Per cent dose	μg Ra excreted	Per cent dose	μg Ra excreted	Per cent dose
1	3.06	6.12	4.05	8.10	7.19	14.40	4.19	8.38
2	4.24	8.48	1.48	2.96	2.475	4.95	1.14	2.28
3	2.52	5.04	1.10	2.20	2.02	4.04	.680	1.36
4	1.325	2.65	.609	1.22	1.30	2.60	.323	.646
5	.915	1.83	.378	.756	.765	1.53	.249	.498
6	.804	1.608	.297	.594	.652	1.34	.159	.318
7	.400	.800	.217	.434	.585	1.17	.144	.288
8	.410	.820	.205	.410	.441	.882	.151	.302
9	.394	.788	.166	.332	.328	.656	.126	.252
10	.239	.478	.142	.284	.264	.528	.087	.174
11	.246	.492	.107	.214	.234	.468	.081	.162
12-13	.424	.848	.202	.404	.344	.688	.110	.220
22	.0652	.134	.0329	.0658	.0713	.143	.0339	.0678
23	.0709	.142	.0336	.0672	.0832	.166	.0245	.0490
38	.0419	.0838	.0184	.0368	.0377	.0754	.0160	.0320
99	.0098	.0196	.0068	.0136	.0126	.0252	.0058	.0116

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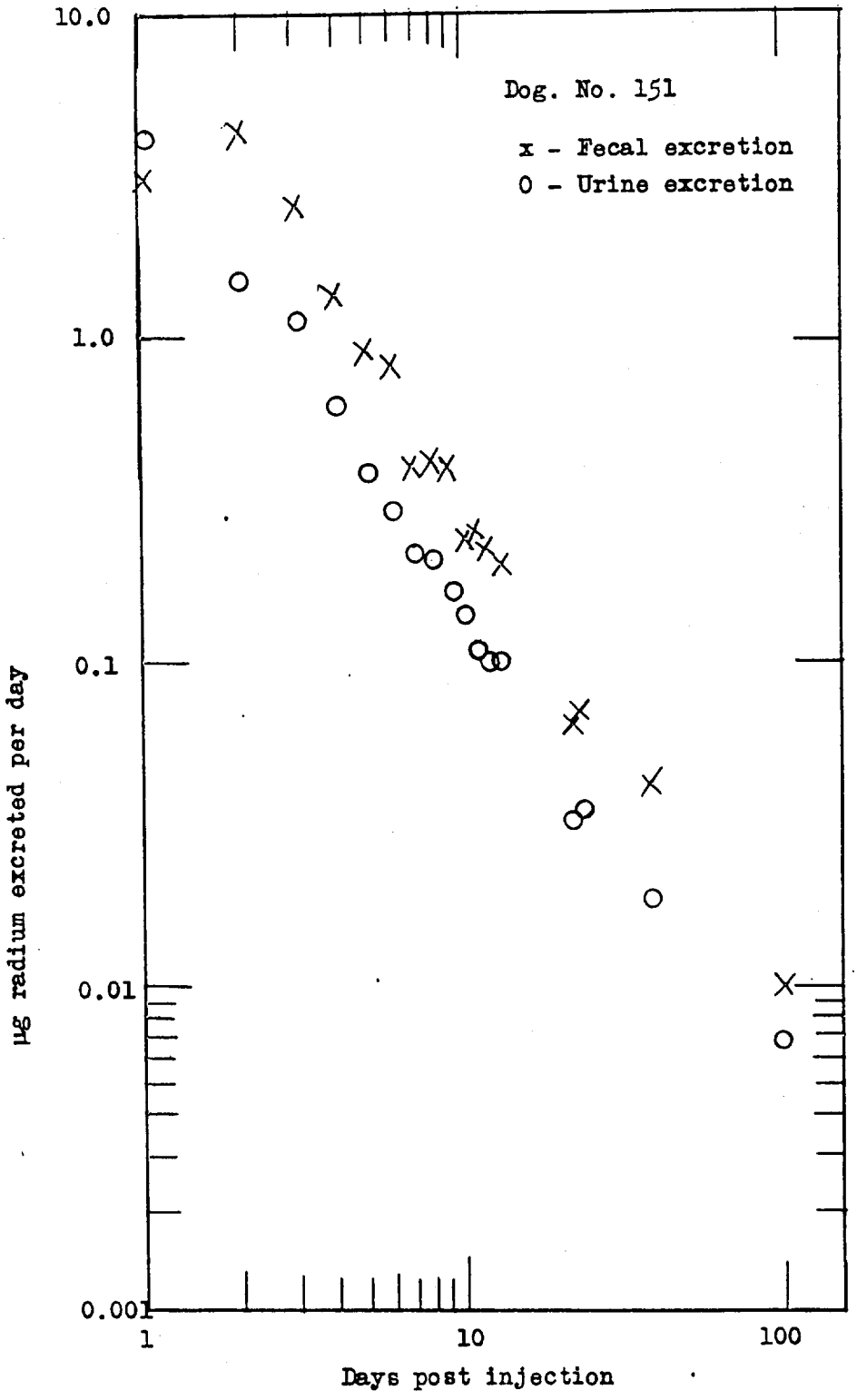

Figure 1

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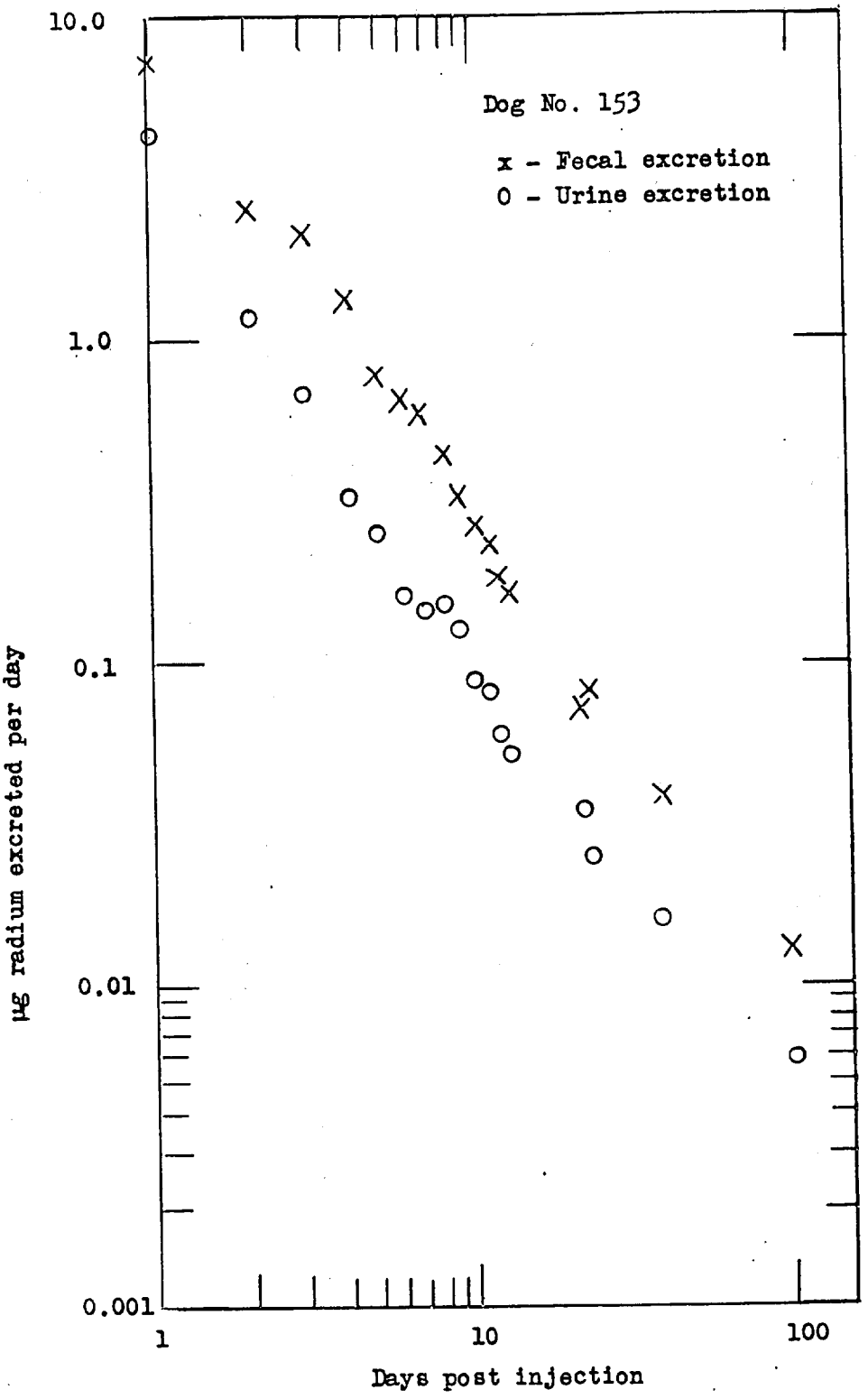

Figure 2

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STUDIES ON THE QUANTITATIVE SEPARATION OF PROTEIN BOUND IODINE FROM IODIDE

by
W. B. Mason, S. Coco and K. Knight

Abstract

Citrated plasma obtained from a patient on the fourth and fifth days after administration of 10 mc. I-131 for hyperthyroidism has been used to study the separation of protein bound iodine from iodide. The data indicate 93% (σ=3) of the plasma activity to be due to labeled PBI. Separation of proteins by dialysis, or by precipitation with cold 5-15% trichloracetic acid, with acid $ZnSO_4$ + NaOH, with $CuSO_4$ + NaOH, or with $CuSO_4$ + Na_2WO_4 results in no appreciable loss in PBI. Precipitation with $ZnSO_4$ + NaOH, with Na_2WO_4 + H_2SO_4 or with trichloracetic acid at room temperature appears to be incomplete. The most satisfactory separation of PBI from iodide is by dialysis. Iodide is easily removed from the trichloracetic acid precipitate by washing with cold 4-10% trichloracetic acid, but after four washings the loss of PBI amounts to approximately 8%. There is little loss of PBI when the $Zn(OH)_2$ or $CuWO_4$ precipitates are washed with water, but after four washings some 10% of added iodide remains.

An excellent opportunity for evaluating various procedures for the separation of PBI from iodide recently arose due to the necessity of performing phlebotomy on a patient receiving I-131 for hyperthyroidism. Roughly 170 ml of blood were removed on the fourth day after oral administration of 10 mc. I-131. The following day an additional 460 ml were removed. In each instance, the blood was drawn into a regular Baxter Bottle containing 2.94 gm dextrose, 1.64 gm sodium citrate, 0.60 gm citric acid and 120 ml water. Due to differences in dilution, the clear supernatants had different absolute activities, but were otherwise indistinguishable so far as the present experiments were concerned. Activity measurements were made with the aid of a sensitive gamma counter recently developed by the Medical Physics Section for measuring the activity of solutions contained in 25x150 mm test tubes. Without this instrument the present study would have been impossible.

Based on the Poisson distribution for counting rates, the standard deviation

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(σQ) for the data reported in the following sections is roughly 3.

I. Dialysis:

Dialysis from a cellulose sausage against a slow stream of distilled water (0.09 I/l) resulted in a 7% loss of activity in two hours. As shown in subsequent sections, precipitation with various reagents carries down 92-93% of the initial activity. It may therefore be concluded that some 93% of the plasma activity represents labeled PBI.

Dialysis for 2 hours removed 90% of the activity due to added iodide containing I-131 as iodide. Removal of iodide is roughly first order with respect to iodide concentration.

Data are summarized in Table 1.

Table 1

Contents of Dialysis Sac	Percentage Initial Activity After Dialysis For 30 min.	60 min.	90 min.	120 min.	180 min.
3.00 ml plasma (Bottle I)	93	93	96	--	--
duplicate	91	97	92	--	--
3.00 ml plasma (Bottle II)	--	--	--	93	93
duplicate	--	96	--	93	91
3.00 ml plasma 375γ iodide*	51	--	19	13	--
duplicate	44	--	17	5	--
3.00 ml plasma 0.11γ iodide*	45	25	15	7	--
duplicate	52	32	21	13	--

* Activity measurements corrected for activity due to labeled PBI

II. Trichloracetic Acid:

Precipitation from cold (5-8°C) trichloracetic acid in concentrations of 5-15% give results in good agreement with those obtained by dialysis. Precipitation is incomplete with cold trichloracetic acid in concentrations of 2-1/2%, 17-1/2% and 20%. Precipitation at room temperature is nearly complete from 5% acid, but incomplete from 10%. The precipitate may be washed equally well with cold 4-10% trichloracetic acid, but the loss of activity totals 8% after four washes. Slightly more activity is lost when the precipitate is washed at room temperature with 5% acid. Activity is rapidly lost when the precipitate is washed with water, or with cold 1-2%

trichloracetic acid.

The initial precipitation carries down 12-22% of added iodide, but this can be nearly completely removed by washing with dilute trichloracetic acid.

Data are summarized in Table II-A and Table II-B.

Table II-A

Temp. (C.)	Trichloracetic Acid Conc. (%) Precipitation	Wash Sol.	Percentage of Initial Activity in Supernatant	Precipitate after being washed 0x	1x	2x	3x	4x
22-25	5	0	-	92	83	45	9	-
	duplicate		-	94	83	57	12	-
22-25	5	5	-	91	85	89	83	-
	duplicate		-	89	91	79	80	-
22-25	10	10	-	87	86	89	81	78
	duplicate		-	86	89	86	83	79
5-8	2 1/2	-	7	-	-	-	-	-
5-8	5	-	4	92	-	-	-	-
5-8	5	1	7	-	-	-	-	55
5-8	5	2	3	-	-	-	-	67
5-8	5	4	3	-	-	-	-	84
5-8	5	8	3	-	-	-	-	85
5-8	7 1/2	6	5	-	-	-	-	84
5-8	10	-	5	93	-	-	-	-
5-8	12 1/2	10	5	-	-	-	-	85
5-8	15	-	5	92	-	-	-	-
5-8	17 1/2	-	7	-	-	-	-	-
5-8	20	-	9	-	-	-	-	-

Notes: Initial activity measurement made on 3.00 ml plasma and 12 ml water in a 25x150 mm pyrex test tube, and 15 ml trichloracetic acid having twice the desired final concentration added slowly with constant mixing. After 1 hour, solution centrifuged and supernatant aspirated into a second test tube for activity measurement. Precipitate washed with 15 ml portions of wash solution. Activity of precipitate measured after resuspension in 15 ml wash solution. Refrigerated centrifuge (5°C.) used for work with cold solutions.

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Table II-B

Temp. (C.)	Trichloracetic Acid Conc. (%) Precipitation	Wash Sol.	Iodide Added (γ)	Percentage Iodide Remaining After Precipitate Washed 0x	1x	2x	3x	4x
22-25	10	10	0.11	12	6	4	2	-
	duplicate			13	4	4	1	-
22-25	5	10	11.	18	4	2	0	-
	duplicate			18	4	2	1	-
5-8	10	8	0.16	22	10	6	4	3
	duplicate			21	9	5	4	3

Notes: Initial activity measurement made on 3.00 ml plasma and 12 ml water in a 25x150 mm pyrex test tube. Iodide containing a known amount of I-131 (as iodide) added and the solution made uniform. 15 ml trichloracetic acid having twice the desired final concentration added slowly with constant mixing. After 1 hour, solution centrifuged and supernatant removed by aspiration. Precipitate resuspended in 15 ml wash solution for activity measurement. Centrifugation, aspiration and activity measurements repeated, using 15 ml portions of wash solution. Refrigerated centrifuge (5°C.) used for work with cold solutions. Measured activities corrected for activity due to labeled PBI.

III. Zinc and Copper Hydroxides:

Precipitation from an acid zinc solution with sodium hydroxide gives results comparable to those obtained with trichloracetic acid and by dialysis. The precipitate undergoes no appreciable loss in activity when washed four times with distilled water. The initial precipitation, however, carries down 36-40% of added iodide, and this is incompletely removed by subsequent washing with water. Two other procedures for precipitation with zinc hydroxide give less satisfactory results, perhaps due, in part at least, to the relatively large amounts of citrate and dextrose present in the plasma. Precipitation with copper hydroxide appears to be complete.

Data are summarized in Table III.

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Table III

Note	Procedure	Iodide Added (γ)	Percentage Initial Activity After Precipitate Washed 0x	1x	2x	3x	4x
A	Acid $ZnSO_4$ + NaOH	--	92	93	90	--	91
	duplicate		88	92	94	--	95
B	Acid $ZnSO_4$ + NaOH	11	36	18	12	--	8
	duplicate		40	20	11	--	8
C	$ZnSO_4$ + NaOH		84	80	78	--	--
	duplicate		82	82	77	--	--
D	$ZnSo_4$ + NaOH	--	81	67	66	66	--
	duplicate		75	69	71	73	--
	duplicate		80	74	73	--	--
	duplicate		80	74	--	--	--
E	$CuSO_4$ + NaOH	--	94	92	87	--	--
			91	--	92	--	--

Notes:

A. 3.00 ml plasma and 24 ml acid $ZnSO_4$ solution (12.5 gm $ZnSO_4 \cdot 7H_2O$ and 125 ml 0.25 N H_2SO_4 per liter) made uniform in a 25 x 150 mm pyrex test tube and 3.0 ml 0.75 N NaOH added slowly with constant stirring. 50.0 ml acid $ZnSO_4$ solution requires 6.72 ml NaOH to the phenolphthalein end point. Solution centrifuged and the supernatant removed by aspiration. Activity measurements made with precipitate resuspended in 15 ml distilled water. Precipitate washed with 15 ml portions of distilled water.

B. Procedure same as in A, except add iodide containing I-131 (as iodide) to serum and make uniform before adding acid $ZnSO_4$ solution. Activity measurements corrected for activity due to labeled PBI.

C. 3.00 ml plasma, 21 ml distilled water, and 3.0 ml $ZnSO_4$ solution (100 gm $ZnSO_4 \cdot 7H_2O$ per liter) made uniform and 3.0 ml 0.5 N NaOH added slowly with constant mixing. 10.0 ml $ZnSO_4$ solution requires 11.0 ml NaOH to the phenolphthalein end point. Balance of procedure same as in A.

D. 3.00 ml plasma, 15 ml distilled water, and 2.0 ml $ZnSO_4$ solution (100 gm $ZnSO_4 \cdot 7H_2O$ per liter) made uniform and 2.0 ml o.5 N NaOH added. 10.0 ml $ZnSO_4$ solution required 10.8 ml NaOH to the phenolphthalein end point. Balance of procedure same as in A.

E. 3.00 ml plasma, 21 ml distilled water, and 3.0 ml $CuSO_4$ solution (10.0 gm $CuSO_4 \cdot 5H_2O$ dissolved in 90 ml H_2O) made uniform and 3.0 ml 0.50 N NaOH added slowly with constant mixing. Balance of procedure same as in A.

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IV. Tungstic Acid and Copper Tungstate:

Precipitation of PBI with copper tungstate is complete and there is little loss when the precipitate is washed with water. The initial precipitation, however, carries down 42-43% of added iodide. One washing with water reduces this to 23%, but 11-12% remains after four washings. Precipitation with tungstic acid appears to be slightly less complete than with copper tungstate. The precipitate can not be washed with water.

Data are summarized in Table IV.

Table IV

Note		Iodide Added (γ)	Percentage Initial Activity After Precipitate Washed				
			0x	1x	2x	4x	6x
A	$CuSO_4$ + Na_2WO_4	--	93	--	90	--	91
	duplicate		94	--	92	--	91
B	$CuSO_4$ + Na_2WO_4	11	43	22	16	12	--
	duplicate		42	24	18	11	--
C	Na_2WO_4 + H_2SO_4	--	90	94	91	70	--
	duplicate		91	91	80	58	--

Notes:

A. 3.00 ml plasma, 21 ml distilled water, and 3.0 ml $CuSO_4$ (5.0 gm $CuSO_4 \cdot 5H_2O$ per 100 ml) made uniform in a 25x150 mm pyrex test tube and 3.0 ml Na_2WO_4 (6.0 gm $Na_2WO_4 \cdot 2H_2O$ per 100 ml) added slowly with constant mixing. Solution centrifuged and the supernatant removed by aspiration. Activity measurements made with precipitate resuspended in 15 ml distilled water. Precipitate washed with 15 ml portions of distilled water.

B. Procedure same as in A except iodide containing I-131 (as iodide) mixed with serum and water before $CuSO_4$ solution added. Activity measurements corrected for activity due to labeled PBI.

C. 3.00 ml plasma and 24 ml distilled water made uniform and 1.5 ml Na_2WO_4 solution (100 gm $Na_2WO_4 \cdot 2H_2O$ dissolved in 1 liter H_2O) slowly added with constant mixing. 1.5 ml 0.66 N H_2SO_4 added dropwise with constant mixing. Balance of procedure same as in A.

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V. Summary:

Citrated plasma obtained from a patient on the fourth and fifth days after administration of 10 mc. I-131 for hyperthyroidism has been used to study the separation of protein bound iodine from iodide. The data indicate 93% (σ=3) of the plasma activity to be due to labeled PBI. Separation of proteins by dialysis, or by precipitation with cold 5-15% trichloracetic acid, with acid $ZnSO_4$ + NaOH, with $CuSO_4$ + NaOH, or with $CuSO_4$ + Na_2WO_4 results in no appreciable loss in PBI. Precipitation with $ZnSO_4$ + NaOH, with Na_2WO_4 + H_2SO_4 or with trichloracetic acid at room temperature appears to be incomplete. The most satisfactory separation of PBI from iodide is by dialysis. Iodide is easily removed from the trichloracetic acid precipitate by washing with cold 4-10% trichloracetic acid, but after four washings the loss of PBI amounts to approximately 8%. There is little loss of PBI when the $Zn(OH)_2$ or $CuWO_4$ precipitates are washed with water, but after four washings some 10% of added iodide remains.

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THE PREVENTION OF X-IRRADIATION INDUCED HYPERGLYCEMIA IN RABBITS BY MEANS OF INSULIN

by
L. T. Steadman and A. J. Grimaldi

Abstract

The hyperglycemia produced in rabbits by whole body x-irradiation reaches a maximum in about 4 hours after the start of the irradiation. For a dose of 2000 r the plasma glucose, which is normally about 130 mg per cent, is elevated on the average to a value of 320 mg per cent with occasional values over 500 mg per cent. When regular insulin is injected subcutaneously, 5 units at the start of the irradiation and 5 units more in 3 hours, the rise in plasma or cell glucose is prevented from occurring in practically every instance. Smaller amounts of insulin are sufficient to prevent the hyperglycemia for lower doses.

Background: In a previous Quarterly Technical Report Thompson and Steadman (1) presented data on the temporary rise in blood glucose levels in the rabbit following whole body x-irradiation. The magnitude of the dose administered varied from 65 to 2000 r. The radiation was delivered by a Picker Industrial X-ray machine operated at 200 kv peak and had a half-value-layer of 1.6 mm of copper. An increase in plasma and red blood cell glucose concentration was observed to occur almost immediately after the start of the irradiation with a maximum elevation in about 4 hours. Thereafter, the hyperglycemia subsided about as rapidly as it arose and in 24 hours the blood sugar level was back to a normal value. For a dose of 1000 r, for instance, the plasma glucose level changed, on the average, from a normal value of about 135 mg per cent to a maximum at 4 hours of about 300 mg per cent. The magnitude of the hyperglycemia increased with

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the dose of irradiation. However, there was considerable individual variation in the response to irradiation and occasionally very much higher values (500-800 mg per cent) were found, especially in subsequent experiments with doses above 1000 r. In some of the instances of large hyperglycemia the animal succumbed immediately.

It is, therefore, possible that an extreme hyperglycemia following irradiation may have some significance in the explanation of the so-called initial shock state and early deaths in animals such as the rabbit.

The appearance of hyperglycemia in other laboratory animals was found by Kohn (2) to develop more slowly and apparently to be not as severe. He found that with rats, irradiated with 600-900 r, the plasma glucose level rose to 210 mg per cent in 2 to 3 days. Guinea pigs behaved much the same. Prosser, Painter, and Swift (3) determined in a few dogs that a dose of 350 r produced in 5 to 9 days a slow increase of only about 20 mg per cent above the normal value.

Since the importance of insulin in carbohydrate metabolism is well known, and it has been stated (4) that the administration of insulin will prevent or reduce a hyperglycemia from whatever cause, it was of interest to determine whether the hyperglycemia induced by irradiation also could be prevented by the use of insulin. Furthermore, should the lipemia which in the rabbit frequently appears on the first day after irradiation be related to the previous disturbance in carbohydrate metabolism, then a study of the effects of insulin might have some bearing on the explanation of the elevation of lipids.

Methods: In order to test the effects of insulin administration the experiments mentioned above (1) were first extended considerably in order to

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have more animals in the control series and to obtain a better evaluation of the magnitude of the hyperglycemia as a function of the dose of irradiation. The methods used were essentially as described in the previous report, and the earlier results have thus been incorporated into the present data.

In the conduct of the first experiments two particular procedures were used which require additional comment, namely, the use of anesthesia and the shielding of the head. It was found initially that, with the rabbit fastened to an animal board and irradiated with small doses (50-100 r), the rise in blood glucose was much higher than was expected from extrapolating the values obtained for higher doses to a zero dose of irradiation, that is, the threshold dose for elevated glucose appeared to be very low. Subsequently, it was found that some animals exhibited a considerable hyperglycemia merely on being tied to the board without being irradiated. Therefore, in order to have a reliable determination of the effect of small doses not complicated by the effects of alarm stimuli, the procedure of anesthetizing with nembutal was instituted, which proved to be satisfactory. However, for a fundamental study of the effects of insulin it did not seem advisable to give insulin and nembutal at the same time and thus tests were made without nembutal for the higher doses of irradiation. The results, to be given later, show that with high doses of irradiation the use of nembutal is not necessary, the magnitude of the hyperglycemia found is not significantly affected by the presence or absence of the anesthetic.

As stated before, the head and neck shield was employed because, otherwise, many of the animals receiving doses of 1500 to 2000 r developed a nasal discharge in the first day or two and died of suffocation, which trouble unduly interfered with the course of the experiment. The secretion

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would dry up around the nostrils and along with material picked up from the bottom of the cage gradually would seal off the nasal passages. Apparently the rabbit has not learned to breathe through the mouth in such an emergency. From subsidiary experiments on irradiating only the head it was concluded that irradiation of the head made little contribution to the hyperglycemia following whole body irradiation. More recently, however, the nasal discharge hazard has been eliminated somewhat by cleaning away the secretion periodically and by placing the animal on a wire mesh floor away from the bedding. The measurements to be given below show that the magnitude of the hyperglycemia is essentially the same whether the head is shielded or not.

Glucose determinations were made on a pre-irradiation sample obtained a day or two before the irradiation and on samples taken at 2, 4, 6, and 24 hours after the start of the irradiation. The duration of the irradiation was approximately one hour for each 1000 r. Blood samples were obtained by heart puncture using heparin as the anticoagulant. Both plasma and red blood cells were separately analyzed.

Measurements of the plasma total lipid were also made on the same animals on samples taken at one and two days as a continuation of the experiments of Steadman and Thompson (5). It is intended, however, that the lipid studies will be discussed in a separate report.

Administration of Insulin

The rationale for the treatment with insulin for the purpose of preventing the hyperglycemia was arrived at from a consideration of the work of Scott and Fisher (6). They found with fasted rabbits that the subcutaneous injection of 2 units of regular insulin per kilogram produced a hypoglycemia which was fully developed in 2 hours (plasma level of 50 mg per cent), but the

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effect of the insulin had about disappeared by 6 hours after the injection. With the injection of the same amount of protamine-zinc insulin the maximum depression of the blood sugar occurred at about 2.5 hours and the effect lasted for about 10 hours.

After some initial experiments using varying amounts of either type of insulin administered by subcutaneous injection in the hind leg and injected at different times relative to the start of the irradiation, it was found that the hyperglycemia could be prevented if the regular insulin were administered at the start of the irradiation. With the higher doses of irradiation the hyperglycemia could be controlled more effectively by increasing the insulin dosage and by giving a second injection at 3 hours after the first. The protamine-zinc insulin seemed to have some effect in lowering the plasma glucose, but its ability compared with the regular insulin was small. Consequently, the insulin studies were carried on principally with the use of the regular insulin. Since all of the rabbits were about the same in weight (7.5 to 9 pounds) the insulin dosage was the same in each case. The dose was 2.5 units for the 1000 r irradiation dose and for the 1500 r and 2000 r doses 5 units were injected at the start of the irradiation and 5 units more were injected 3 hours later. The preparation used was Insulin Squibb, 40 units per cc, made from zinc-insulin crystals. The protamine-zinc insulin was also made by the same company.

Results and Discussion: The data on plasma glucose levels following different irradiation doses for the control rabbits and the insulin-treated animals are presented in the main in summary form as averages for each experiment. The number of animals in each experiment is given and the conditions of the experiment are designated. However, the individual results for all of the

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animals in a series are shown in two instances to illustrate the typical individual variation.

From table 1 (page 27) it is seen that the hyperglycemia has a maximum at 4 hours and that the magnitude of the rise in glucose increases with the dose. The accompanying graph, figure 1 (page 26), shows a plot of the maximum elevation versus r dose. The maximum value for Experiment 3 wherein the animals were not anesthetized is indicated by a delta, Δ, and it is observed to fall on the curve for the other experiments showing that with relatively high doses of irradiation it is not necessary to anesthetize the animal when determining the effect of irradiation on the blood glucose levels.

A comparison of Experiments 3 and 4 shows that shielding the head gives results which are not significantly different from irradiation of the whole body. Also a comparison of the data from Experiments 5 and 6 indicates further that anesthesia is not important as a depression of alarm stimuli when relatively high doses of irradiation are employed. Table 2 (page 28) gives the results for the individual animals in Experiment 6 which was for a dose of 2000 r, the head being shielded and the animal not anesthetized. Although the simple numerical average gives the maximum glucose elevation as occurring at 4 hours, an inspection of all of the individual data, including as well the 500, 1000, and 1500 r values, indicates that the maximum effect may be shifting somewhat toward an earlier time as the dose is increased.

In table 3 (page 29) is presented the results obtained with insulin treatment. With the single injection of insulin at the start of the irradiation (Experiments 7 and 8) there is the indication from the slightly elevated values on the sixth hour that the insulin is losing its effect by this time. The individual data and a few results obtained with single doses of insulin

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with 2000 r show this insufficiency more strikingly. When, however, the insulin dosage is prolonged there seems to be practically complete protection.

The individual values for Experiment 10 are given in table 4 (page 30). With one exception, the No. 969 value at the second hour, the hyperglycemia is under control and in many instances a hypoglycemia was produced. It may have been that one or two of the animals in the experiments died in hypoglycemic shock but the evidence is mainly circumstantial. They had plasma sugar levels below 50 mg per cent but died unobserved during the night without any other apparent cause of death. On the other hand, no convulsions due to hypoglycemia have been seen in any of the experiments when irradiation was involved.

Since a hypoglycemia has been produced in several instances with the insulin after the fourth hour, it is postulated that the liver glycogen must have been depleted during the first 4 hours and was the source of the excess plasma glucose because ordinarily a demonstration of hypoglycemia by insulin is effected with a fasting animal. As a matter of fact, Rother (7) measured the glycogen content of the liver in the rabbit following irradiation and found it to have been markedly reduced. It seems plausible therefore that the irradiation causes, somehow, a mobilization of the liver glycogen into glucose and that the normal supply of insulin in the animal is not sufficient to prevent the rapidly developing hyperglycemia although it is effective in restoring the blood glucose to normal levels in 24 hours, otherwise there would be a permanent elevation as in a diabetic individual.

The average values for the insulin-treated animals are also plotted in figure 1 (page 26) for comparison. Three other animals were also treated with insulin and irradiated with 2000 r dose without anesthesia and without a head shield. The hyperglycemia was also prevented. This test serves as

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a check on the procedure in the case of whole body irradiation. The red blood cell glucose levels in the above experiments with insulin were similarly found to be maintained at a normal level. Thus the effect of the insulin on the course of the hyperglycemia induced by whole body x-irradiation seems to be well in accordance with its properties of reducing hyperglycemia caused by other conditions or agents.

The effect of the insulin administration on the appearance of the lipemia is not as definite or as marked. There seems to be a reduction in the total lipids but the variability of the data obtained in the above experiments does not permit a reliable conclusion as yet.

The present experiments were not very well adapted to proving whether or not the use of insulin, with the resulting absence of hyperglycemia, has a beneficial effect on the clinical appearance or early mortality of the animals. This is because several blood samples were taken in a short time, and with the large doses of irradiation, particularly, there was a high incidence of death within 2 days due to damage from the heart punctures. In these cases of early fatality a severe hemorrhage into the pericardium or the chest cavity was found which presumably was related to a long blood-clotting time.

Acknowledgement

The technical assistance of Thelma Carrol, Lucy Johnson, Tracy White, and Florence VanSlyke is gratefully acknowledged.

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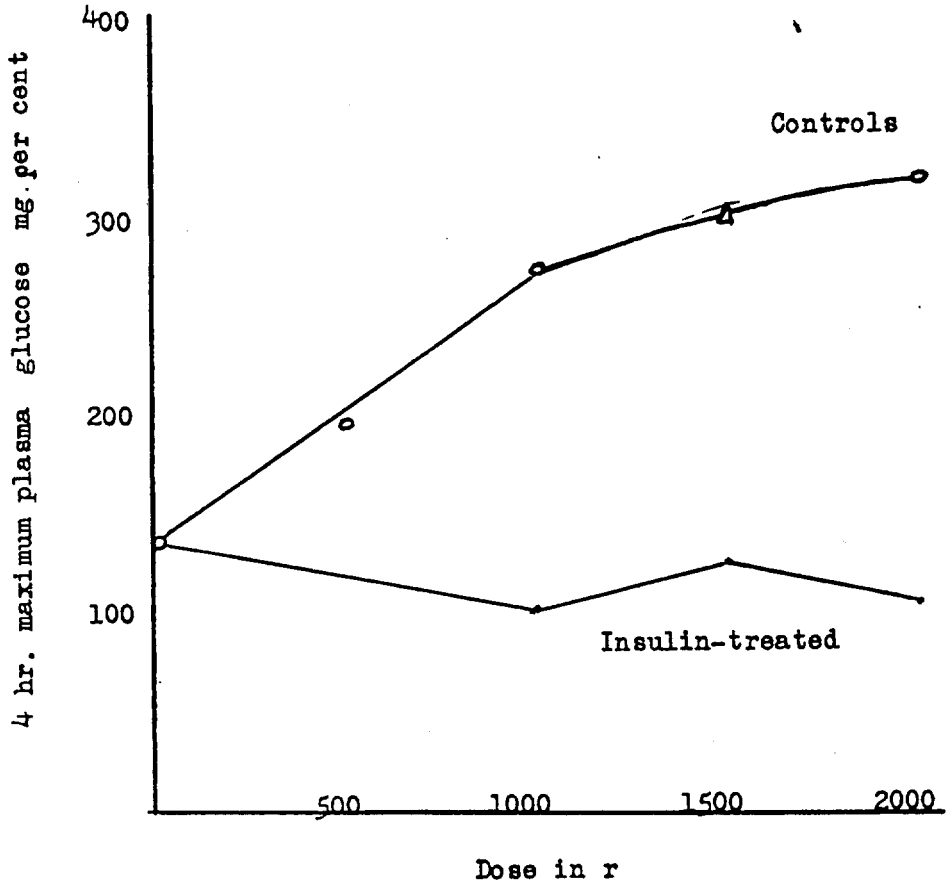

Figure 1

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TABLE 1

AVERAGE PLASMA GLUCOSE IN MILLIGRAMS PER CENT
200 KVP X-RADIATION HVL 1.6 MM COPPER - CONTROLS

Exp. No.	Dose in r	No. of Animals	Time After Start of Irradiation in Hours Pre-Irrad.	2	4	6	24
1	500, head shielded, anesthetized	6	136	187	196	170	141
2	1000, head shielded, anesthetized	9	130	240	273	206	130
3	1500, head shielded, not anesthetized	10	144	263	298	255	127
4	1500, head not shielded, not anesthetized	10	148	258	286	221	117
5	2000, head shielded, anesthetized	7	138	282	322	234	129
6	2000, head shielded, not anesthetized	10	132	294	307	241	130

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TABLE 2

PLASMA GLUCOSE IN MILLIGRAMS PER CENT
200 KVP X-RADIATION HVL 1.6 MM COPPER

(Controls - Experiment 6 - 2000 r)

Animal No.	Time After Start of Irradiation in Hours Pre-Irrad.	2	4	6	24
953	137	360	375	160	106
955	115	264	286	272	80
958	160	210	214	152	105
959	122	222	202	135	104
962	122	230	236	234	214
966	120	340	305	230	190
970	135	256	216	260	145
974	162	365	540	510	94
976	107	273			
982	142	415	385	218	130
Average	132	294	307	241	130

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TABLE 3

AVERAGE PLASMA GLUCOSE IN MILLIGRAMS PER CENT
200 KVP X-RADIATION HVL 1.6 MM COPPER

(Insulin Treated - Head Shielded - Not Anesthetized)

Exp. No.	Dose in r	No. of Animals	Time After Start of Irradiation in Hours Pre-Irrad.	2	4	6	24
7	1000, 2.5 units regular insulin at start	6	137	128	99	140	131
8	1500, 5 units regular insulin at start	5	148	164	164	184	147
9	1500, 5 units at start plus 5 units in 3 hours	7	141	148	124	110	173
10	2000, 5 units at start plus 5 units in 3 hours	10	132	130	104	100	96

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TABLE 4

PLASMA GLUCOSE IN MILLIGRAMS PER CENT
200 KVP X-RADIATION HVL 1.6 MM COPPER

(Insulin Treated - Experiment 10 - 2000 r)

Animal No.	Time After Start of Irradiation in Hours				
	Pre-Irrad.	2	4	6	24
954	149	116	110	86	121
956	107	102			
957	117	130	50	54	51
960	131	140	135	140	56
961	110	82	67	53	
965	119	104	85	102	145
969	120	248	160	129	59
973	187	126	86	123	121
975	110	118	120	95	97
981	173	135	118	117	118
Average	132	130	104	100	96

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PROTECTION OF THE-X IRRADIATED DOG WITH EXTERNAL ABDOMINAL SHIELDING

by
M. Coulter and R. Miller

Abstract

Sixteen adult dogs that were survivors of 525 r 1 MEV x-irradiation were exposed 3 months later to 525 r of 250 KV x-irradiation. One-half of the dogs had their entire abdomen shielded with 1/8 inch lead during exposure. None of the shielded dogs died, while all of the control dogs were dead by the 23rd day after irradiation. The WBC count fell in the customary fashion in the control dogs, but none of the shielded dogs developed WBC counts of less than 1500. It is evident that abdominal shielding will still protect a dog that has survived a previous LD 90 of total body radiation.

- - -

Lead shielding of the exteriorized spleen during exposure increases the survival rate of animals exposed to a lethal dose of x-irradiation. Jacobson has shown this to be true in mice, rats and guinea pigs (1,2,3). A previous report from this laboratory indicates that lead shielding of the exteriorized spleen was effective in reducing x-irradiation mortality in dogs (4).

It was felt that external shielding of the whole abdomen might provide a more practical approach to the prevention of the acute radiation syndrome. Further, with this method, one could determine the total cumulative dose of irradiation from repeated exposures which a shilded animal could survive and study the hematologic changes accompanying repeated doses.

In this study 16 adult dogs which were survivors of 525 r 1 MEV x-irradiation were re-exposed 3 months later to 525 r of 250 KV x-irradiation. One-half of the dogs had their entire abdomens shielded with 1/8 inch lead during the exposures. Animals were radiated in pairs comprised of one non-shielded and one shielded dog. The dogs were anesthetized and laid on their backs during the radiation.

Results: None of the shielded dogs died, while all of the control dogs were dead by the 23rd day post-radiation. The gross pathology in the control dogs that

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died was typical of that found in the acute radiation syndrome; severe hemorrhage of the lungs and/or gastro-intestinal tract being the predominant cause of death.

The average white blood cell count of the dogs at the beginning of this experiment was lower than that obtained before the dog had received any radiation. The average count was approximately 11,000 when the dogs were received into the colony and approximately 7,000 before the 2nd radiation; which was 3 months after the first radiation. Therefore, it may be possible that hematopoietic recovery was still incomplete 3 months after the first exposure.

Following the second exposure, the white blood cell count fell to lower levels in the control than in the shielded dogs as shown in Graph I (page 34). None of the shielded dogs had a white blood cell count below 1500 in the post-irradiation period. There was no significant difference in the platelet values between the 2 groups, Graph II (page 34). No significant differences in the hematocrit values were noted between the groups for the first 9 days post-radiation. From this time on the values were much lower in the control group. During this period the greatest mortality in the control dogs occurred.

Discussion: This method of external abdominal shielding does not give one any definitive data regarding the mechanisms and organs involved in the protection afforded the animal. Using this technique, some vertebral marrow, as well as the abdominal organs, is shielded. However, it will be of practical interest to determine how many exposures to a large dose of x-irradiation a dog can survive. It is planned to re-expose the survivors of this study at approximately 2 to 3 months intervals to the same dose of x-irradiation and to protect them with abdominal shields.

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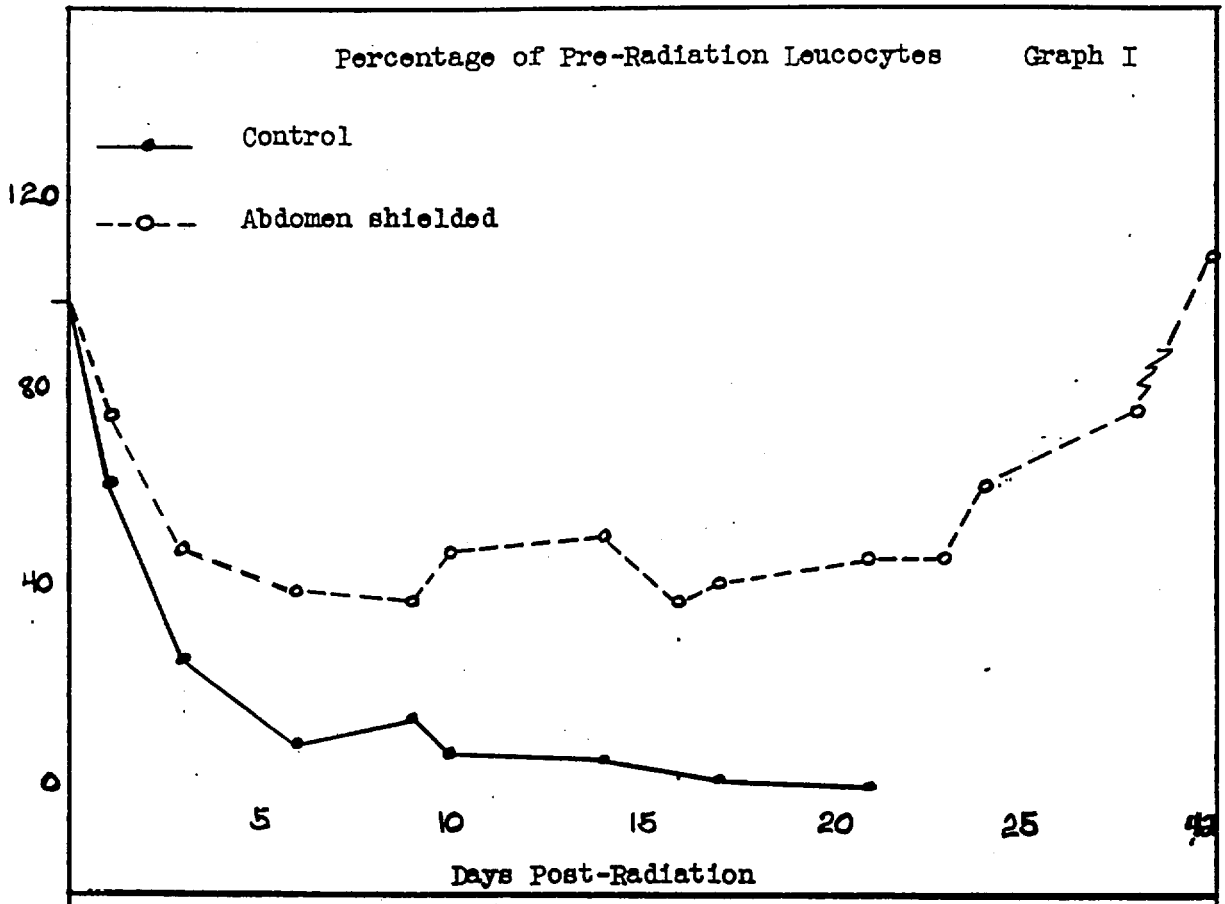

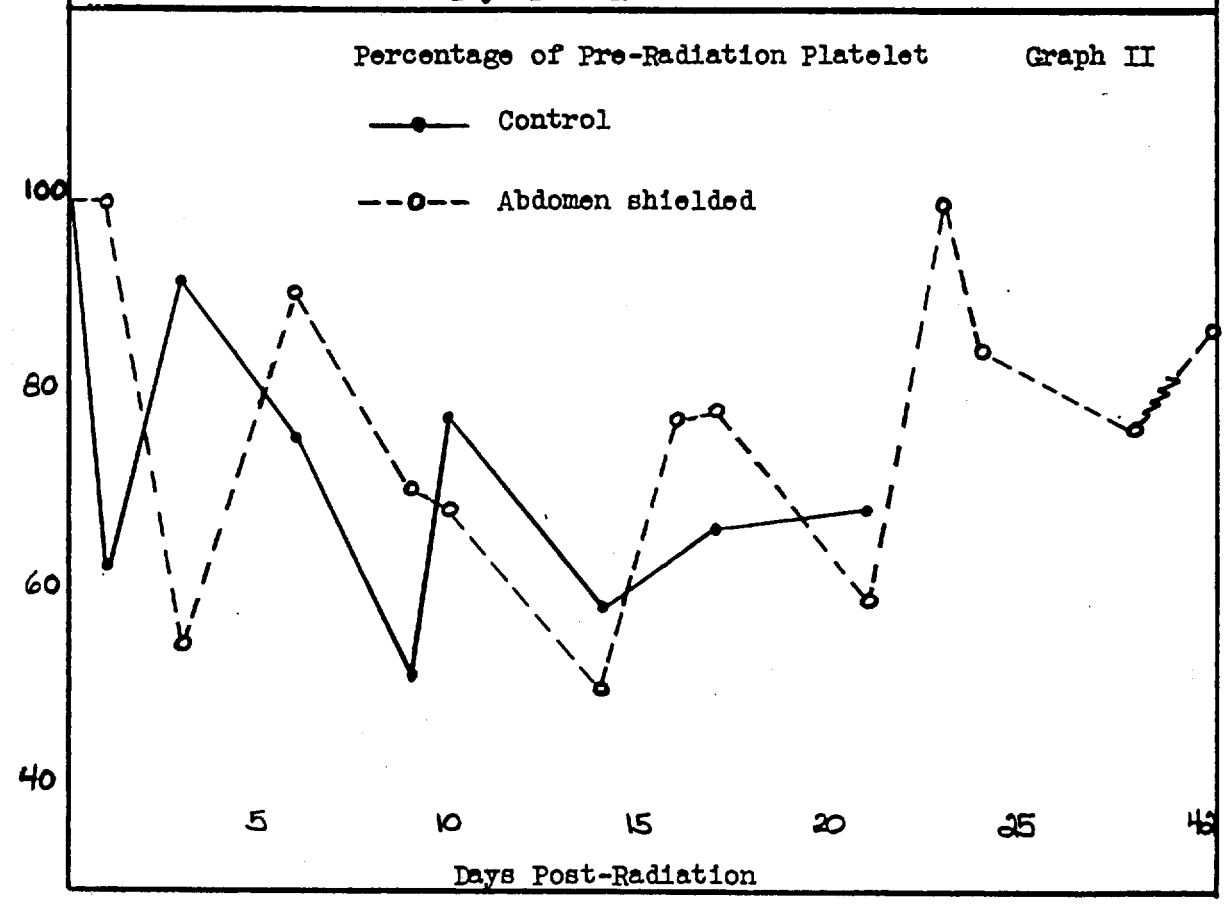

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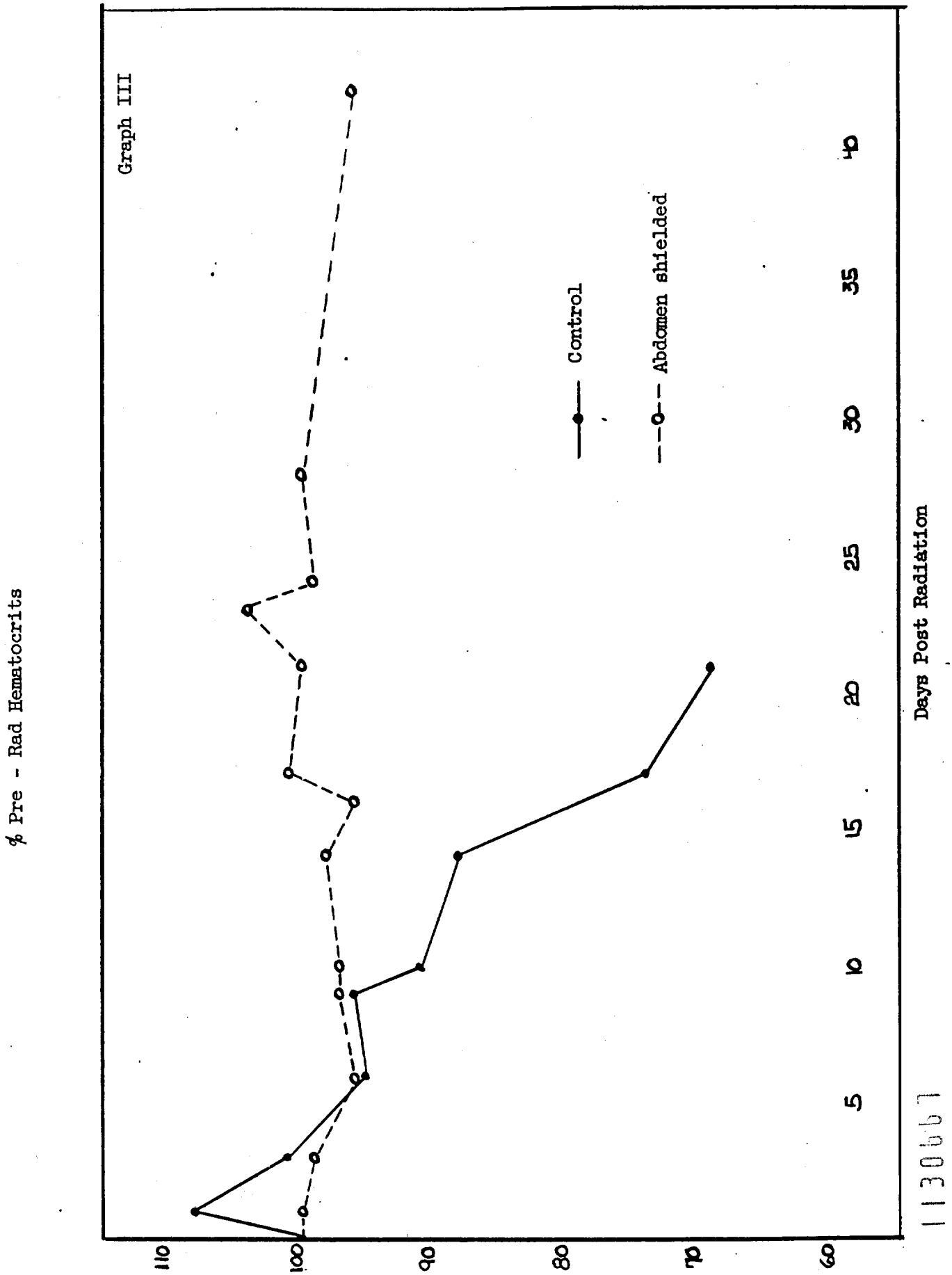
Graph III
% Pre - Rad Hematocrits
110
100
90
80
70
60
5
10
15
20
25
30
35
40
Days Post Radiation
Control
Abdomen shielded

Preliminary Report on some Clinical Features of the Radiation Syndrome Complicated by Induced Infection

By

Miller, R. W. and Coulter, M. P.

ABSTRACT

A preliminary study of the effects of induced sepsis and peritonitis in the radiated (LD50) dog is presented. 100% of the animals infected died and the morbidity rate was very high. The WBC count, and hematocrit did not reflect the presence of infection. A high incidence of terminal unrestrained growth of bacteria in the blood stream occurred. The gross pathology of the animals with sepsis showed changes indistinguishable from those of the uncomplicated radiation syndrome except for the severe hemorrhages in the kidney cortex. This anatomic area was much more extensively involved than usual. The value of a prophylactic broad spectrum antibiotic is discussed.

- - -

The human radiation casualty is apt to suffer concomitant infection as a consequence of wounds, burns, epidemics, or from dormant pre-existent foci of infection. The purpose of these studies is to determine some of the clinical and pathologic features of the radiation syndrome when combined with infections of various types.

A. Induced sepsis:

Methods: A group of mongrel dogs consisting of 12 males and 13 females was divided and treated as follows:

(1) 9 dogs: x-irradiated plus intravenous staphylococcus aureus intermittently post-irradiation.

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(2) 9 dogs: radiated controls.

(3) 7 dogs: injected as in group (1). No irradiation.

X-ray factors: 250 KV 15 ma planoconvex aluminum filter with 0.5 mm copper, 7.6-8.0 r/minute, 450 r total dose.

Bacteriologic factors: 4.5-18.0 x 10^{10} bacteria* were injected into a jugular vein on the 2,6,9,13 and 16th days following irradiation. One cc of blood was drawn for culture before the bacteria were injected and 1 cc of blood was obtained for culture 24 hours later. Colonies were counted 24 hours after the pour plates were made.

Hematology:

3 control hematocrits and white blood counts were secured. These determinations were repeated on the 2,6,9,13 and 16th days post-radiation.

The white blood count was determined 1/2, 1,2,3,4, and 24 hours after the first two bacterial injections (2 and 6th days) in 4 radiated dogs and 4 non-radiated animals.

Pathology:

Autopsies included an examination of all organs but the brain. Sections were taken for microscopic study but have not yet been reviewed.

EKG's:

Leads used were AVR, AVL, AVF, and two precordial leads: one from the 3rd interspace just to the left of the sternum and the other was in the 5th interspace in the midclavicular line. 3 control records were obtained. Following the day of irradiation all dogs had daily EKG's until all the infected radiated animals had succumbed. Then EKG's were secured every other day on the radiated controls.

* Staphylococcus aureus, American type culture collection #9801.

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Results: Mortality:

Treatment	No. of dogs treated	# died	Day of death
Radiation + sepsis	9	9	6 - 18
Radiation	9	3	7, 13, 27
Sepsis	7	0	- - - -

Bacteriology:

All dogs receiving the intravenous bacteria had positive blood cultures during the experiment. 4 of the radiated infected animals had 500 colonies or more per cc of blood, usually just before death, while only one control had more than 200 colonies (500). 3 radiated infected dogs died 36-60 hours after their last blood culture had been obtained. The possibility exists that their colony count would have been higher had the blood been cultured at a time closer to their deaths.

Hematology:

(a) Hematocrit: There was a gradual linear decline in the hematocrit levels beginning the 6th day post-irradiation (Graph I). The values of the infected non-radiated animals fell too, so that until the 9th day, at least, no difference could be found in the hematocrit levels for any of the three groups. However, there was no summation of depressant effects in the dogs who were both irradiated and infected.

(b) WBC: The usual exponential decline in white blood cell counts of both the radiated controls and the radiated infected animals was demonstrated (Graph II). No difference between the two groups was detectable.

Neither the infected controls nor the infected radiated dogs displayed a leukopenia or leukocytosis within the first four hours of the injection of the bacteria. The infected controls did have a marked

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leukocytosis 24 hours later, which was not displayed by the radiated infected animals (Graph III).

EKG:

The table summarizes the markedly positive findings. More subtle changes will be reported after further studies of the records obtained have been made.

EKG changes	Radiated-infected dogs	Radiated dogs	Infected dogs
S - T displacement	4/9	3/9*	6/7
Increased QRS time	2/9	0	0

* 2 of these dogs had gross pathologic changes of severe infection.

Pathology:

All but one infected radiated dog had very marked hemorrhages in the renal cortex, a finding that is rare in radiated animals in which sepsis has not been induced. The gross cardiac lesions in the radiated and infected dogs were no more extensive than they are in non-septic radiated animals.

B. Induced peritonitis:

Methods: A group of 12 mongrel dogs were divided and treated as follows:

(1) 6 animals were irradiated. On the 7th day after irradiation an intraperitoneal injection of staph aureus was given.

(2) 6 non-irradiated dogs received staph aureus as did those animals in Group I.

(These animals had survived a previous total body irradiation (350 r LD10) administered two months previously.)

X-ray factors: Same as dogs in the sepsis experiment except that the dose rate was 8.2 r/minute.

Bacteriologic Factors: Same as in sepsis experiment except that 5.28×10^{10} bacteria were injected into the left upper quadrant of the abdomen

Bacteriology:

Treatment	Dog	Colonies per cc of blood following i.p. injection: Control	4 hr.	1 hr.	5 hr.	24 hrs.	48 hrs.	P.M.* heart Blood culture	P. M.* Abd. culture
	1	0	5	2	264	594	____	Staph	Staph
	2[a]	0	17,820	2970	330	28	2970	Staph + Strep	Staph + strep
Radiated +	3	0	35	15	5940	26,136	____	Staph	Staph
l.p.	4[b]	0	0	0	1	2	9	Staph + gm neg rod	Staph + gm neg rod
bacteria	5	0	0	0	1	____	____	Staph	Staph
	6	0	32	22	7028	____	____	Staph	Staph
	1	0	1056	792	528	198	17	____	____
Non-	2	0	0	0	2	0	0	____	____
radiated +	3	0	264	1386	924	60	2	____	____
l.p. bacteria	4	0	2	2	2	0	0	____	____
	5	0	0	0	16	7	0	____	____
	6	0	0	1	0	0	0		

a - probably i.v. instead of i.p.
b - probably i.m. instead of i.p.
* - post mortem

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instead of the I.V. injections. Blood cultures were taken before injection and ½, 1, 5, 24 and 48 hours after injection. Heart's blood and cultures of the serosal surface of the gut were obtained at post mortem.

Hematology:

2 control white blood counts were obtained on each dog. This determination was repeated following radiation on the same schedule as the blood cultures.

Pathology:

Post-mortem exams were done on the animals as in the sepsis experiment.

Results: Mortality;

Treatment	# dogs treated	# died	Day of Death
Radiation + peritonitis	6	6	1,1,1,1,2*,3*
Peritonitis	6	0	---

* Gross pathology showed no peritonitis. Blood cultures showed that 2 were probably injected I.V. while pathology showed that 3 were injected into the rectum muscles.

Hematology:

Graph IV shows the WBC counts of the 2 groups of dogs on the 7th day post-irradiation following the intraperitoneal injection of staph aureus.

Pathology:

Changes in all radiated animals were typical of the usual pathology found in the radiation syndrome except that the four animals in which I.P. injections were accomplished , showed erythema of the entire bowel serosa, with bloody fluid free within the abdomen. The one animal had gangrene of the terminal ileum. The animal which was presumed to have been injected intravenously showed no gross evidence of peritonitis. Another dog had a hemorrhagic necrosis of the muscles

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and subcutaneous tissue covering the left side of the abdomen and lower chest suggesting that the intended I.P. injection had actually been deposited intramuscularly. This dog had no gross pathology indicative of peritonitis.

Discussion:

Aureomycin has recently proved in our laboratory to be ineffective in the treatment of the uncomplicated radiation syndrome in dogs (1) . The increased lethality of the syndrome when infection is superimposed, as shown above in the studies on induced infection, points out the benefit that may come from the prophylactic use of a broad spectrum antibiotic for the human victim of an atomic catastrophe. Thus, even though such a drug may not decrease the mortality of the pure bred radiation disease, it may prevent the superimposition of infection, the severity of which is heightened when the host has been irradiated. Experiments are now under way to determine if an antibiotic will reduce this induced infection - radiation mortality.

The presence of infection in these experiments was not reflected in the white blood count even on the second day post-irradiation. The hematocrit was equally unrevealing. The gross pathology of the septic radiated dogs was similar to that of non-infected radiated dogs. These findings suggest that the signs of infection might be masked by the radiation sickness to some extent, and that delayed recognition of infection would compromise the individual's chances for survival.

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Summary:

A preliminary study of the effects of induced sepsis and peritonitis in the radiated (LD50) dog is presented. 100% of the animals infected died, and the morbidity rate was very high. The WBC, and hematocrit did not reflect the presence of infection. A high incidence of terminal unrestrained growth of bacteria in the blood stream occurred. The gross pathology of the animals with sepsis showed changes indistinguishable from those of the uncomplicated radiation syndrome except for the severe hemorrhages in the kidney cortex. This anatomic area was much more extensively involved than usual. The value of a prophylactic broad spectrum antibiotic is discussed.

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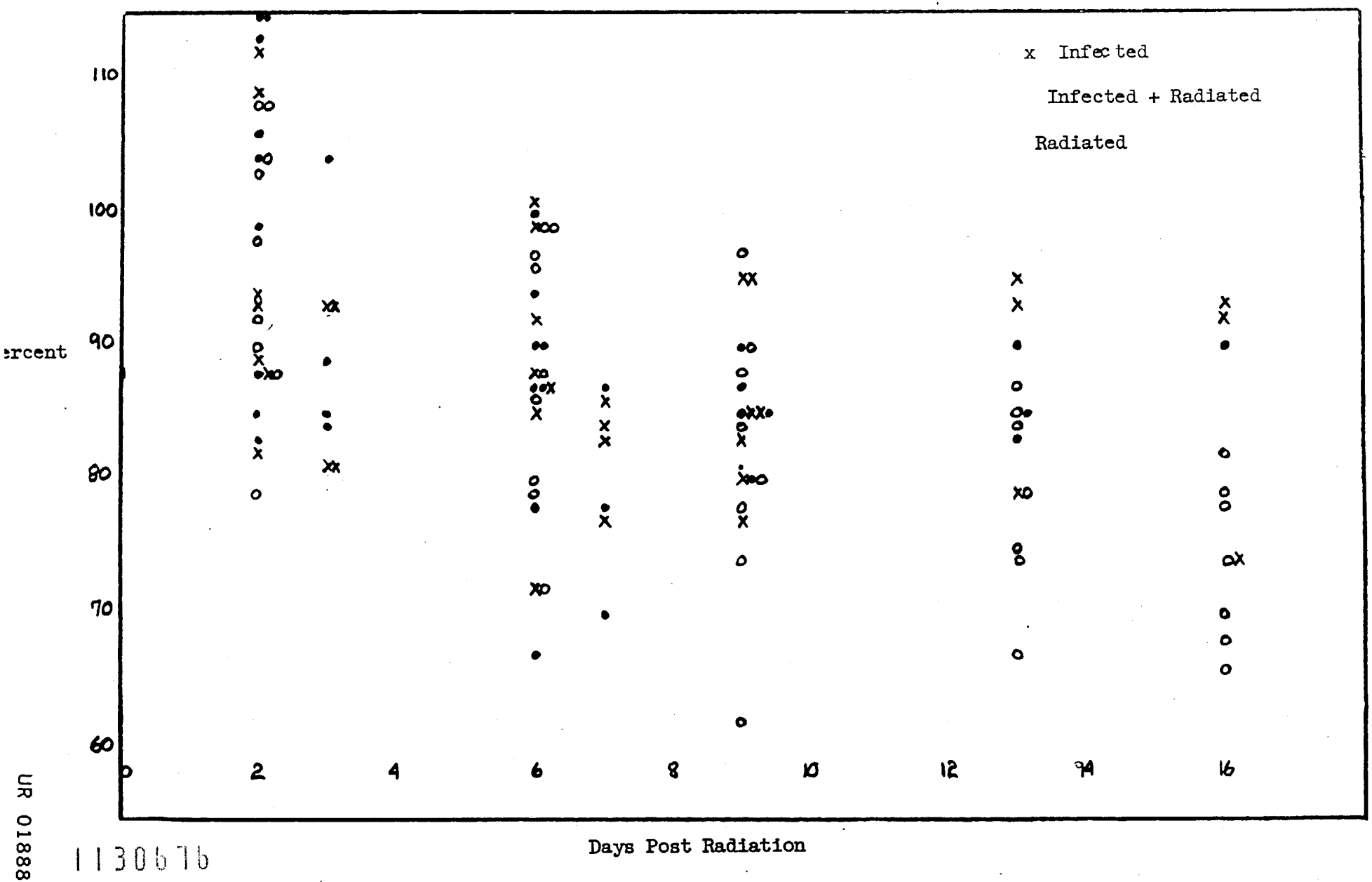
Sepsis

Graph I

Hematocrit Values



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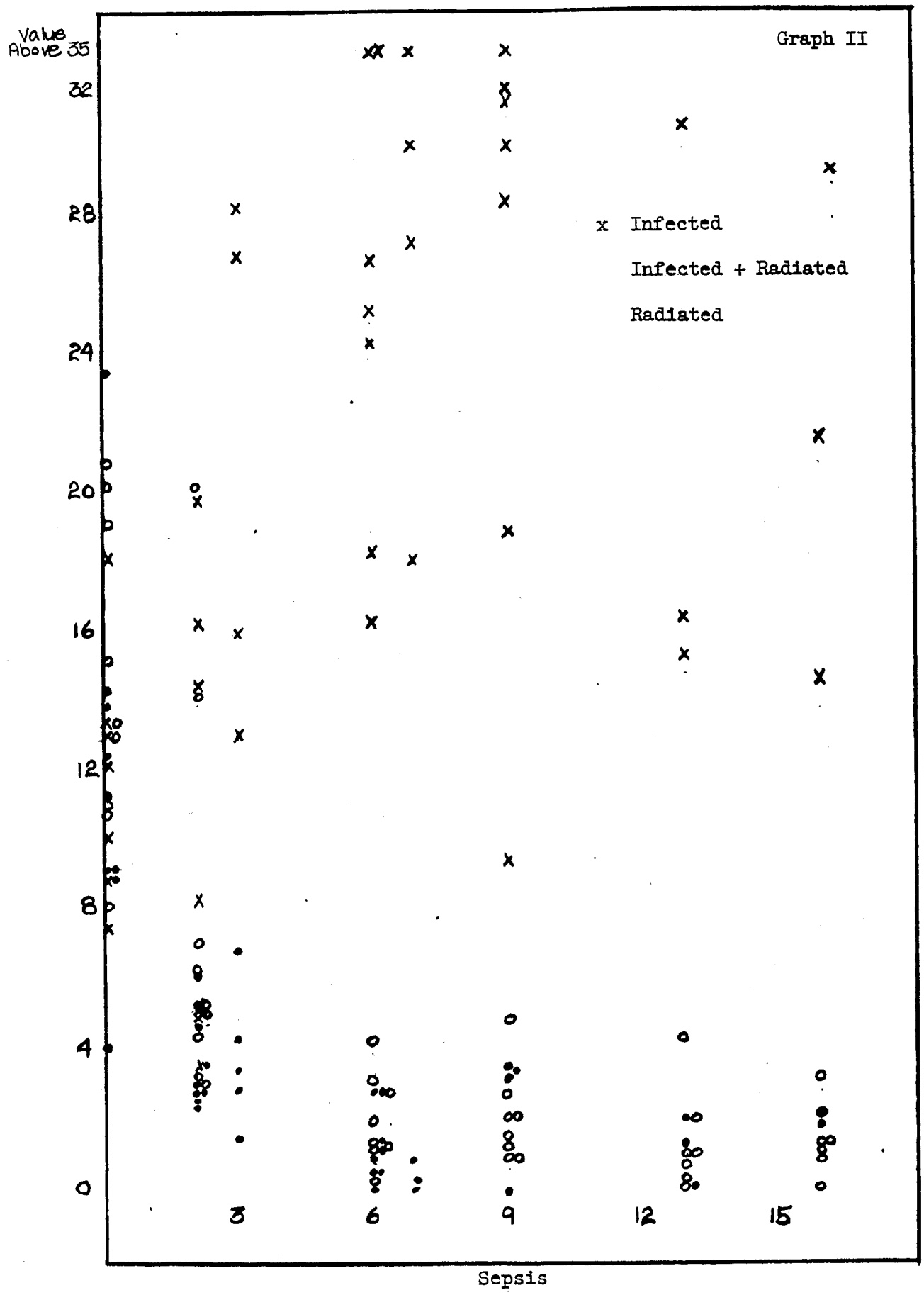

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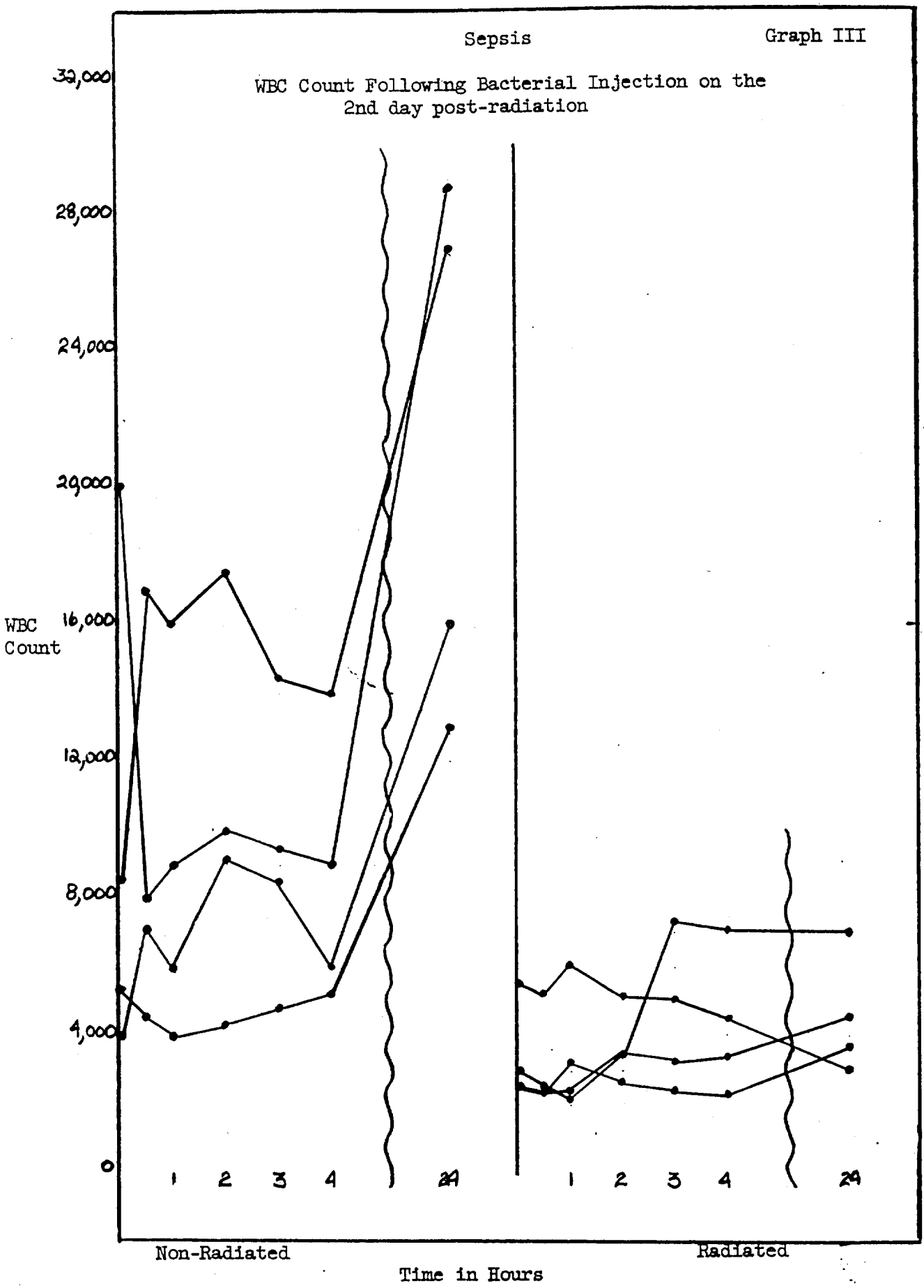
Sepsis
Graph III
WBC Count Following Bacterial Injection on the
2nd day post-radiation
32,000
28,000
24,000
20,000
16,000
12,000
8,000
4,000
0
WBC
Count
1
2
3
4
24
1
2
3
4
24
Non-Radiated
Radiated
Time in Hours

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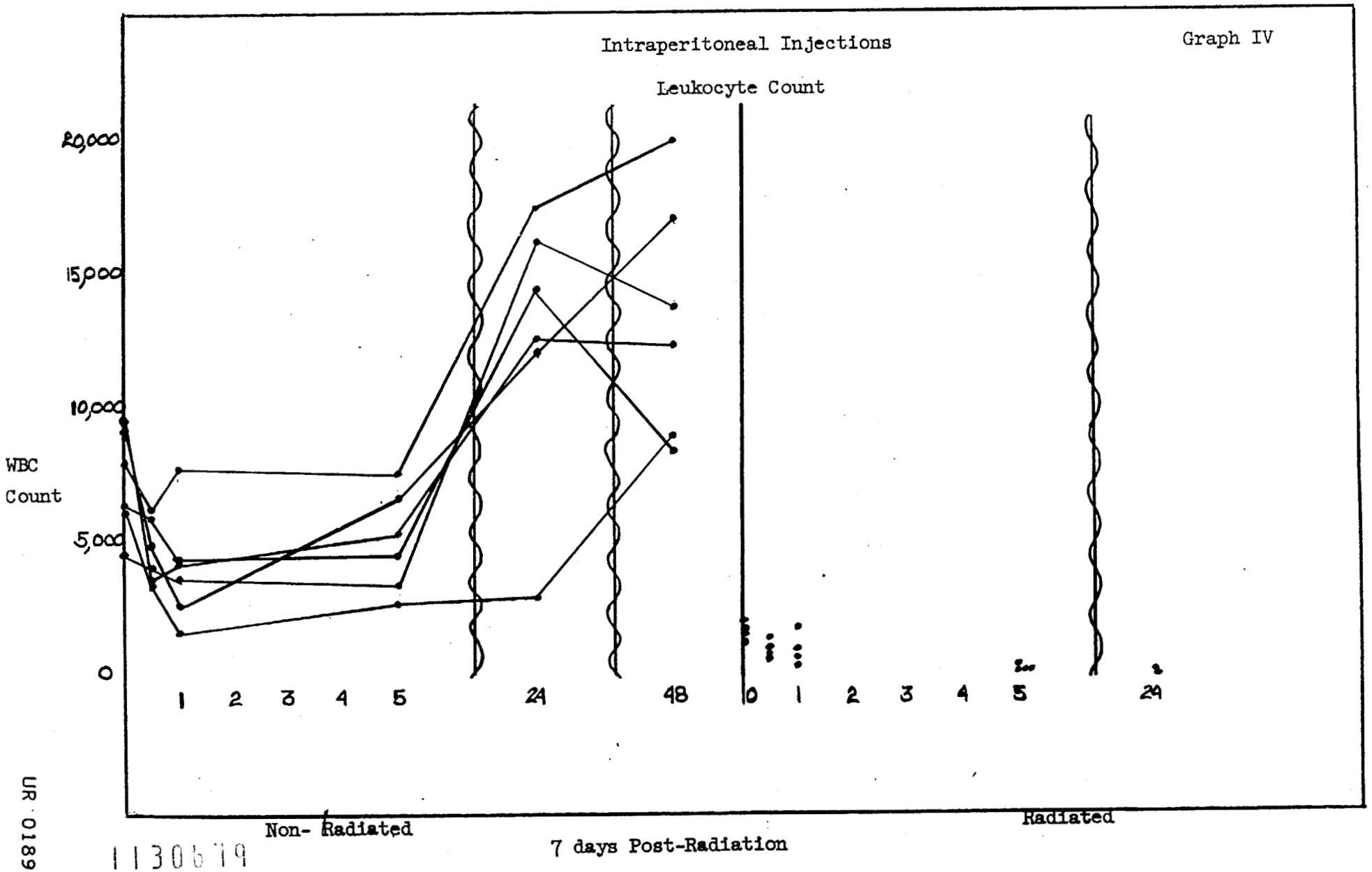

Time in Hours Following I. P. Injections of Staph Aureus

1130679

UR 01891

STUDIES RELATIVE TO THE PHAGOCYTIC ACTIVITY OF LEUKOCYTES IN IRRADIATED DOGS

by
M. Ingram and M. Adams

Abstract

A convenient in vitro method of studying the phagocytic activity of leukocytes by incubation whole heparinized blood with saccharated iron is described.

Preliminary interpretation of studies relative to the phagocytic activity of leukocytes of irradiated dogs and irradiated dogs treated with Pyromen after exposure indicates that:

1. Irradiation with 300 r produces a decrease in phagocytic activity of leukocytes one week after exposure. This effect is not observed in Pyromem treated dogs exposed to a like dose.

2. Phagocytic activity returns to normal before the beginning of hematopoietic recovery as indicated by leukocyte counts.

3. The magnitude and direction of post-exposure trends of the leukocyte counts are similar in the two groups of dogs.

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The phagocytic activity of leukocytes is generally considered to be of central significance in combatting infection and tissue damage, and in clearing away debris, whether it be produced by normal wear and tear or by sudden trauma. In this respect there has been considerable speculation regarding 1) the effects of whole body irradiation on phagocytosis and 2) the possible contribution of altered phagocytic activity to the general picture of radiation induced damage and subsequent recovery. The present report is intended primarily as a description of a convenient method for direct study of the phagocytic activity of leukocytes. Certain preliminary observations which have been made in initial experiments are presented briefly.

Methods of studying phagocytosis generally fit into one of three categories, namely 1) histological studies in which tissues are observed with special regard for the presence of phagocytes containing debris or injected material, 2) studies of the fate of injected particulate materials which may or may not be labeled with radio-

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active isotopes and which are known to be readily phagocytized, and 3) studies of phagocytes incubated with bacteria or similar particles, as in the opsonocytophagocytic test. The method described in this report belongs in the third category and appears to be unusually well suited to direct evaluation of phagocytic activity of white blood cells.

Specifically the method consists of incubating freshly drawn heparinized whole blood with saccharated iron* which is readily taken up by granulocytes and monocytes. This material has several advantages of which the most notable are 1) low toxicity for leukocytes, 2) the rate and extent of uptake are not influenced by prior specific sensitization of the animal to the phagocytized substance, and 3) the presence of saccharated iron in the cells may be beautifully demonstrated by the Prussian Blue reaction.

The procedure is carried out at 37.5° C. ± approximately 0.1° C. A water bath equipped with an electric stirrer and cold water cooling coils as well as electrical heating units and a thermoregulator permits maintenance of the temperature within these limits. The reaction vessel is a 10cc volumetric flask with a snugly fitting rubber stopper. A solid glass rod runs from the bottom of the flask through the stopper and fits into the chuck of an electric stirrer. A long #20 needle with stylet also runs through the stopper and is long enough to reach the bottom of the flask. A second short #20 needle without stylet is inserted through the stopper to permit excape of air when blood is injected into the otherwise closed system.

Before a run is made, the water bath is brought up to the desired temperature and observed for a period of time sufficient to assure the observer that temperature is maintained within the desired limits. During this warm-up period the stirrer holding the volumetric flask is lowered so that the flask is immersed approximately to the calibration mark in the water bath. A small 5cc bottle containing dried heparin

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* The commercial preparation Proferrin, kindly supplied by Sharp and Dohme Co., has been used in these studies.

and a measured amount of saccharated iron is also suspended in the water bath. When the temperature has stabilized at 37.5° C., 10cc of venous blood is drawn quickly using a syringe and needle which have been previously warmed to body temperature in a hot air oven. This blood is immediately discharged into the heparin-saccharated iron bottle which in turn is held in the water bath where mixing is accomplished by rotating the bottle by hand in the horizontal plane for 30 seconds. The mixture is then aspirated into a second warmed syringe and transfered quickly to the volumetric flask through a long needle. The stylet is replaced, the stirrer holding the flask started, and set at the lowest speed indicated on the rheostat control dial. Blood can be sampled at any time by stopping the stirrer, removing the stylet and attaching a syringe to the long needle. The sampling procedure requires 15-20 seconds. The equipment and procedure are illustrated in Figures I and II.

Coverslip blood smears are pulled from the blood sample aspirated from the flask. These smears are later stained by immersion for approximately 12 seconds in a mixture of equal parts of freshly prepared 10% potassium ferrocyanide and 10% HCL at 55-60° C. A 0.3% solution of basic fuchsin in 95% ethyl alcohol is used as a counterstain. The basic fuchsin stains nuclei deep pink, erythrocytes and cytoplasm of leukocytes pale pink. The ingested iron is identified by the vivid Prussian Blue color which makes even single granules of ingested iron stand out sharply.

Cells which contain iron are classed as "marked", those which do not as "unmarked". In reading smears, the lymphocytes are considered to be non-phagocytic, all other leukocytes as phagocytic. Leukocytes are classified as 1.) marked phagocytes, 2.) unmarked phagocytes, or 3.) lymphocytes. The extent of phagocytosis is then expressed as the percent of phagocytes which contain ingested iron.

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Preliminary observations utilized the blood of a dog with 20,000 - 30,000 leukocytes per cubic millimeter. Three tenths of a cc of saccharated iron was found to be sufficient to mark a large percentage of the phagocytes in 10cc of this blood in half an hour of incubation. Larger amounts of saccharated iron resulted in negligible increases in the uptake. Accordingly, this proportion of 0.3cc saccharated iron/10cc blood has been used in all experiments carried out to date.

Although interpretation of the results of initial studies of phagocytic activity of the leukocytes of normal and irradiated dogs is not yet completed, certain interesting observations can be made on the basis of a preliminary survey of results. Representative preliminary data from an experiment utilizing four dogs are presented in Table I.

In this experiment, 4 dogs received whole body exposure to 300 r of 250 KVP x-rays. Two of the dogs received 2 micrograms of Pyromen* i.v. three times a week. The other two dogs received no Pyromen. Phagocytosis studies on each dog's blood were done once a week for 4 weeks, and one last run was made six weeks after exposure. These studies consisted of serial sampling of incubated blood-saccharated iron preparation over a period of 40 minutes. The protocol called for classification of 1000 leukocytes for each dog for each interval sampled. Table I, however, represents only the final interval (i.e. after 40 minutes incubation) and the figures are subject to moderate

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* Pyromen is a non-protein bacterial component which, among other effects, is believed to stimulate the reticulo-endothelial system. The Pyromen for these experiments has been supplied through the courtesy of the manufacturers, Travenol Laboratories, Inc.

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revision since in some instances fewer than 1000 cells have been classified.

The more noteworthy aspects of results obtained to date are as follows:

1. Phagocytic activity of the leukocytes of heavily irradiated dogs is depressed transiently during the first post-exposure week.
2. Phagocytic activity returns to approximately normal levels two weeks after exposure despite the fact that leukocyte counts remain markedly depressed until much later and are still below normal six weeks after exposure.
3. The post-exposure administration of 0.2 cc of Pyromen three times a week appears to have prevented the post-exposure depression of phagocytic activity of dog leukocytes.
4. The post-exposure administration of 0.2 cc of Pyromen three times a week did not alter the direction or magnitude of the trend of leukocyte counts during the post-exposure period. Specifically, the production of granulocytes appears to have had no higher priority in Pyromen-treated dogs than in untreated dogs.

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Table I

Leukocyte Phagocytic Activity Expressed as % of Pre-exposure Phagocytic Activity

(Preliminary Survey of Results)

	Pre-exposure	Post Exposure			
		1 wk	2 wks	3 wks	4 wks
Radiated (2 dogs)	100%	63%	114%	108%	111%
Radiated plus Pyromen (2 dogs)	100%	107%	123%	121%	107%

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Table II

Phagocytic Activity of Leukocytes of Irradiated Dogs

		Irradiated	Irradiated + Pyromen
Pre-exposure	% granulocytes*	78%	84%
	Phagocytic activity**	100%	100%
1 wk. post-exposure	% granulocytes	84%	85%
	Phagocytic activity	63%	107%
2 wks. post-exposure	% granulocytes	71%	79%
	Phagocytic activity	114%	123%
3 wks post-exposure	% granulocytes	64%	75%
	Phagocytic activity	108%	121%
4 wks post-exposure	% granulocytes	83%	80%
	Phagocytic activity	111%	107%

* As determined on routine differential WBC counts.

** Expressed as % of pre-exposure phagocytic activity, or

$$\frac{\text{\% phagocytes containing ingested iron after 40 minutes incubation during post-exposure period}}{\text{\% phagocytes containing ingested iron after 40 minutes incubation during pre-exposure period}} \times 100$$

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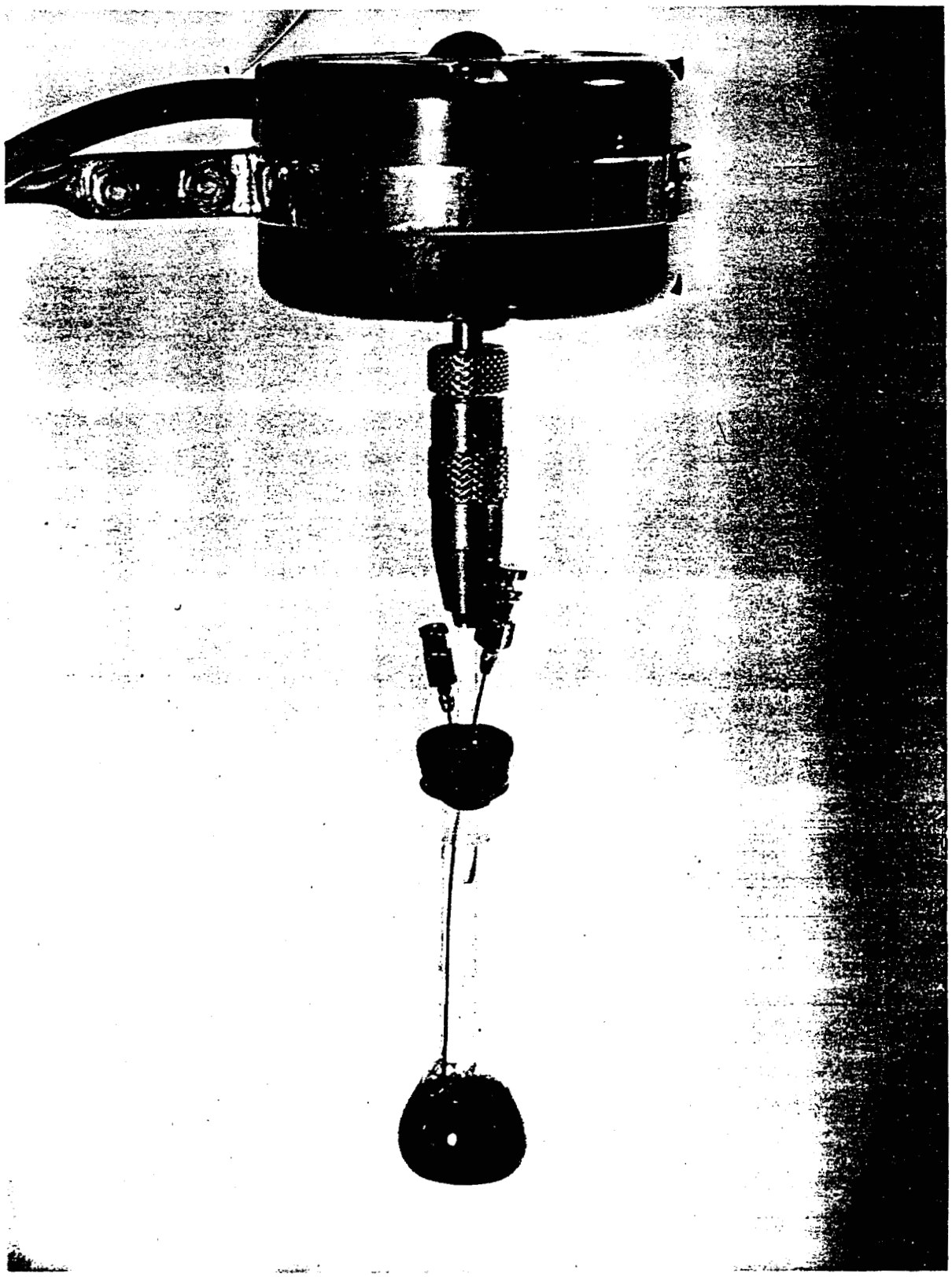

Figure 1.

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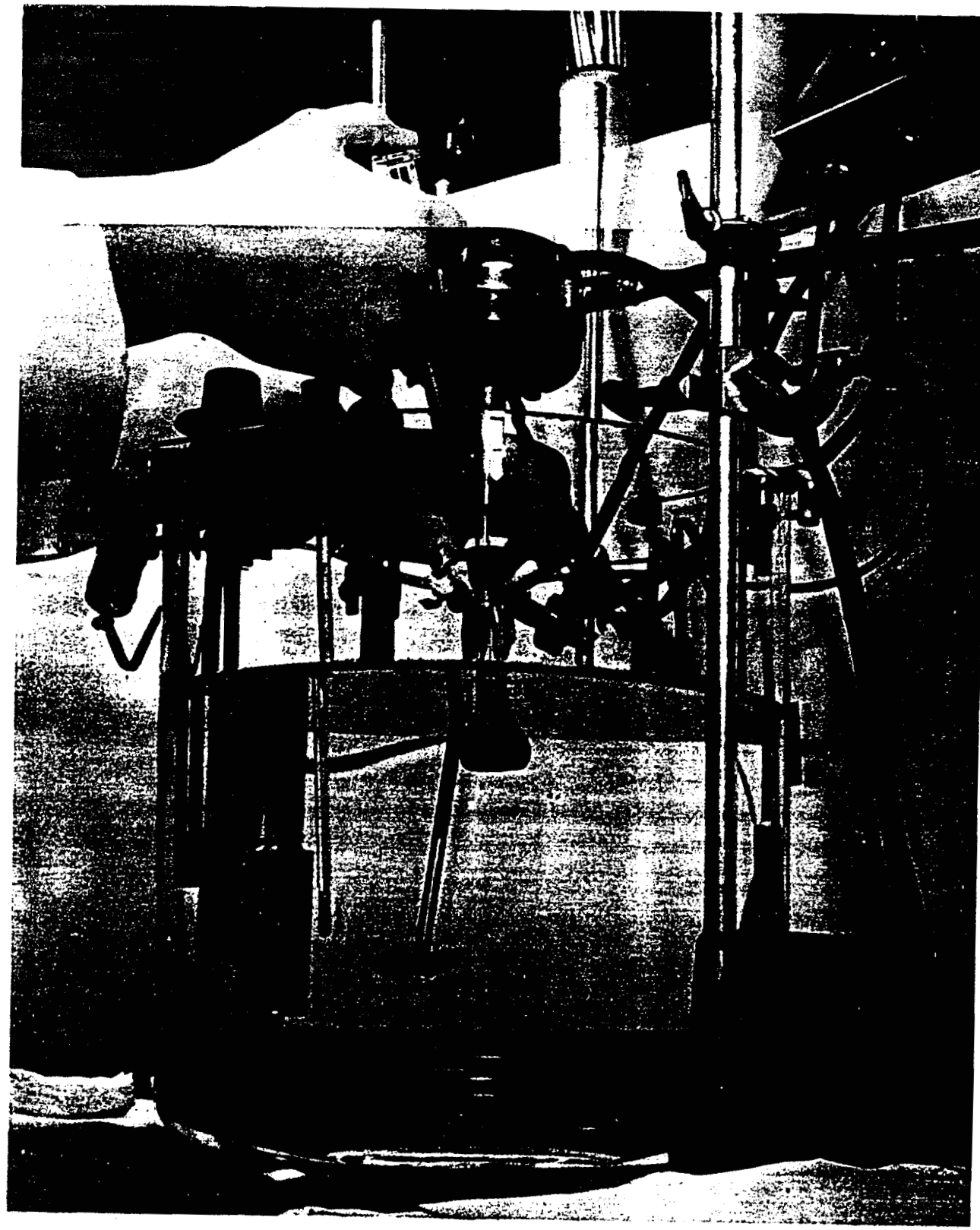

Figure 2.

1130688

EFFECT OF RUTIN ON THE X-IRRADIATED DOG

by
M. Coulter and F. Furth

Abstract

A group of 24 normal dogs were exposed to 450 r of 250 KV x-irradiation. This dose has been producing a mortality of 60-70% in dogs in this laboratory. The dogs were divided into four groups of 6 animals each.

Group	Mortality
I Controls	100%
II Rutin, 50 mgm t.i.d. orally for 1 week prior to radiation	83%
III Rutin, 50 mgm t.i.d. orally for 4 weeks post-radiation	83%
IV Rutin, 50 mgm t.i.d. orally for 1 week prior and 4 weeks post-radiation	83%

There was no significant differences in the rates of mortality or in the post mortem findings between the groups.

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One of the major factors contributing to the mortality of the acute radiation syndrome is an hemorrhagic diathesis. Several factors may play a role in the production of this hemorrhagic tendency including bone marrow depression with resulting thrombocytopenis, hyperheparinemia, and loss of vascular integrity.

There is conflicting evidence in the literature regarding the effectiveness of the "Vitamin P" compounds in protecting animals exposed to ionizing radiation from this bleeding tendency.

Field and Rekers (1) report a mortality of 60% in control dogs exposed to 350 r of 250 KV x-irradiation. Rutin administered following the irradiation had no effect on mortality, whereas its administration of 1 week prior to irradiation reduced the mortality in 6 dogs to 33%, and when given for 1 week prior and 4 weeks post-radiation reduced the mortality in 27 dogs to 11%. When a dose of 450 r was used, this therapy was ineffective; the mortality in 5 control dogs being 80% and in 4 dogs treated before and after radiation with rutin, 75%.

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Sokoloff has reported a reduction in mortality in rats exposed to 800 r x-irradiation when vitamin P substance was administered in a dose of 5 mgm/day for 30 days (2). The control mortality was 80% and in the treated animals 10%.

In this laboratory a group of 60 rats were exposed to 750 r of 250 KV x-irradiation. One half of the rats received 5 mgm rutin orally daily for 1 week pre and 4 weeks post-irradiation. The 28 day mortality was 47% in both control and treated animals. However, the incidence of diarrhea post-radiation was markedly diminished in the rutin treated rats as compared with the controls. The effect has been noted frequently with antibiotic therapy of the irradiated rat (3).

Kohn and his associates have reported on the failure of rutin to protect rats exposed to an LD 50 of 250 KV x-irradiation (4), and Cronkite has reported on the ineffectiveness of this drug in protecting mice which had been exposed to lethal doses of ionizing irradiation (5).

In view of the conflicting reports concerning the effectiveness of vitamin P compounds in the therapy of the acute radiation syndrome, it was decided to repeat the studies on dogs as done by Field and Rekers. This is a preliminary report and we are aware that the number of dogs used in this study is too small to be statistically significant.

A group of 24 normal dogs were exposed to 450 r of 250 KV x-irradiation. This dose has been producing a mortality of 60-70% in dogs in this laboratory. The dogs were divided into four groups of 6 animals each.

Group	Mortality
I Controls	100%
II Rutin, 50 mgm t.i.d. orally for 1 week prior to radiation	83%
III Rutin, 50 mgm t.i.d. orally for 4 weeks post-radiation	83%
IV Rutin, 50 mgm t.i.d. orally for 1 week prior and 4 weeks post-radiation	83%

There was no significant differences in the rates of mortality between the groups. Minimal subcutaneous hemorrhage was noted, with no apparent difference between the groups. The white blood cell and platelet counts and the hematocrits

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declined in a similar manner in each of the groups. Gross pathological findings revealed no differences in the extent of hemorrhage between the groups. As typically seen in this syndrome, those dogs dying at the end of the first week post-radiation frequently showed severe pulmonary hemorrhage while those dying in the 3rd and 4th weeks frequently had severe gastro-intestinal hemorrhage.

Discussion: Rutin therapy had no beneficial effect on the irradiated dogs in this study. It should be pointed out that Field and Rekers found no striking benefit from this drug when the irradiation dose was 450 r. However, as noted previously in this paper, this dosage was chosen as it has been found to produce an LD 60-70 in this laboratory during the past 3 years. Further studies with this drug and larger groups of dogs are contemplated.

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1130691

A METHOD FOR COMPLETE EXTRACTION OF EMBEDDING MATERIAL FROM TISSUE SECTIONS FOR THE ELECTRON MICROSCOPE

by
Michael L. Watson

Abstract

A method for complete extraction of embedding material from butyl methacrylate embedded sections for the electron microscope is described. The extraction is carried out in the microscope by electron bombardment at high beam intensities made possible by removing the condenser aperture. Little or no distortion due to the method of extraction is observed. Extraction is not the result of simple heating but may be due to a depolymerization resulting from electron bombardment.

Shrinkage normal to the section occurs and is greater in some tissue components than in others. It is suggested that this observation may make possible an estimate of the relative water contents of different tissue components.

Background: Claude and Fullam (1, 2) reported on a study of embedding materials which might be removed from tissue sections by sublimation, and in this work recognized the necessity for and difficulty of removal of embedding material without distortion.

An obvious method for removal of embedding material consists in extraction with a suitable solvent. Surface tension effects during drying and possibly during the dissolving introduce serious distortion as pointed out by Hillier (3).

Partial sublimination in the microscope of paraffin-celloidon and of butyl methacrylate has been noted (4). Under ordinary conditions, however,

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clearing is incomplete. In this laboratory near dirt particles on butyl methacrylate embedded sections, it has been observed that there is often complete clearing on exposure to the electron beam with little or no distortion. It was thought that heat developed by the particle due to electron absorption was reponsible for the extraction. Exposure of sections to an incandescent tungsten filament in vacuo removed the embedding material adequately (5). However, this often introduced distortion which is presumably due to boiling of the plastic. The presence of distortion in many sections cleared by simple heating and its absence in sections cleared by the electron beam near dirt particles suggested a difference in the mechanism of clearing.

Since incomplete extraction occurred on exposure to operating beam intensities in the microscope, it appeared that gradual changes took place in the plastic during exposure to the beam which prevented all of it from being extracted. Accordingly, much higher beam intensities were tried. Removal of the condenser aperture from the microscope (RCA EMU-2A) increased the available beam intensity by about 100 times. Exposure of sections to the highly intense beam resulted in almost instantaneous removal of embedding material. Little or no distortion could be detected on comparison with unextracted sections (figures 1 and 2, pages 63 and 64).

<u>Methods</u>: To carry out this type of extraction the condenser aperture is removed*. The specimen is placed in the usual position. The condenser current is set at maximum before turning on the beam. This is necessary because reflections of the beam apparently from the objective pole-piece

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*The microscope should be monitored for excessive x-ray production. Our microscope produced about 80 mr per hour at 4 inches from the viewing chamber with the beam at cross-over when the condenser aperture was removed.

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make it difficult to control the illumination on the specimen if cross-over is below the specimen. With the microscope in operation an area is selected for clearing. The condenser current is reduced at such a rate that the image of the filament is brought into focus in about one-half a second. Before this focus is reached the section should clear. The condenser current is immediately returned to maximum.

Clearing occurs at lower beam intensity in the middle of a square and is most satisfactory with 100 mesh rather than 200 mesh specimen grids. Silica-coated colloidon substrates are used to provide adequate strength for the extraction process. A certain amount of trial is necessary to find the minimum amount of silica required. Experience has shown that thick sections are much more apt to tear than thin ones.

Results: Figures 1 and 2 (pages 63 and 64) show views of a section before and after electron extraction. It is apparent that minute details barely visible in the unextracted section are easily seen in the same section after extraction.

An important advantage of complete electron extraction over partial electron extraction lies in the fact that the section may be effectively shadow-cast. Figure 3 (page 65) shows a view of the male rat germinal epithelium after shadowing. It is apparent from this picture that shrinkage normal to the substrate has occurred since some structures which originally extended beyond the limits of the section cast shorter shadows than others. These include the nuclear wall, the cell wall, and the microsomes. The mitochondria are about three times as high as the microsomes. Thus we are offered the possibility of estimating relative water contents of tissue components on the basis of their relative shrinkage on electron extraction.

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Figure 1. A portion of the alveolus of the rat before removing the embedding material. Details are rather hazy and contrast is poor. 10500 X.

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Figure 2. The same field as shown in figure 1 after extraction by electron bombardment. In the original print the 640 A banding of collagen fibers can easily be made out, whereas it cannot be seen at all in figure 1. 10500 X.

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Figure 3. A view of the male rat germinal epithelium. A portion of a young resting spermatid lies on the left and on the right is a portion of the Sertoli cytoplasm. Details of the spermatid cell wall and nuclear wall are clearly visible. This section was shadow-cast with uranium after extraction of the embedding material. Relatively greater shrinkage of microsomes (m) over that of mitochondria (M) in a direction normal to the section is apparent. 29200 X.

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UR 01909

Electron extraction has been in use in this laboratory for about a year with satisfactory results. No trouble is experienced with poor resolution due to inadequate extraction or due to distortion arising from the extraction method.

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1130698

A NEW MICROTOME FOR THIN-SECTIONING FOR ELECTRON MICROSCOPY

by
Michael L. Watson

Abstract

A new microtome is described for the cutting of thin sections for the electron microscope. The specimen is mounted on an arm attached to a constantly rotating shaft. The advance mechanism for the knife consists of a stiff member supporting the knife which is bent by stretching a relatively flexible spring attached to it. Very reproducible motion is claimed. Ribbons with a thickness of less than 300 Å have been obtained.

Background: A conventional microtome adapted for thin-sectioning for electron microscopy in this laboratory proved inadequate where extremely thin sections were desired. A simplified mechanism more reproducible in operation was designed and built.

Description: The tissue block is mounted on an arm attached to a rotating shaft. The cutting face is perpendicular to the axis of the shaft. The shaft rotates at constant speed and end-play is satisfactorily controlled by a disk attached to the rotor and turning in a spring-loaded thrust bearing. Details may be seen in figures 1 and 2 (pages 68, 69).

The knife is mounted with its cutting edge perpendicular to the axis of the rotor. It is supported at the centers of two parallel brass strips which are in turn attached to the base-plate of the microtome at their ends (see figure 3, page 69). The knife is advanced by bending of the brass strips due to the tension on the feed spring attached at their center line. The tension on this spring is varied through a worm-gear coupling to the rotor. Relative motion of the two ends of the feed spring is a function of the

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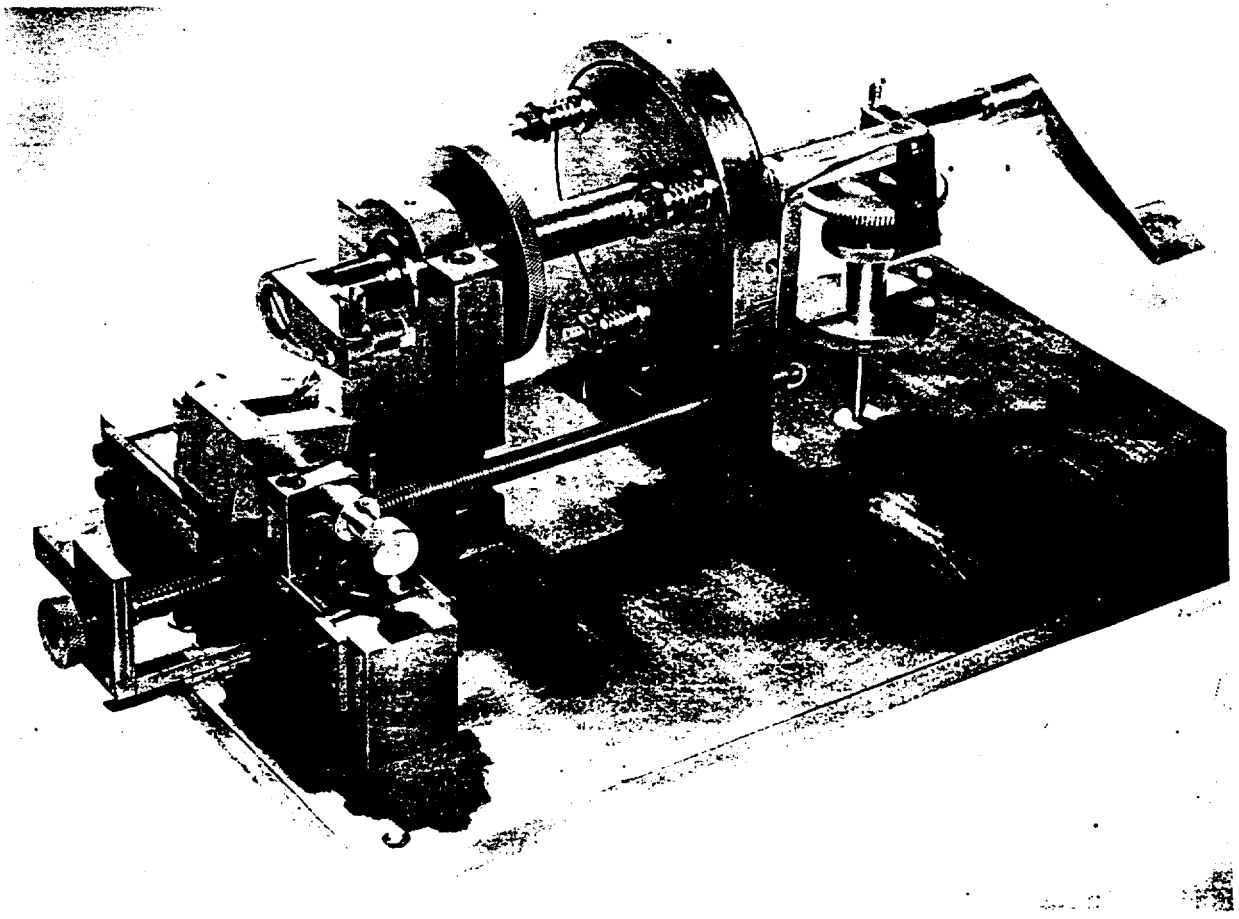

Figure 1. An over-all view of the microtome.

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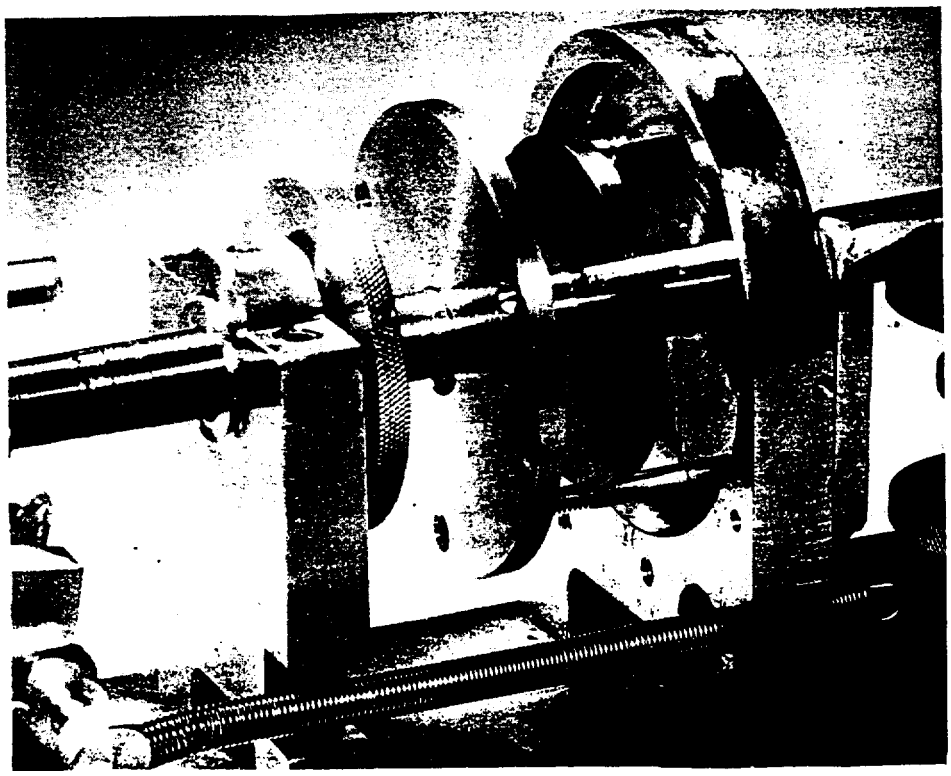

Figure 2. The thrust bearing is disassembled to show the thrust plate.

Figure 3. A view of the motor coupling to the microtome.

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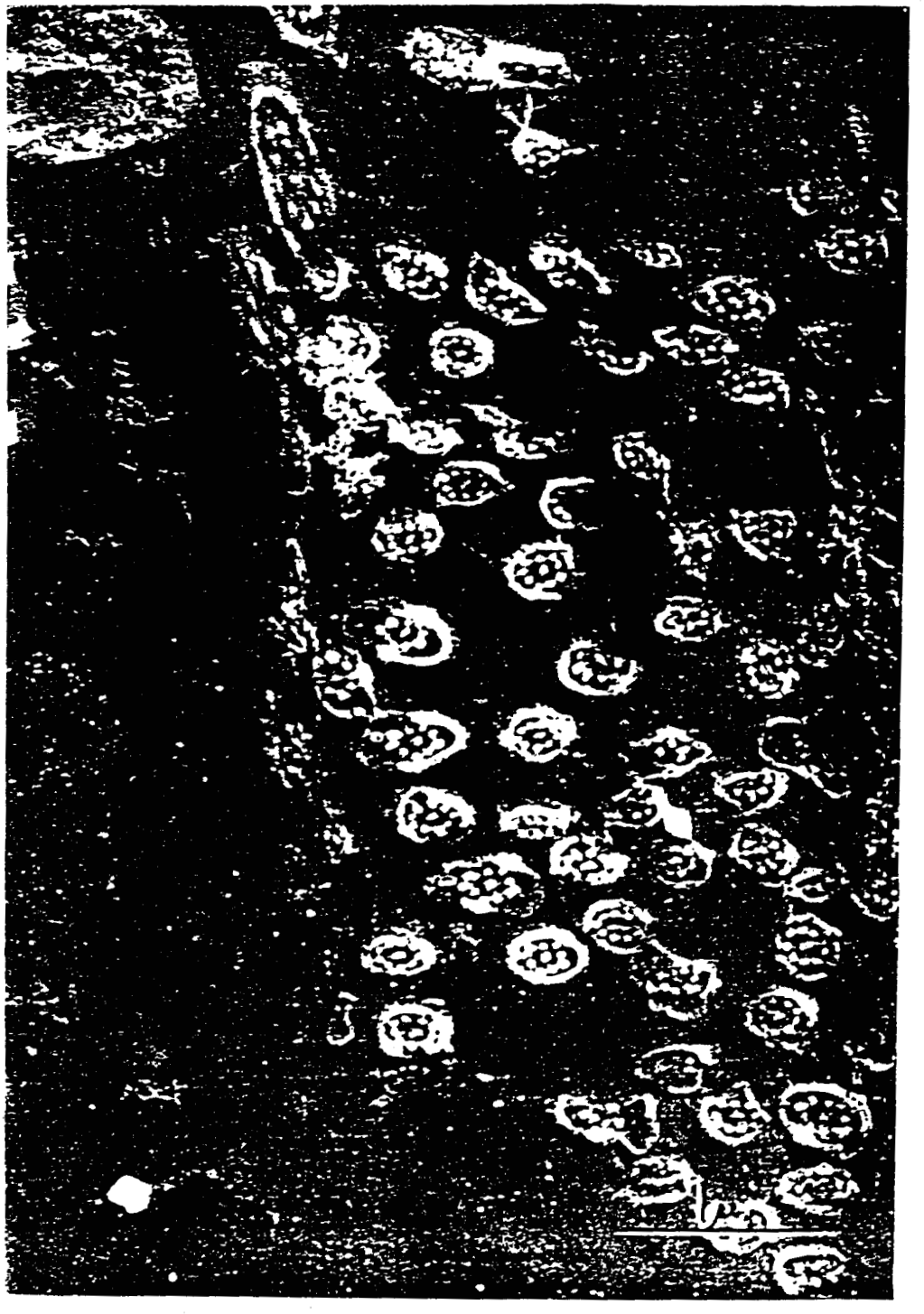

Figure 4. Transverse sections of sperm tails in the male germinal epithelium of the rat. Uranium shadowed at an angle of 5:1. 29200 X.

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stiffness of the brass strips and the characteristics of the spring. On the unit described the relative motion is about a million to one. Damping of the knife feed is necessary to prevent vibration and is obtained by the use of an oil-soaked cotton stuffing in the feed spring and oil-soaked blotting paper between the brass strips and the block supporting the knife holder.

The specimen arm is screwed onto the end of the rotor-shaft which is split to permit friction adjustment. A coarse setting of the specimen relative to the knife may thus be obtained. For finer adjustment a spring is held in compression against the brass knife-mounting strips opposite the feed spring. The degree of compression is controlled with a thumb screw.

The motor drive coupling (figure 3, page 69) is designed to reduce transmission of longitudinal vibrations to the rotor shaft. The rotor is driven at about 60 rpm.

Results: The microtome has been in use in this laboratory for about six months. Using a glass knife we have been able to cut ribbons under favorable conditions in which sections were too thin for tissue details to be readily seen in the microscope without complete removal of embedding material and shadow-casting. Cross-sections of sperm tails (figure 4, page 70) which contain rather dense fibrils were made. Shadow-casting and subsequent measurement of the shadows indicated a thickness of about 180 Å. Allowing for shrinkage during the extraction of the embedding material we estimate that the initial section thickness was probably less than 300 Å.

The constant motion of the rotor allows much greater reproducibility of motion of the specimen relative to the knife than can be obtained with reciprocating mechanisms. The type of advance used eliminates all of the trouble experienced in conventional microtomes with starting friction. A microtome of this type is relatively simple and easily built.

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UR 01915

TECHNICAL REPORTS ISSUED FOR DISTRIBUTION

January 1, 1952 thru March 31, 1952

Report No.	Title	Authors	Subject Category
UR-185	Spermatogenesis in the Adult Albino Rat as Revealed by Tissue Sections in the Electron Microscope (UNCLASSIFIED) Issued: March 7, 1952	Watson	Health and Biology
UR-191	Some Potential Hazards of the Widespread Use of Roentgen Rays in Pediatrics (UNCLASSIFIED) Issued: March 24, 1952	R. Miller	Health and Biology
UR-192	The Toxicity and Urinary Excretion of Crystalline Metaphosphates (UNCLASSIFIED) Issued: March 24, 1952	Gosselin Maynard Downs	Health and Biology
UR-200	Quarterly Technical Report October 1, 1951 thru December 31, 1951 (UNCLASSIFIED) Issued: March 4, 1952	Blair	Health and Biology

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