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Director

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INTRODUCTION

The scientific work presented herein has been coded at the program and problem levels according to the scheme given on Pages 7 and 8. In the report all contributions to a given problem have been assembled together without regard to the administrative organization except that the number of the section which did the work is prefixed in each case. By using this number, it can be found on Page 12 what administrative officer can be approached for information about particular work.

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 a final report.

EXPLANATION OF PROGRAM AND PROBLEM CODES

The scientific work at The University of Rochester Atomic Energy Project has been coded at the program and problem levels. The programs, in general, indicate broad fields of investigative or service activities while the problems indicate divisions of these fields. Although no consistent method of division in problems was possible, an attempt was made to achieve a natural division in the sense that each problem would encompass a subject normally written up and generally considered as a unit. The program or chemical toxicity of uranium, for example, has been broken down into problems according to the divisions commonly employed by toxicologists.

The problem codes are not related directly to the administrative organization of the Project. Consequently, the smallest administrative unit, the section, may work on more than one of the coded problems. Conversely, more than one section may work on the same coded problem. The administrative organization will be ignored in making this quarterly report of our research and service activities, all material being assembled according to the program and problem codes. The contribution of each section to a Quarterly Technical Report will be prefixed by the section number, however, to permit reference to the administrative organization if necessary.

It has not been possible to code the problems sufficiently broadly to avoid all overlapping. In cases in which various parts of a given investigation might be coded differently, the whole work was coded according to its principal subject matter as long as the minor subjects were relatively unimportant. Otherwise, the work was divided under appropriate codes.

PROGRAM AND PROBLEM CODES

I. X.R. BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (X-RAYS AND γ RAYS)

- X.R.1 Tolerance Studies (dose levels, surivial time, gross and histo-pathology)
- X.R.2 Mechanism of Effects (physiological and biochemical)
- X.R.3 Therapy (measures against radiation effects)
- X.R.4 Hematology
- X.R.5 Genetics (histogenetics)
- X.R.6 Embryology
- X.R.7 Bacteriology and Immunology

II. I.R. BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (INFRA-RED & ULTRA-VIOLET)

- I.R.1 Flash Burns

III. R.M. BIOLOGICAL EFFECTS OF RADIOACTIVE MATERIALS (CONTACT, INGESTION, ETC.)

- R.M.1 Polonium
- R.M.2 Radon
- R.M.3 Thoron
- R.M.4 Miscellaneous Project Metals

IV. U. URANIUM

- U.1 Physical and Chemical Properties
- U.2 Toxic Effects (description of acute and chronic toxicity)
- U.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)
- U.4 Fate (distribution and excretion)
- U.5 Mechanism of Toxic Effects
- U.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

V. Be. BERYLLIUM

Be.1 Physical and Chemical Properties

Be.2 Toxic Effects (description of acute and chronic toxicity)

Be.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

Be.4 Fate (distribution and excretion)

Be.5 Mechanism of Toxic Effects

Be.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

VI. Th. THORIUM

Th.1 Physical and Chemical Properties

Th.2 Toxic Effects (description of acute and chronic toxicity)

Th.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

Th.4 Fate (distribution and excretion)

Th.5 Mechanism of Toxic Effects

Th.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

VII. F. FLUORIDE

F.1 Physical and Chemical Properties

F.2 Toxic Effects (description of acute and chronic toxicity)

F.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

F.4 Fate (distribution and excretion)

F.5 Mechanism of Toxic Effect

F.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

VIII. Zr. ZIRCONIUM

Zr.1 Physical and Chemical Properties

Zr.2 Toxic Effects (description of acute and chronic toxicity)

Zr.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

Zr.4 Fate (distribution and excretion)

Zr.5 Mechanism of Toxic Effect

Zr.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

IX. S.M. SPECIAL MATERIALS

S.M.1 Physical and Chemical Properties

S.M.2 Toxic Effects (description of acute and chronic toxicity)

S.M.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

S.M.4 Fate (distribution and excretion)

S.M.5 Mechanism of Toxic Effect

S.M.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

X. I.S. ISOTOPES

I.S.1 Tracer Chemistry

I.S.2 Radioautography

I.S.3 Therapy

XI. O.S. OUTSIDE SERVICES

XII. P.H. PROJECT HEALTH

XIII. H.P. HEALTH PHYSICS

H.P.1 Research and Development

H.P.2 Service

XIV. C.S. SPECIAL CLINICAL SERVICE

XV. I.N. INSTRUMENTATION (SPECTROSCOPY, ELECTRON MICROSCOPY, X-RAY AND NUCLEAR RADIATION DETECTORS, X-RAY DIFFRACTION, ELECTRONICS)

I.N.1 Research and Development

I.N.2 Service

I.N.3 Instrumentation for Outside Organizations

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3170	Radiation Toxicology	J. Newell Stannard
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UNCLASSIFIED

PROGRAM X.R.

BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (X-RAYS AND γ RAYS)

Problem Code: X.R.2 (Mechanism or Effects)

Section Code: 3140

Authors: B. W. Gabrio and K. Salomon

The Distribution of Total Ferritin in the Intestine and Mesenteric Lymph Nodes of Horses After Iron Feeding.

Background. Ferritin is an iron protein complex compound which is used as an iron storage protein and which has been postulated also to play a role in iron absorption. It occurs mainly in spleen, bone marrow and liver. It also occurs after iron feeding in the intestinal tract. Because of the great sensitivity of the intestinal mucosa as well as of the hemopoietic system to radiation, more information about the role of ferritin in iron metabolism is desirable. Due to recent work carried out in this laboratory* as well as in the laboratory of Shorr and Mazur, a quantitative serological method for the determination of ferritin and apoferritin, its protein moiety, has been made available. Consequently, a quantitative distribution study of this protein has become possible. A thorough knowledge of the distribution of ferritin under normal conditions is essential before pathological conditions can be investigated.

It has been postulated that a mechanism exists in the gastrointestinal mucosa for the regulation of iron absorption. Hahn et al (1) suggested that ferritin in the gastrointestinal mucosa was involved in such a way that the protein moiety, apoferritin, could accept the iron and eventually, when saturated, prevent further absorption thus establishing a "mucosal block". Granick (2)

*B. W. Gabrio, Thesis for Degree of Doctor of Philosophy in Biochemistry

modified this postulate by contending that the mucosal cells were "physiologically saturated" with respect to the ferrous ions instead of ferritin, but that the ferrous ions were in equilibrium with ferritin iron.

In 1946 Granick (3) presented evidence which seemed to substantiate the hypotheses that ferritin exerted a regulatory action in the absorption of iron. Using the semi-quantitative technique of counting ferritin crystals formed on a slide after teasing a piece of tissue in the presence of 10% $CdSO_4$, he found ferritin only in trace amounts in normal duodenal mucosa of guinea pigs. After iron feeding, however, there was a rapid and profound increase in the amount of ferritin in the intestinal mucosa, and about 3-6 days after iron feeding, ferritin content had dropped to the level of the control animals. This increase and decrease of ferritin content paralleled the phenomenon of the "mucosal block".

Gillman and Ivy (4) were unable to demonstrate consistently this increase in crystallizable ferritin in guinea pig intestine after iron feeding, but Granick's results could be reproduced in our laboratory*. However, Gillman and Ivy presented histochemical evidence for the demonstration of iron which indicated that there was a progressive increase in the amount of iron in the intestinal epithelial cells, the lamina propria and tunica propria of the villi, the submucosal lymph vessels, and the mesenteric lymph gland after iron feeding, followed by a gradual decrease in the histologically demonstrable iron. This phenomenon might well be a morphological expression of the "mucosal block".

In the experiments to be reported here we were concerned primarily with the determination of total ferritin**, using a quantitative immunochemical

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*Gabrio, B. W. (unpublished work)

**Hereafter, unless specified otherwise, the term, ferritin, as applied to these experiments, will refer to total ferritin, which includes ferritin, apoferritin, and intermediate forms.

technique, in the intestine and mesenteric lymph nodes of the horse after iron feeding. We were interested to observe if ferritin was involved in the absorption of iron through the intestine of the horse, an animal from which ferritin can be obtained with ease and in quantity, and also to determine whether the increase in iron noted by Gillman and Ivy (4) in the mesenteric lymph nodes after iron feeding might be attributed to ferritin.

Methods. Three eight year old male horses* were used in these experiments. They were sacrificed by being shot through the head. Horse 1 served as the normal, untreated control and was fasted about 18 hours before it was sacrificed. Horse 2 and Horse 3, after an 18 hour fast, were fed gelatin-coated capsules of ferrous ammonium sulfate (containing a total of 30 gm. of iron) with the use of a capsule gun. Horse 2 was sacrificed 24 hours after the administration of iron while Horse 3 was sacrificed 48 hours after iron feeding. A very small amount of undissolved ferrous ammonium sulfate was found in the stomachs of both horses after they were sacrificed.

The following tissue samples were taken from each horse for analysis: three successive portions of intestine beginning just below the pyloric sphincter, 2.5 feet, 5 feet, and 4 feet, in order; the last 3 feet of intestine; and the mesenteric lymph nodes. Each sample was washed with isotonic saline.

The amount of ferritin in the tissue extracts was determined quantitatively by an immunochemical technique worked out in this laboratory (5) and also described by Mazur and Shorr (6,7). The antigenic properties of ferritin were utilized in the following way. Horse spleen ferritin, prepared according to the procedure

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*The authors gratefully acknowledge the kind cooperation of Mr. L. W. Bennett, Bennett and Sons Fox and Mink Ranch, Victor, N. Y., in these investigations which required the use of horses.

of Mazur and Shorr (6) with slight modifications*, was injected into rabbits in increasing amounts over a four-week period. A total of 4.5 mg. of antigen nitrogen was thus injected into each rabbit. The rabbits were bled five days after the last injection and the antisera were collected.

The tissue extract to be assayed for ferritin was prepared by grinding the tissue in a Waring blendor with two times its weight of water. The suspension was heated to 75-80° C., and the coagulum was centrifuged and discarded. Sufficient acetic acid (50% by volume) was then added with stirring to adjust the pH to 4.6 - 4.7, and the solution was allowed to stand for 2 hours at room temperature (6). Any precipitate which formed was centrifuged and discarded. The ferritin was then precipitated by adding 35 gm. $(NH_4)_2SO_4$/100 ml. solution, and after approximately 8 hours, the precipitate was centrifuged down and dissolved in a small amount of water. The solution was dialyzed against isotonic saline, and the clear dialyzed solution, containing ferritin, was used for the quantitative immunochemical test.

The antigen-antibody reaction was measured by the precipitin test (8). The tissue extract was reacted with the horse spleen ferritin antiserum. The total nitrogen content of the specific precipitate formed was determined by the micro-Kjeldahl method, and the amount of total ferritin nitrogen present was read from a standard curve prepared with known amounts of ferritin. The colors of the specific precipitates were noted, since it has been shown (7) that the iron is precipitated quantitatively with the antigen.

Results. The distribution of total ferritin in the intestine and mesenteric lymph nodes of normal and iron fed horses is presented in Table 1 (Page 18). In the normal horse, ferritin was found to occur in the first 2.5 feet of

*Mazur, A., personal communication

TABLE 1

THE DISTRIBUTION OF TOTAL FERRITIN IN THE INTESTINE AND MESENTERIC LYMPH NODES OF NORMAL AND IRON FED HORSES

Animal and Treatment	Successive portions of intestine beginning just below the pyloric sphincter			Last 3 ft. of intestine	Mesenteric Lymph Nodes
	2.5 ft. Total ferritin N, µg. per ft.	5 ft. Total ferritin N, µg. per ft.	4 ft. Total ferritin N, µg. per ft.	Total ferritin N, µg. per ft.	Total ferritin N, µg. per gm. wet tissue
Horse 1 Normal, fasted	351	132	131	205	15
Horse 2 Fasted. Fed equivalent of 30 gm. iron. Sacrificed after 24 hours.	4225	320	79	270	57
Horse 3 Fasted. Fed equivalent of 30 gm. iron. Sacrificed after 48 hours.	2592	1546	1717	783	77

intestine beginning just below the pyloric sphincter, and in the following 5 feet and 4 feet of intestine to a lesser extent. The presence of ferritin was also noted in the normal animal in the last 3 feet of intestine, and a small amount of ferritin was found in the mesenteric lymph nodes. The colors of the specific precipitates obtained from the antigen-antibody reactions involving the normal horse tissue were considerably paler than the deep red-brown precipitates obtained with the tissue extracts from iron-fed horses.

Twenty-four hours after the oral administration of ferrous ammonium sulfate, containing 30 gm. of iron, a decided increase was noted in the amount of ferritin in the portion of intestine beginning just below the pyloric sphincter and extending for 2.5 feet (4225 µg. of ferritin N/foot of intestine in the iron fed horse; 351 µg. of ferritin N/foot of intestine in the control horse). The following 5 feet contained perhaps somewhat more ferritin than that of the control. The 4 feet of intestine following that, as well as the last 3 feet of intestine, showed essentially the same amount of ferritin as the control. However, the ferritin content of the mesenteric lymph nodes of Horse 2 had increased almost fourfold over that present in the untreated horse.

Forty-eight hours after the feeding of an equivalent amount of 30 gm. of iron, the amount of ferritin in the first 2.5 feet of intestine had increased considerably in comparison with the control animal (2592 µg. of ferritin N/foot of intestine in the iron fed horse; 351 µg. of ferritin N/foot of intestine in the control), but the ferritin content was less than that noted in the horse 24 hours after iron feeding. However, unlike the values of the ferritin content in the animal 24 hours after iron feeding, the amount of ferritin in the succeeding 5 feet and 4 feet of intestine of the horse 48 hours after iron administration was found to be appreciably increased over that of the untreated animal. In

addition, 783 ug. of ferritin N/foot were found in the last 3 feet of intestine 48 hours after iron feeding in comparison with the values of 205 µg. in the control horse and 270 µg. in Horse 2. Furthermore, the ferritin content of the mesenteric lymph nodes 48 hours after the feeding of iron was determined as 77 µg. of ferritin N/gm. of wet tissue, while 24 hours after iron feeding the value was 57 µg., and the control horse contained 15 µg. of ferritin N/gm. of wet tissue.

Discussion. Since the antibody produced during the process of immunization with ferritin is directed toward the protein moiety of ferritin, and since both apoferritin and ferritin react identically with the antiserum, the results in this investigation are designated as total ferritin, a term which implies various possibilities of combinations of the two substances. Since the colors of the specific precipitates obtained with the tissue extracts of the normal horse were paler in color when compared to those of the iron fed horses, it is likely that in the tissues of the untreated horse the ratio of ferritin to apoferritin was very low, or else that a ferritin low in iron was being precipitated.

The procedure for extracting and precipitating ferritin has not been proved quantitative over the entire range of ferritin concentrations studied, but it was the only method recorded in the literature (9) at the time this work was carried out. Recently the procedure has been modified by Mazur and Shorr (7).

The increase in the total ferritin content of the intestine of the horse after iron feeding would indicate that ferritin is involved in the phenomenon of iron absorption in this animal. Twenty-four hours following the administration of iron, it appeared that the iron was absorbed largely through the first 2.5 feet of intestine as demonstrated by the profound increase in the amount of total ferritin in this portion of the intestine. However, 48 hours after iron feeding, the iron was absorbed into the first 11.5 feet of intestine as well as the last

3 feet. Essentially then, these results are similar to those obtained by Granick (3) in the guinea pig, and the process of iron absorption, involving ferritin, appears to be comparable in the two species.

Since there was a notable increase in the amount of total ferritin in the mesenteric lymph nodes of the horse after iron feeding, the increase in histologically demonstrable iron reported by Gillman and Ivy (4), following the administration of iron, might be attributed to an increased ferritin content of this tissue. Thus these results suggest that the lymphatic system is involved in iron absorption and that, here again, ferritin assumes a role.

Summary. The distribution of total ferritin in the intestine and mesenteric lymph nodes of normal and iron fed horses was investigated using a quantitative immunochemical technique for determining the ferritin in tissue extracts.

Ferritin was found to be present normally in the first 11.5 feet of intestine of the horse as well as in the last 3 feet of intestine. Small amounts of ferritin were detected in the mesenteric lymph nodes of the normal animal.

Twenty-four hours after the oral administration of ferrous ammonium sulfate (containing 30 gm. of iron), the total ferritin content was appreciably increased in the first 2.5 feet of intestine of the horse, while 48 hours after iron feeding, there were notable increases in the amount of total ferritin in the first 11.5 feet and in the last 3 feet of intestine.

Twenty-four hours after iron feeding there was approximately a fourfold increase in the amount of total ferritin in the mesenteric lymph nodes over the control value, while after 48 hours over a fivefold increase was noted.

The quantitative data indicate that ferritin is involved in the phenomenon of iron absorption through the intestine of the horse. Furthermore, it appears that the lymphatic system is concerned with iron absorption and that ferritin is

involved in this process also.

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Problem Code: X.R.3 (Therapy)

Section Code: 2606

Authors: Frank W. Furth and Molly Coulter

The Effect of Aureomycin on the Radiation Syndrome in Dogs.

Background. During the past nine months the Therapy Section has been studying the role of infection as it affects the morbidity and mortality of the x-radiated animal and various methods of combating these infections. One of the potential reservoirs of bacterial infection, the flora of the intestinal tract, has been studied in both the normal and radiated animal. Various antibiotics have been tested singly and in combination for their effect on animals receiving a large dose of whole body x-radiation. Numerous experiments with rats have

been done and have been partially reported elsewhere*. This report concerns observations made on a group of dogs which received a large dose of whole body x-radiation and were treated with a single antibiotic, aureomycin. Studies of the hematology, clotting mechanisms, bacteriology and pathology of radiated dogs have been carried out concurrently.

Methods. The dogs used were adult, healthy mongrels averaging 11.3 kilograms. They were housed in separate cages and fed a soft mash of Purina Dog Chow, Kibbled meal. As the animals became anorexic for the mash following radiation they were offered milk and fresh ground beef.

The dogs were divided into two groups of five dogs each. An attempt was made to have pairs of dogs of approximately the same weight and body build in each group, so that the groups were comparable.

Radiation was administered with a 250 Kv. Picker machine at 15 ma. using a parabolic aluminum filter with 0.5 mm. of copper. The target skin distance was 40 in., and the total target skin dose was 450 r administered at the rate of 7.15 r/min. In this laboratory this approximates an LD 90, 30 days, of whole body x-radiation for the dog. The dogs were radiated in pairs composed of one dog from each group.

Within 2 to 6 hours following radiation the 5 dogs in the treated group each received a 250 mgm. capsule of aureomycin orally, and this medication was continued every 6 hours, day and night for 28 days. The capsule was given in a small ball of hamburg; the 5 control dogs receiving a similar ball of hamburg without aureomycin.

Bacteriological studies included blood, autopsy, and quantitative stool

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*Howland, Joe W., Furth, Frank, Bennett, L. R., Coulter, Molly, McDonnel, G. M., UR-94 Studies on Factors Effecting the Radiation Syndrome. I. The Effect of Aureomycin and Antibiotics on Whole Body Irradiation October 14, 1949

cultures. During the course of the experiment venous blood was obtained every 2 to 4 days for aerobic and anaerobic culture. Cultures of heart's blood, liver, spleen and lung for aerobic and anaerobic culture were obtained at autopsy. All autopsies were done within 4 hours after death and in most cases within 2 hours. Complete taxonomic studies were not done on all the bacteria so that a positive identification is available on only some of the organisms.

In order to evaluate more fully the effects of the antibiotic therapy, the sensitivity of the various organisms isolated before and after death to aureomycin, streptomycin and penicillin were determined, using a tube dilution method.

Quantitative studies of the bacterial flora of the stool were done before and after radiation. Three types of organisms were studied; the coliforms, staphylococci and streptococci. Pour plates, using various dilutions of a uniform saline suspension of fresh stool, were made with Mannitol Salt media for staphylococci and with EMB for the coliforms. For streptococci isolation, the saline suspension was spread on the surface of Mitis Salivarius media. The colonies on the plates were counted after 24 hours incubation at 37 C. The stools were also plated on SS media for the isolation of pathogens.

A complete autopsy examination, excluding the cranial cavity, was done on each dog that died. Tissue for microscopic examination was taken from all major organs but the description of the microscopic pathology is not complete at the present time.

The hematological, bacteriological and clotting mechanism studies and clinical observations were recorded for a 2 week control period prior to radiation.

Results. Hematological studies including counts of the red blood cells, white blood cells and platelets were done by a member of the hematology section under the direction of Dr. M. Ingram. The findings are shown in graphs I, II

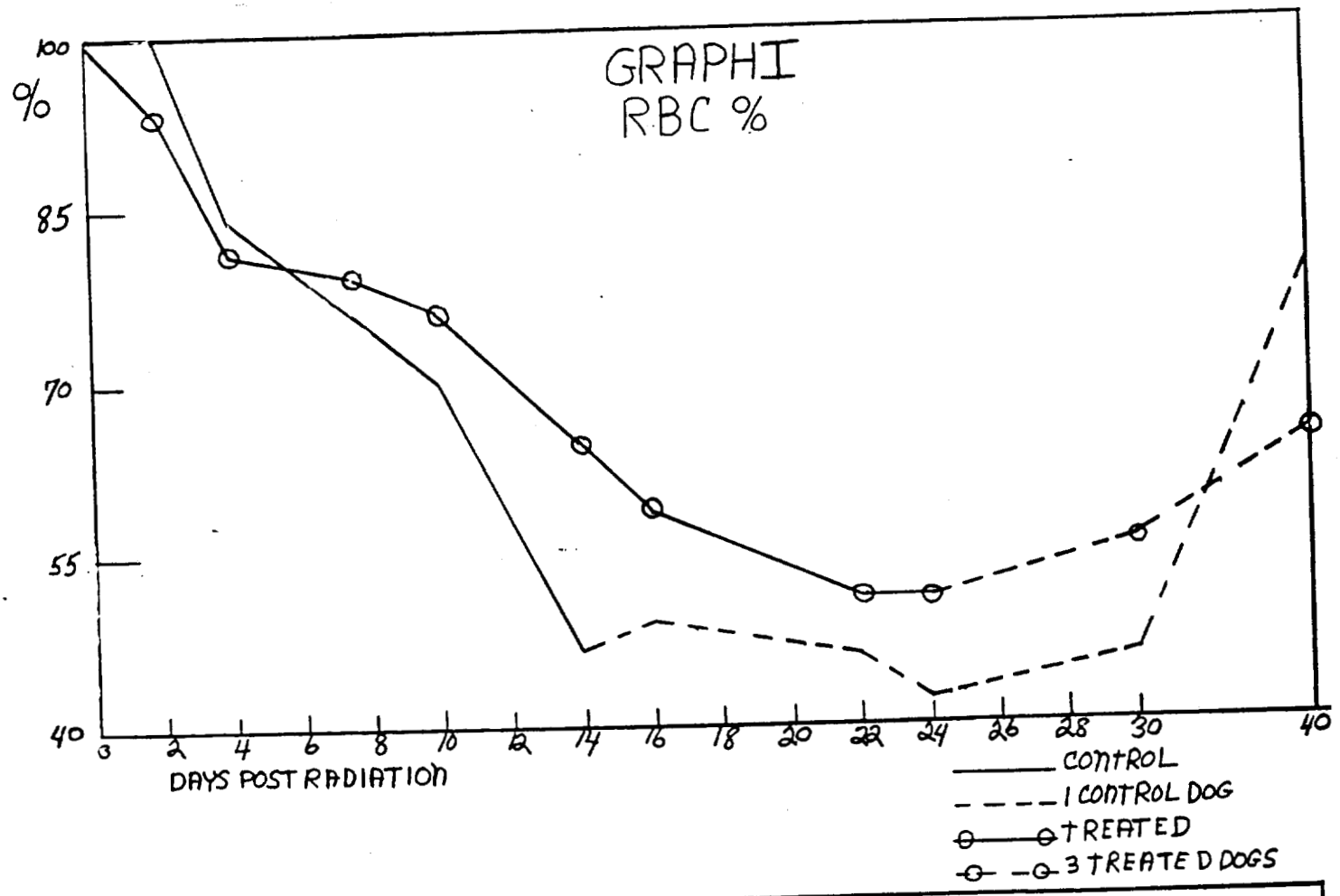
(Page 26), and III (Page 27). The lower red blood cell values in the control group after the 10th day can be accounted for in part by the higher incidence of gross gastro-intestinal hemorrhage in these dogs. There was no significant difference in the degree of leucopenia in either group. A correlation between the white blood cell count and the time of death was noted in the dying animals in both groups. In the 24 to 48 hours preceding death the white blood cell counts fell to 300 cells/mm^3 or less. No significant difference between the two groups was noted in the blood platelet counts. The first evidences of bleeding tendency occurred when the counts dropped below 30% of the pre-radiation value on the 12th to 14th days.

In order to study some aspects of the defect in the coagulation mechanism, whole blood coagulation times and prothrombin determinations were done. The coagulation times were done by a modified Lee White method. Following radiation, the coagulation times increased in each group, but there was no significant difference between the groups, Table 1 (below). The coagulation times for the animals which died were not above the average for the group during the 24 to 48 hours preceding death. The degree of clot retraction was diminshed concurrently with the drop in platelet count. It has been reported that aureomycin decreases

TABLE 1

COAGULATION TIMES IN MINUTES

	Pre-Radiation Value	Days Post-Radiation							
		2	4	8	10	14	16	22	24
Control Averages	5.9	10.8	10.2	13.2	12	10.5	9	9	12
Treated Averages	6.3	7.3	12	12	13.2	11.4	12	6.8	11


GRAPH I
RBC %
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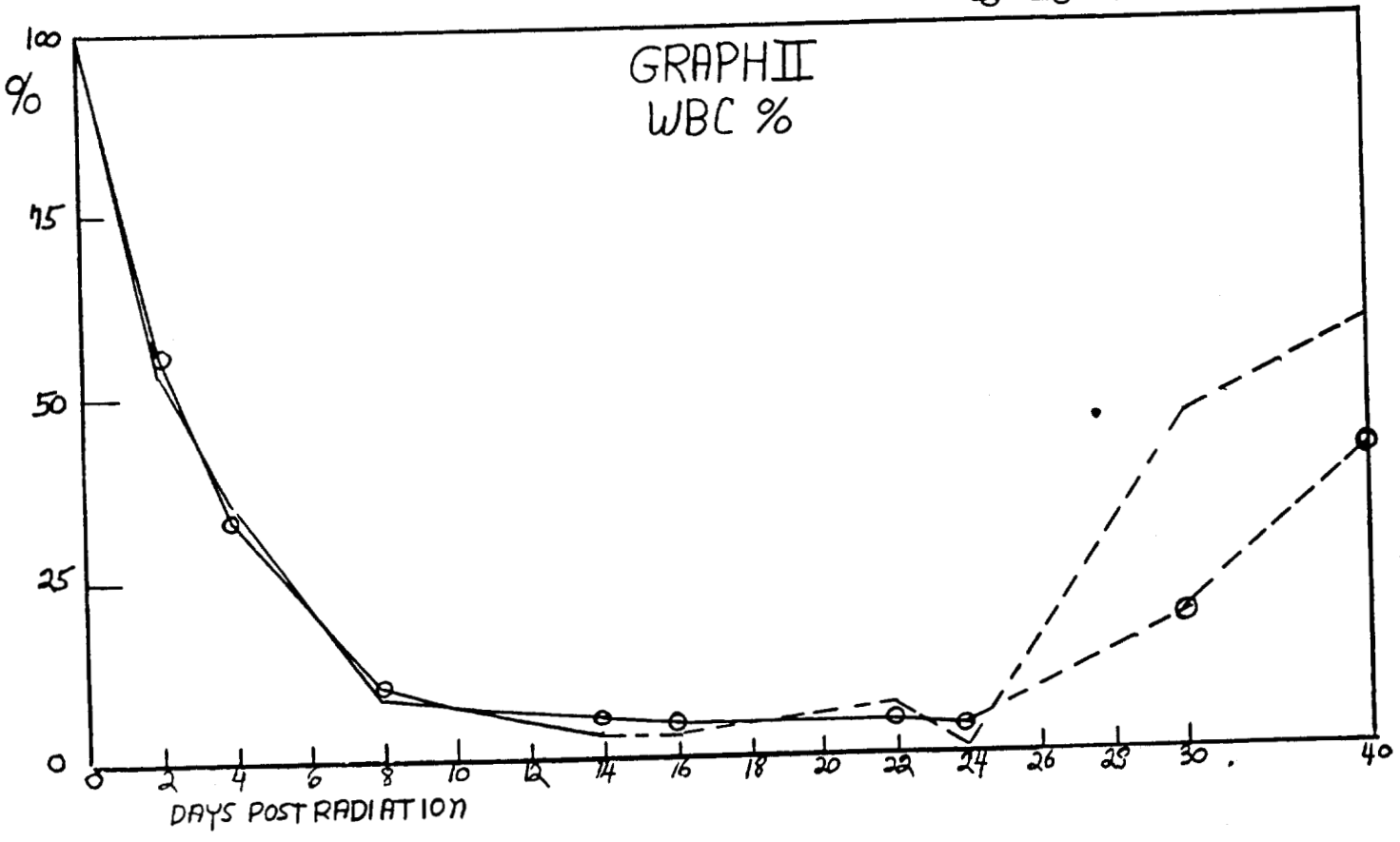
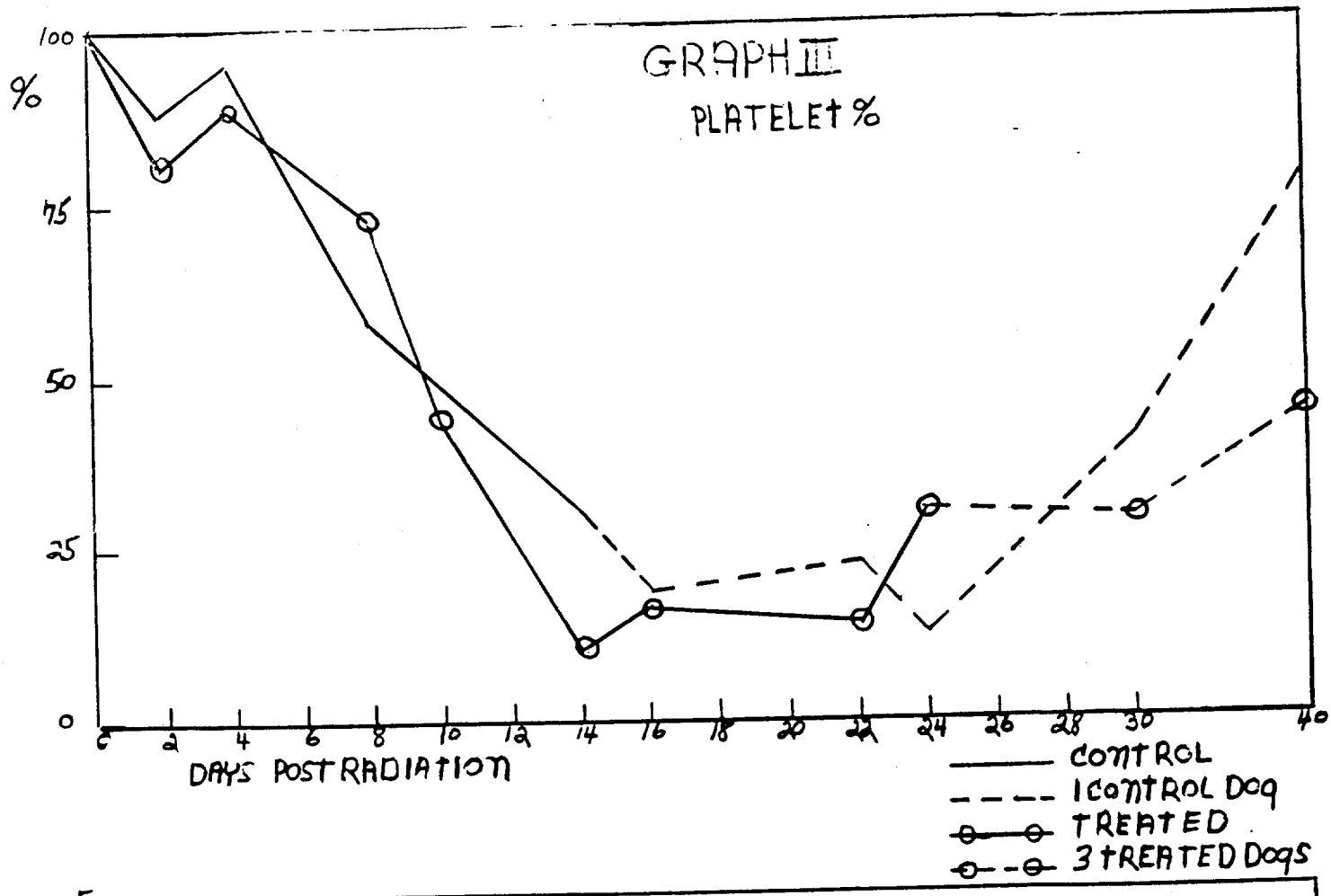
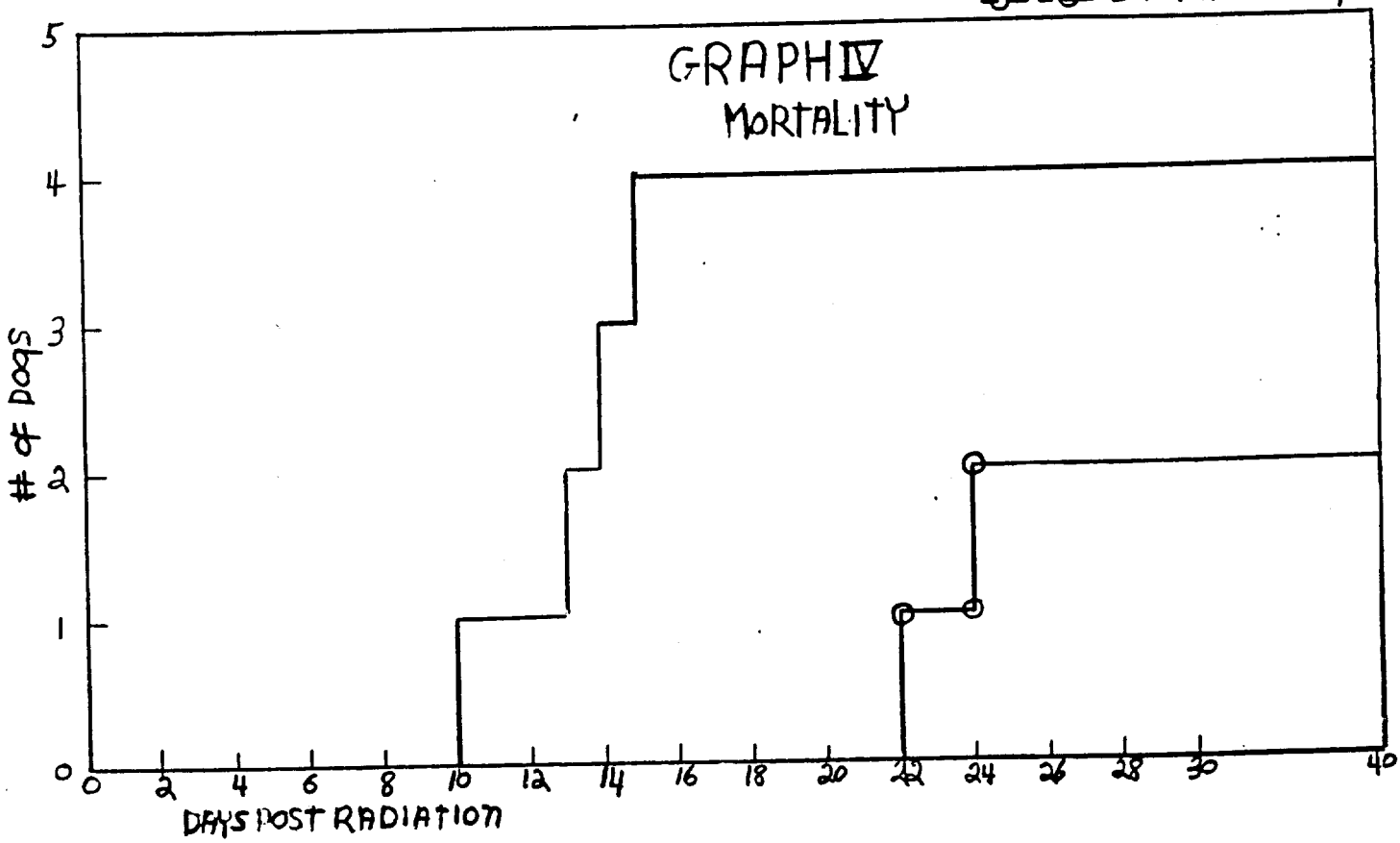
DAYS POST RADIATION
CONTROL
1 CONTROL DOG
TREATED
3 TREATED DOGS


GRAPH II
WBC %
100
%
75
50
25
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
40
DAYS POST RADIATION


GRAPH III
PLATELET %
%
100
75
50
25
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
40
DAYS POST RADIATION
CONTROL
1 CONTROL DOG
TREATED
3 TREATED DOGS


GRAPH IV
MORTALITY
# of DOGS
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
40
DAYS POST RADIATION

the coagulation time in normal dogs*. This was not noted in the x-radiated dogs.

The prothrombin studies done in the laboratory of Dr. James Scott of the Pharmacology Division included three types of determinations, the recalcification time, the one stage prothrombin time and the two stage prothrombin time (Table 2, Page 29). These studies were carried out on three dogs in each group. Because of the death of two of the control dogs, the values after the 14th day for this group are the determinations on the single surviving dog. There was no essential difference in the values for the two groups, the highest levels being reached on the 14th to 16th day. As with the coagulation time, no significant variation occurred immediately preceding death in the two dogs that died.

Four blood cultures were obtained from each animal before radiation and all were negative. Following radiation, a total of 35 aerobic and 24 anaerobic cultures were done on the treated group; on the controls 23 aerobic and 14 anaerobic cultures were done (Table 3 below). Of the 59 cultures in the treated group 8

TABLE 3

BACTERIOLOGY

Treated Group	Blood Cultures		Autopsy Cultures	
Dog No.	Aerobic	Anaerobic	Aerobic	Anaerobic
I		Gm. + coccus	Pseudomonas	Gm. - rod
II	Diphtheroids	Gm. - rod	Pseudomonas	Gm. - and + rods
	Pseudomonas	Gm. + rod		
		Gm. + coccus		
III		Gm. + coccus		
		Gm. + rod		
IV		Gm. + rod		
Control Group				
I		Gm. - rod	A.Aerogenes	Gm. + rod
			Gm. + coccus	
II		Gm. - rod	E. Coli	Gm. + rod
		Gm. + coccus		
III		Gm. + coccus	E. Coli	Gm. + and - rods
IV	A.Aerogenes	Gm. + coccus	E. Coli	Gm. + rod

*Macht, D. I., and Farkas, R., Science, 110, 305 (Sept. 23, 1949). Aureomycin and Blood Coagulation

TABLE 2

Day Post - Radiation	Recalcification Time In Seconds		One Stage Prothrombin Time In Seconds		Two Stage Prothrombin Time In Units		Two Stage Prothrombin Time Percentage	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Average Pre-rad. Val.	105	107.5	11.8	11.8	258	253	100	100
1	115	115	11	10	253.3	255	97	100.1
3	120	110	18	10	195	255	76	100
7	155	160	19	16	162	217	62	86
9	157	140	16	16	135	207	53	82
14	225	170	29.5	20	110	120	42.5	48
16	255	180	32	21	80	107	32	42
21	240	185	39	24.6	90	117	36	47
23	195	165	27	20	120	130	48	52
30	150	125	20	22	140	170	56	67
44	135	133	20	15	220	220	88	86

UNCLASSIFIED

(14%) were positive, and of the 37 cultures in the control group, 6 (16%) were positive. A greater number of facultative and obligative anaerobes than strict aerobes was isolated from these cultures. Blood cultures were obtained from two of the control dogs within 48 hours of death and both were negative. A blood culture taken from one of the treated dogs approximately 12 hours before death was

were more resistant. The relative sensitivity of the organisms to streptomycin indicates that this antibiotic used in conjunction with aureomycin may prove more effective.

Quantitative analysis of the bacterial flora of the stool showed that the coliform organisms increased markedly following radiation, reaching a peak on the 4th day, and returning to pre-radiation values by the 7th to 10 days. The coliforms reached higher levels in the control group than in the treated group, which may have occurred because of the sensitivity of some of the coliform strains to aureomycin. Both the stool staphylococci and streptococci counts in the treated group rose to high levels following radiation, whereas the counts in the control group remained unchanged. Presumably, aureomycin caused a shift in bowel flora, but similar studies on normal dogs receiving aureomycin have not been done to confirm this. No organisms ordinarily considered pathogenic were isolated on SS media, although it was noted that the incidence of Proteus in the treated group increased following radiation.

Immediately following radiation the dogs began to lose weight, and continued to do so until the 20th day post-radiation. There was no significant difference in weight loss between the two groups except between the 10th and 15th day. During this period the weight loss and incidence of mortality was highest in the control group. The maximum average weight loss in both groups was about 15% of pre-radiation weight.

Anorexia was first noted in the control dogs on the 9th or 10th day post-radiation and by the 12th to 13th day none of these dogs was eating and all appeared very lethargic. This anorexia and lethargy preceded death in 4 of the control dogs. In marked contrast, the treated group during this period appeared outwardly normal; all were eating well and were lively. Anorexia and lethargy began to appear in the treated group on the 17th day post-radiation and continued

to the 30th day in the 4 surviving dogs.

One of the most striking observations was the difference in the time of death and total mortality in the two groups (Graph IV, Page 27). The first control dog died on the 10th day post-radiation, and by the 15th day 4 of the control dogs were dead. The first death in the treated group did not occur until the 22nd day post-radiation, with one other treated dog dying on the 24th day. The total mortality 4 months post-radiation is 80% for the control group and 40% for the treated group.

The most prominent gross finding at autopsy was evidence of a hemorrhagic diathesis with areas of extravasation of blood occurring in the lungs, spleen, kidneys and mucosa of the gastro-intestinal tract and beneath the pleura and peritoneum. Bleeding in the control group was more extensive and occurred in more organs than in the treated group. Three of the 4 dogs in the control group had large amounts of blood in the gastro-intestinal tract at autopsy, associated with ulcerations of the mucosa of the stomach and small intestine. The two treated dogs which died had no ulceration of the intestinal mucosa, and no blood within the lumen of the intestine. Both of these treated dogs had a few subcutaneous hemorrhages and hemorhages into the cortex of the kidney. Extensive hemorrhage into the retroperitoneal space and the tissues of the oral cavity occurred in one of the treated dogs. The first control dog which died showed a minimum of petechiae, and grossly no obvious cause of death was found. The difference in degree of the hemorrhagic tendency between the two groups may be explained by the time after radiation at which death occurred. The treated animals died at a later time when the bleeding tendency was decreasing as is indicated by the clotting and prothrombin times reported above.

Gross evidence of pneumonia was found in 2 of the control dogs, and in 1 of the treated dogs.

Discussion. Since the antibiotic, aureomycin, was the only specific therapeutic agent administered in this experiment, presumably the delayed morbidity and reduced mortality of the radiation syndrome in dogs can be attributed to it. As a corollary, bacterial and possibly viral infection may play a role in the toxicity associated with large doses of radiation, since aureomycin is a potent anti-bacterial and anti-viral agent. This concept of the role of infection in the mortality associated with radiation is supported by the bacteriological studies at autopsy reported above, as only aureomycin-sensitive organisms were recovered from all the control dogs, while aureomycin-resistant organisms were isolated from the two treated dogs which died.

Both the occurrence of ulceration of the gastro-intestinal mucosa associated with massive gastro-intestinal hemorrhage in three of the four control dogs which died, and the absence of ulceration and gastro-intestinal hemorrhage in the 2 treated dogs which succumbed, suggest that the changes in bacterial flora of the bowel, occurring during the administration of aureomycin, tended to decrease the derangements in the intestinal mucosa ordinarily found in the radiation syndrome. This may be one of the major factors in reducing the mortality in the treated group.

Both groups of dogs exhibited the signs of radiation sickness, with anorexia and lethargy, but these occurred much sooner in the control group. Equally severe depressions of the blood elements, and alterations in the coagulation mechanisms occurred in both groups. Despite these findings, typical of severe radiation sickness in both treated and controls, aureomycin apparently reduced the total mortality by 50%.

Numerous studies on the effect of other antibiotics, singly and in combination on the radiation syndrome in dogs and other species have been done

and will be reported later. Further work involves more detailed studies on the immunity mechanisms, bacteriology and metabolism of x-radiated animals treated with antibiotics.

Summary.

1. Five dogs given an LD 90 (450 r) of whole body radiation were treated with antibiotic, aureomycin. Five similarly x-radiated dogs served as controls.

2. Identical alterations in blood cell counts and coagulation mechanisms were observed in both groups following radiation.

3. Bacteriological studies, including quantitative bacteriological analysis of the stool, were done.

4. The total mortality in the control group was 80% while the aureomycin treated group had a total mortality of 40%. There was a difference of 12 days between the first death in the control group and the first death in the treated group. A similar delay in the onset of the clinical signs of radiation sickness was noted.

5. Gross autopsy findings revealed no gastro-intestinal ulceration and minimal evidence of hemorrhage in the treated group. Marked gastro-intestinal ulceration and hemorrhage were found in the control dogs which died.

Problem Code: X.R.4 (Hematology)

Section Code: 3351

Author: Maylou Ingram

The Incidence of Unusual Lymphocytes in the Blood of Dogs Exposed to Radiation from the 130 inch Cyclotron.

Previous Quarterly Reports have described the occurrence of lymphocytes

with bilobed nuclei in the peripheral blood of cyclotron personnel who have had no known exposure to radiation in amounts above the tolerance level. In order to check the relationship between the increased incidence of these cells and exposure to radiation from the cyclotron, detailed studies have been made on dogs repeatedly exposed for short periods. These exposures, thirty minutes each, were made on August 22, 1949, October 4, 1949, and December 12, 1949. Interpretation of the results is currently being completed; however, it is possible at this time to report definitely that a marked increase in the incidence of lymphocytes with bilobed nuclei occurred after each exposure with a maximum incidence during the first two post-exposure weeks.

Because the incidence of lymphocytes with bilobed or hourglass nuclei is extremely low, data now seem best interpreted according to whether or not a given smear contains one or more of the abnormal cells. Of the 180 smears examined during the control period, one smear or 0.55% was positive on this basis. By contrast, of the 180-200 smears examined during the two weeks after each exposure, approximately 20% were positive. These figures for the post-exposure period are approximate; however, it is unlikely that any major revision will be indicated.

Interpretation of the initial findings in the blood of cyclotron personnel on this same basis indicates that during the period before the cyclotron was operating, 3.75% of 80 smears were positive. Of 100 smears from the same group during the first three months of cyclotron operation, 23% were found to be positive. Additional information relative to the incidence of the cells in later periods of cyclotron operation will be made available in subsequent reports.

Studies of the relationship of the bilobed lymphocyte response to various kinds and intensities of ionizing radiation are in progress, and it is

expected that investigation of factors which might influence the nature and/or magnitude of the response will also be undertaken.

PROGRAM U.

URANIUM

Problem Code: U.3 (Toxic Limits)

Section Code: 3210

Authors: S. Laskin, L. Leach and R. H. Wilson

Experimental Production and Studies of Uranium Dioxide Fumes.

Uranium metal industrially is a difficult material to process and also exhibits a marked tendency to be pyrophoric. Under certain practical conditions, therefore, such processes as machining and grinding of the metal are known to produce quantities of fumes as atmospheric contaminants. Available data indicate that these fumes are in the form of one of its oxides (presumed to be uranium dioxide). The nature of its production and the small particle sizes characteristic of fumes suggested that this industrial hazard if typical of other metals may represent a problem differing from that of exposure to dusts of the same material. The lack of biologic data and other information pertaining to physical and chemical differences of uranium and uranium oxide fumes has prompted studies of such materials. The information was also required to extend the spectrum of the particle size toxicity studies of uranium oxides to sizes significantly below those obtained from dust atmospheres (H. B. Wilson, et al.). The purpose of this report is to present a method for the production of uranium dioxide fumes from uranium metal, the results of preliminary studies at maintaining continuous atmospheres and the results of a preliminary, short-term inhalation study with animals.

Prior to the present studies numerous attempts at continuous production of fumes from uranium metal or its compounds were unsuccessful because of complex

practical or contaminant difficulties. Among the methods explored were induction furnaces, burning of salt dissolved in organic solvents, conventional arcs and evaporation from tungsten filaments. The arc method appeared most feasible but practically the electrodes were found to corrode too rapidly to maintain burning. Evaporation from tungsten filaments under conditions of high vacuum or inert gas enclosure was successful if all traces of air were removed to prevent burning of the filament. Under conditions required for continuous feeding of an animal exposure unit, however, excessive contamination from the filament was obtained. The problem appeared insurmountable until it was recognized that the major difficulty with previous attempts was the rapid reactivity of uranium metal with oxygen and under conditions of the temperatures required with water, vapor, nitrogen, carbon and other materials. Introducing the principles of controlled heating or arcing of uranium metal within an inert gas chamber and a controlled rate of oxidation resulted in the development of a simple fume feed which could be maintained indefinitely.

The most successful method to date simply consists of a uranium metal arc burning in an air-contaminated argon atmosphere. The apparatus illustrated diagramatically in Figure 1 (Page 39) is constructed of conventional laboratory equipment representing at its present stage only a crude model of the fume feed required for inhalation studies. The arc is formed between a 1/8" diameter uranium rod as a movable electrode and a 1x1x1/8" uranium plate held in position by a brass support. The arc is hand fed and inclosed in an arcing chamber constructed from a 4-liter vacuum filtering flask. The movable electrode is guided by means of a short length of glass tubing inserted in the flask stopper and held in place by means of a tight rubber sleeve. Current is supplied to the electrodes from a 110V AC line passing through a resistance of 8 ohms. Regulation

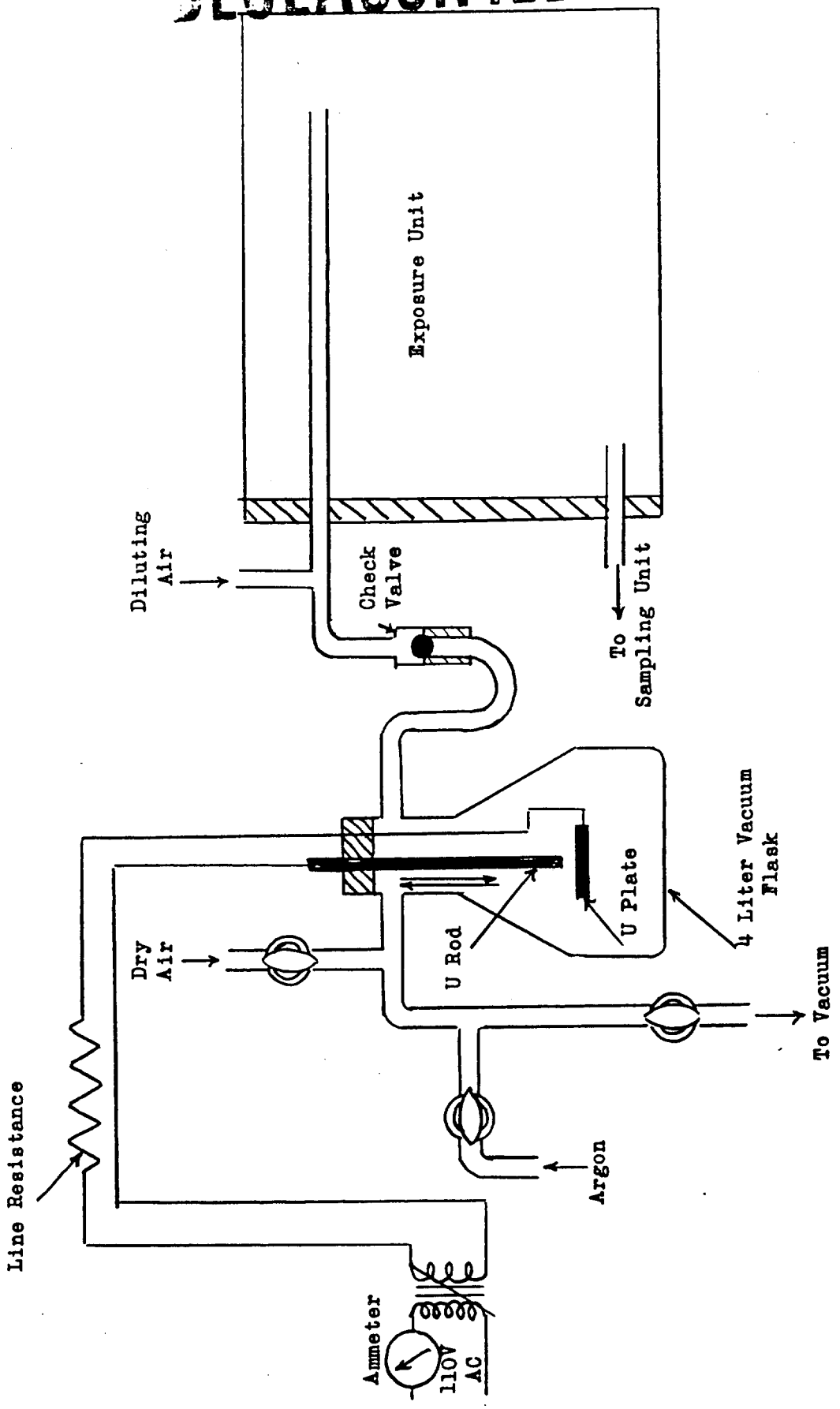


Figure 1. Uranium Fume Exposure Unit and Feed

is obtained by means of a powerstat and the current is measured at the 110V side. A current of 12 amps and an arcing distance of 1/4" resulted in a steady arc which could be maintained for several hours with minimal adjustment.

The arcing chamber connects to a small exposure unit constructed from a 12 x 12" cylindrical battery jar. This jar is mounted horizontally in a wooden support and sealed by means of a plywood and rubber gasket cover. A check valve serves to isolate the arcing chamber during gas filling and preliminary evacuation.

For operation the flask is evacuated with a Welch Duoseal Pump flushed with argon and re-evacuated. This process is repeated several times to eliminate traces of moisture and residual air. The chamber is then filled with argon and a continuous flow rate of 4 liters per minute established. Increased gas pressure forces the check valve open flushing the exposure unit. Clean dry air is admitted to the arcing chamber by means of a glass valve and manometer system at a metered rate of 0.1 liters per minute. The atmosphere of the exposure unit is continuously sampled at a rate of 14 liters per minute from its exhaust port. The resulting negative pressure mixes diluting air with the fume-contaminated argon just before entering the exposure unit.

Several preliminary studies of the operating characteristics of this system were made. Figure 2 (Page 41) illustrated the concentrations obtained during a one-hour run. Concentrations were determined from filter paper samples collected at 5 minute intervals and analyzed by the ferrocyanide method. Points plotted on the graph represent concentrations for the mean time interval of sampling. A mean concentration of 14.9 mg UO_2/m^3 with a standard deviation of 3.0 was obtained. Spectrographic* and chemical analysis of the samples from this atmosphere proved the fume to be of high purity. Typical samples containing 1-2 mg of uranium

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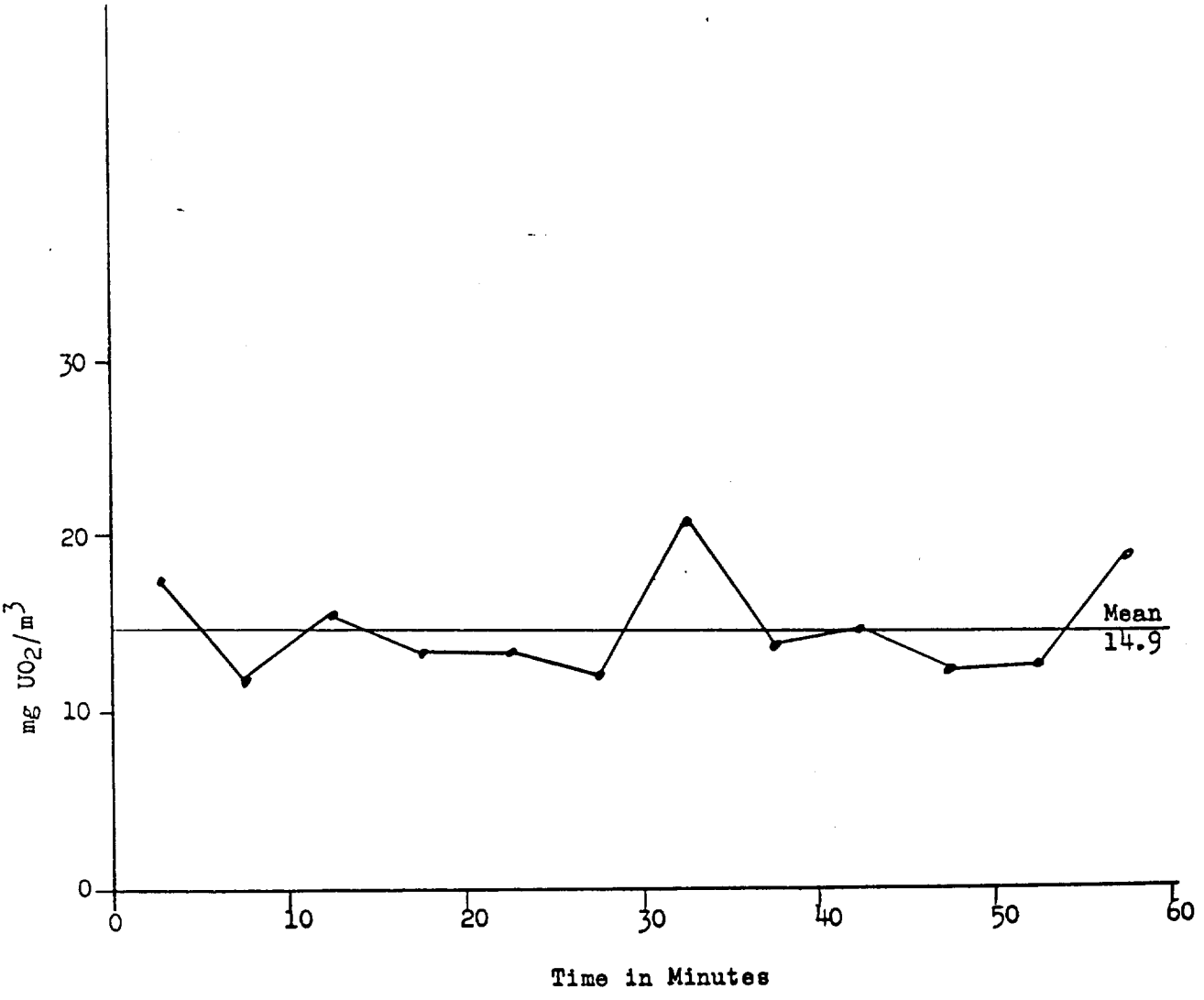
* Dr. L. T. Steadman

Figure 2. Preliminary Concentration Study of UO_2 Fume Feed



showed only traces of copper, lead and beryllium (1 μg or less of each). X-ray diffraction* analysis of samples collected with an electrostatic precipitator confirmed the fume composition as uranium dioxide. Only negligible traces of contaminants were found which may have been the contaminants reported in the spectrographic analysis or other oxides of uranium.

In order to characterize physically the fume produced, electron micrographs were made of samples collected by means of the oscillating thermal precipitator. The material, illustrated in Figure 3 (Page 43), was photographed at an initial magnification of 7,100 times. The fume appears to consist of extremely small unit particles approaching the resolution limit of the photograph (0.01 μ). Crystal structure and particle shape are therefore difficult to identify. The fume exhibits a marked tendency to form irregular branched chains or agglomerates ranging in size from 0.1 to greater than 1 micron in diameter. Increased agglomeration and chain sizes are observed in other photographs taken from atmospheres of higher fume concentration.

These results were sufficiently encouraging to attempt a preliminary pilot study of exposure to animals. In order to provide comparative toxicity data with other results reported for uranium dioxide atmospheres (1), the output of the arc was increased to produce an atmosphere of approximately 80 mg UO_2/m^3. This increased output was achieved simply by decreasing the arcing distance to 1/8". The resultant increased heating although productive of fume was more difficult to maintain and as later observed resulted in the changes of particle characteristics.

Animal Exposure Study. A mean concentration of 80.7 mg UO_2/m^3 was maintained for a total of 17 hours over a period of 4 days. The animal exposure schedule consisted of 5 hours the first day followed by 4 hours each successive

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* Mr. H. Mermagen

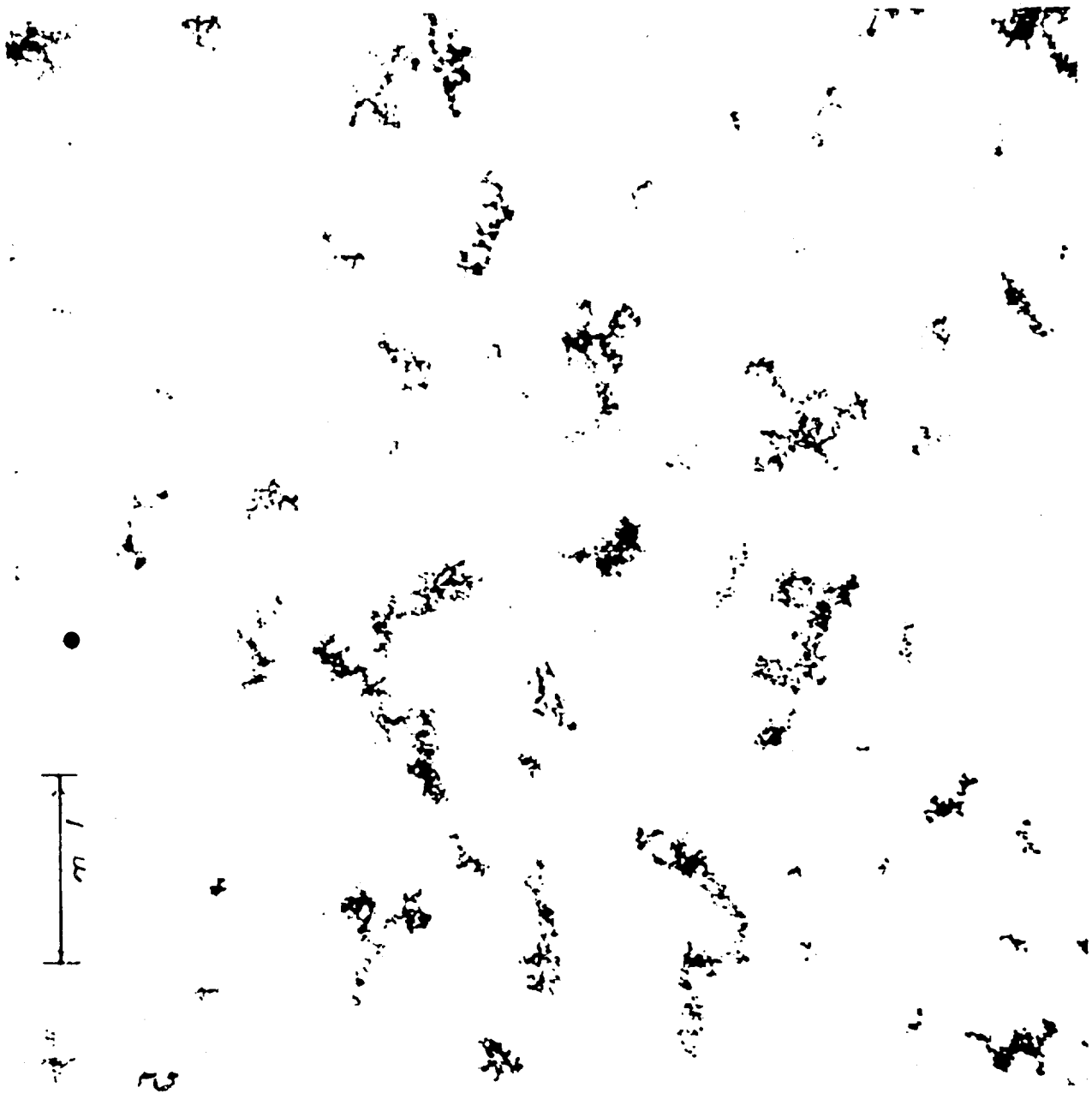

Figure 3. Electron micrograph of a uranium dioxide fume produced at a concentration level of 14.9 mg/m^3

day. The concentration results illustrated in Figure 4 (Page 45) show a much wider range of values obtained in comparison with those of the 14.9 mg UO_2 level. A standard deviation of 41 mg UO_2/m^3 and extreme variations from 5.4 to 186 mg UO_2/m^3 were obtained.

Spectrographic analysis of a sample of this atmosphere showed slightly higher contamination with 20 micrograms of copper and 100 micrograms of zinc found in a sample containing 2.66 milligrams of uranium. This increased contamination was attributed to the heating effect of the arc on the brass supports. The differences, however, were not considered significant because the purity was of the same order of magnitude as those of the 14.9 mg/m^3 level (99.6 to 99.9% UO_2). Electron micrographs of the material illustrated in Figure 5 (Page 46), showed that the increase in concentration resulted in increased particle sizes. Chains and agglomerates are less complex, being composed of few larger units. Two major categories of unit particles were found averaging 0.1 and 0.05 μ, respectively. In addition, numerous individuals and short chains of sizes several tenths of a micron were found.

Six albino male rats were used for this study. Rats were selected on the basis of size and also as a typically resistant species to emphasize any observed toxicity. The animals were divided into two age groups, 4 young averaging 184 grams and 2 mature animals averaging 302 grams. Two controls selected on a weight basis were provided for each age group. Half of each age group including controls were sacrificed on the 8th day after the start of exposure and the remainder sacrificed on the 22nd day. Terminal blood NPN's were obtained on each of the animals and sections of lung, liver, spleen and kidney taken for micropathology. Other criteria included body weight on a daily basis and hematology weekly. Following gross autopsy, tissues (lung, femur and kidney) were also

Figure 4. Concentration of UO_2 Fume Maintained During a 4-Day Preliminary Inhalation Study

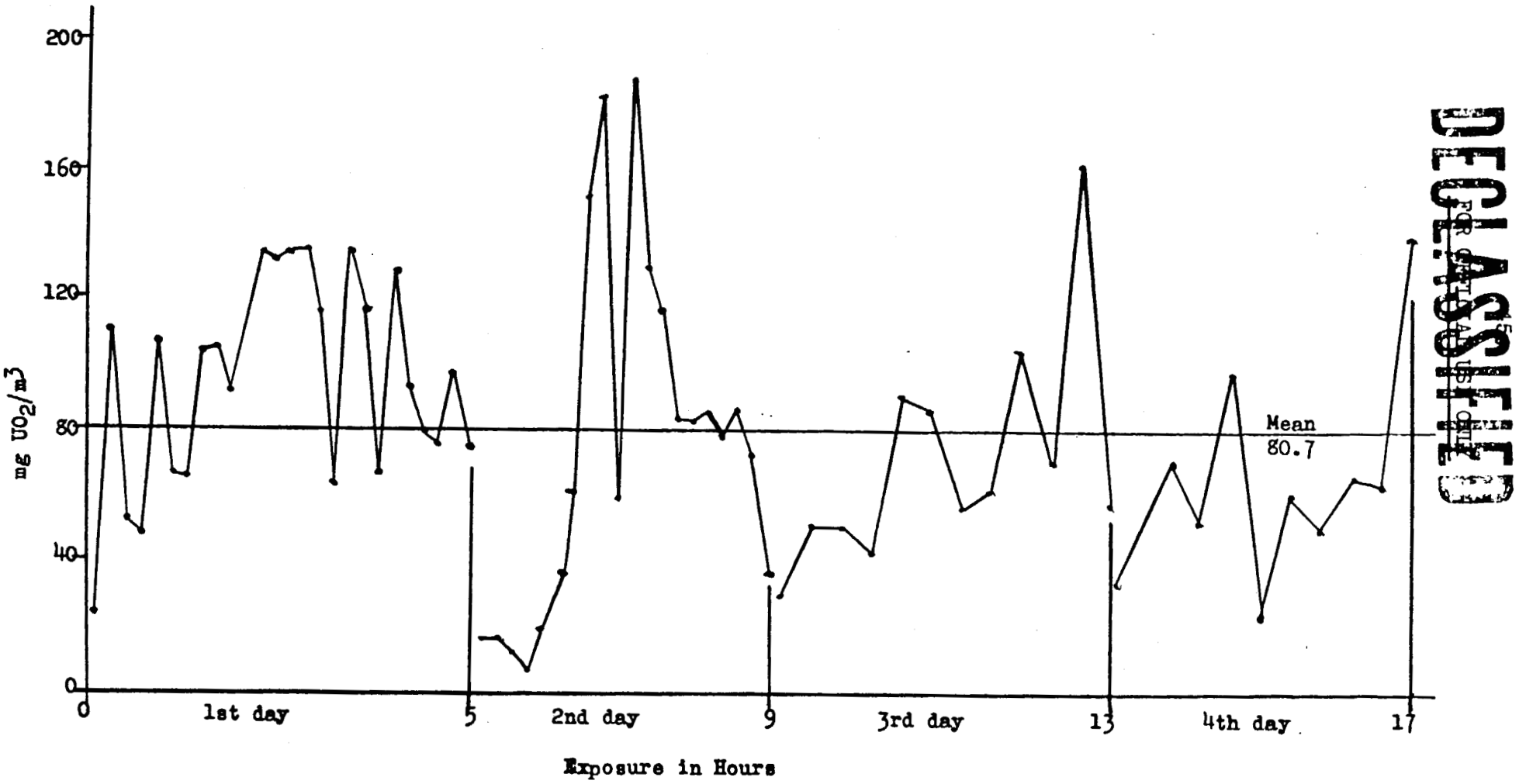

Figure 5. Electron micrograph of a uranium dioxide fume produced at a concentration level of 80.7 mg/m^3

sampled for subsequent uranium analyses.

During the exposure and pre-sacrifice periods little or no changes of significance in the body weights or condition of the animals were found. The hematologic results were inconclusive because of the wide variation of such data and limited number of animals exposed. Some changes appeared in the white cell counts indicating a tendency toward increased number in the young exposed rats. Two of these animals showed increases well above the variations of the others (above 2000-5000). Although these appeared to correlate with the third exposure day decreasing in the subsequent period, similar results were also observed in one of the control animals of each group. A decrease of similar magnitude was found in one of the mature exposed rats and also in a mature control. Differential counts indicate some questionable changes in neutrophil and lymphocyte percentages, but not in the eosinophil values. No changes were found in the erythrocyte count.

The significant findings in this study were made in the blood NPN determinations and micropathology*. All of the exposed animals sacrificed on the 4th day after exposure showed the renal lesions observed in uranium poisoning. A small zone of tubules lying just at the junction of the cortex and medulla exhibited necrotic cells and typical regenerating epithelium. The changes were classified as minimal in the mature rat and in one of the young group. Those of the second young animal were classified as a trace. An increased terminal blood NPN value of the mature rat (52 mg%) was confirmed as a significant minimal change; the values for the two young animals, however, did not show significant increases being 34 mg% compared with 30 mg% for the control.

All of the exposed animals sacrificed on the 19th day following exposure

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* Dr. J. K. Scott

indicated minimal kidney damage from the blood NPN results, values of 46, 52 and 52 mg% being obtained. Although no changes confirming the damage was found in examination of the kidney sections, the post-exposure period was considered of sufficient duration to have allowed complete regeneration. At the levels of damage observed diagnosis of complete regeneration is difficult to make. One of the mature control animals showed a renal lesion classified as probable interstitial nephritis. No damage was reported for the other controls.

Gross autopsy of the exposed animals indicated only slight damage to the lungs in the form of small areas of hemorrhage and edema. Some consolidation was observed in both of the experimental and control mature animals. The micropathology, however, did not show pulmonary lesions specifically attributable to the uranium dioxide fume. No significant differences between the age groups were indicated.

The renal damage observed in this study suggest a level of toxicity comparable to that found in the fine particle-size studies of uranium dioxide dust (0.45 $\mu \cdot M_g$) (1). This agreement might be expected from the electron micrographs obtained on the fume (Figure 5, Page 46). Larger particle sizes present in the same order of magnitude as in the 0.45 μ dust study would represent the bulk of fume mass. Although the concentration levels are comparable, the shorter duration of exposure required to produce renal effects in this study indicates a more rapid absorption of the retained fume. Tissue analysis for uranium content required to confirm this suggestion, however, are incomplete and will be reported later.

Further studies are in progress on the toxicity of the more characteristic fume produced at the 14.9 mg UO_2/m^3 level. Studies are also in progress on the physical and chemical characterization of uranium dioxide fumes and in the

development of higher capacity automatic feeds.

Bibliography

1. Wilson, H. B., et al, The Relation of Particle Size of Uranium Dioxide Dust to Toxicity Following Inhalation by Animals, J. Ind. Hyg. & Tox., 30, 6 (1948)

Problem Code: U.4 (Fate)

Section Code: 3210

Author: C. W. LaBelle*

The Distribution and Transport of Uranium After Deposition in the Rabbit by Inhalation.

In a series of experiments which have been reported (1) a study was made of the various factors that affect the gross deposition of uranium dusts in rabbits by inhalation. In a related paper (2) an experiment was described in which the movement of uranium was studied in the rat following intratracheal injection. The data presented here shows the distribution and transport of uranium dioxide in rabbits after deposition by inhalation. The "retention"** apparatus described in (1) was used to provide for the inhalation of uranium dioxide dust averaging 0.5 micron in diameter over a period which varied from

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* The technical assistance of Clarence Booth and Gertrude Melville is acknowledged.

** Since this apparatus was originally dubbed, it has been found convenient to make a distinction between retention and deposition, deposition being reserved to indicate the amount of a particulate deposited in the lung (or respiratory tract) in inhalation over a short period of time (5-30 minutes), whereas retention has been reserved to refer to the amount remaining some period after deposition when the physiologic processes of removal from the lung or respiratory tract have had opportunity to act. Deposition thus is governed by physical factors whereas retention is governed chiefly by physiologic and chemical processes.

one to four hours, during which time a number of measurements of gross "retention" rate were made. The rabbits studied were sacrificed either immediately upon termination of the inhalation period, or at stated intervals thereafter, and the uranium content of the various tissues determined.

It was found that the uranium was confined, at least during the first twenty hours, to three well-defined physiologic regions: (1) the upper respiratory system, including the oral cavity, the larynx, the external and internal nares, and the nasal sinuses; (2) the gastrointestinal system, including the esophagus, the stomach, and occasionally a small portion of the upper intestine; and (3) the lower respiratory system including the tracheobronchial tree and the lungs. Check analyses for urinary uranium indicated that the fraction reaching the kidney at this stage was negligible, being less than 0.5% of the total.

It was found that the total uranium content of individual rabbits varied widely under identical conditions of exposure, due chiefly to variations in the respiratory pattern of different animals, as had been found to be true of the gross deposition rates measured by the retention apparatus. It was accordingly necessary to reduce all the analytic data to a percentage basis by dividing the total found at any site by the total found in the entire animal to yield a figure which is the analog of the commonly employed "percentage of original dose". When this is done, it becomes possible to demonstrate certain consistent trends in the data as described below.

Results.

1. Gross Retention. Although the gross deposition of dust in rabbits has been more completely studied by the use of the retention apparatus, it is possible as in the present experiment by direct tissue analysis to obtain an independent assay of the accuracy of the data obtained with the retention apparatus. It is

possible to calculate the apparent gross retention of uranium in each rabbit during the inhalation period and to compare this figure with that obtained by adding together all the analytic recoveries of uranium in the same rabbit at autopsy.

This was done in eight rabbits exposed for four hours to a mean concentration approximating 20 micrograms per liter as UO_2 dust. The mean uranium recovery from these animals was 497 micrograms of uranium per kilogram of body weight, with a standard deviation of 209 micrograms per kilogram, or 42% of the mean. The variation between observed and computed retention, on the other hand, leads to a standard error of only 45 micrograms per kilogram, or 9% of the mean. It thus appears that the rather large variations (of the order of 50-100%) found in the gross deposition studies are in fact real variations associated with individual animal characteristics, and that the accuracy of the results from the retention apparatus is of the order of 10%.

2. Initial Distribution of Uranium during Inhalation. Five of the rabbits in the series* were sacrificed immediately upon cessation of the inhalation, and the percentage distribution at each of the three sites determined. The results are as follows:

TABLE 1

DISTRIBUTION OF URANIUM BETWEEN THREE MAJOR PHYSIOLOGIC SITES DURING INHALATION

Animal No.	Hours Exp.	PERCENTAGE OF URANIUM IN		
		Upper Respiratory Tract	Gastro-Intestinal	Lower Respiratory Tract
51	2	36%	38%	26%
53	3	60	18	22
35	4	32	33	35
57	4	32	46	22
52	4	41	29	30
Mean		40	33	27
		12	10	6

* A total of ten rabbits were studied. The groups described overlap so that one animal may be included in several places.

It will be seen that after from 2-4 hours the uranium is relatively evenly divided among the three sites. The relative quantity found in the lung is somewhat more constant than that found in either of the other sites (since its standard deviation is about half that found elsewhere), and amounts to about one-third or a little less, of the total amount of uranium deposited.

3. The Transport of Uranium after Deposition. In order to show the changes in the distribution of the uranium after deposition, a further transformation of the data is useful. From the mean values derived above, the mean uranium content at each site at the end of a four-hour exposure was calculated. The analytical recoveries were then expressed as a percentage of this figure, so that the figures obtained during the exposure indicate the rate at which the uranium level builds up to its four-hour value, and figures obtained after the exposure period reflect subsequent changes at each site. The following results are obtained.

TABLE 2

MOVEMENT OF URANIUM IN RABBITS DURING AND AFTER INHALATION RELATIVE LEVELS WITH RESPECT TO THOSE FOUND AFTER FOUR-HOUR EXPOSURE

Animal No.	Hrs. of Exposure	Hrs. Post-Exposure	RESPIRATORY TRACT		Gastro-Intestinal
			Upper	Lower (lung)	
		INHALATION PERIOD			
51	2	0	28%	13%	22%
53	3	0	160	66	39
35	4	0	91	118	91
57	4	0	91	79	130
52	4	0	118	103	79
4-hour mean	4	0	100	100	100
		POST-INHALATION PERIOD			
50	4	1	54	67	75
54	4	2	5	44	162
37	4	4	22	41	145
56	4	8	8	72	188
55	4	16	18	67	175

The data in this table may be summarized as follows. During the inhalation period a deposit of uranium is built up more or less uniformly in all three sites. During the first day following the inhalation, the uranium content of the upper respiratory passages falls rapidly to about one-sixth its maximal value, the uranium content of the lung falls to from one-half to two-thirds its maximum, while the gastrointestinal tract continues to gain uranium by transfer from both these sites to nearly double its original value after intake has ceased.

Discussion and Conclusions. The data presented here, while limited in extent, are of considerable importance in that they offer independent confirmation of the conclusions reached in the two earlier and more complete papers. First, the accuracy of the retention apparatus has been evaluated and shown to be adequate to justify the conclusions derived from its use. Second, the movement of appreciable quantities of uranium from the lung to the gastrointestinal tract, offered as a hypothesis in the second paper mentioned above, has been given some measure of direct experimental confirmation.

The concept of the process of deposition of uranium by inhalation may be crystallized as follows: uranium dioxide builds up in all exposed sites during inhalation of uranium dust, after which most of the uranium in the upper respiratory passages, and from one-third to one-half that in the lung, is pooled in the gastrointestinal tract, from which it is presumably eliminated. It must, however, be borne in mind that this represents an extreme simplification of the actual process. In particular it is necessary to point out that the line of demarcation between exposure and post-exposure periods is not sharply defined in all respects. Thus, the movement of uranium from the upper respiratory passages into the gastrointestinal system does not begin suddenly at the end of the exposure, but rather it is a process which has been going on continuously

throughout the inhalation period, beginning when the first dust particles are deposited on the nasal mucosa.

Summary.

1. Results obtained by the use of the retention apparatus have been confirmed by direct chemical analyses of animal tissues.

2. The general pattern of the process of retention during inhalation of fine insoluble particulates has been established.

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PROGRAM Be.

BERYLLIUM

Problem Code: Be.3 (Toxic Limits)

Section Code: 3210

Authors: R. H. Hall, H. E. Stokinger, R. E. Root, L. T. Steadman, C. Stroud, E. Alling, F. A. Smith, and J. K. Scott

Animal Inhalation Exposure of BeF_2 Mist - 2 mg/m^3.

Possible exposure to inhalation of beryllium fluoride mist constitutes a hazard in present methods of producing beryllium metal. In UR-103 a preliminary account was given of the effects observed in laboratory animals exposed to beryllium fluoride mist at a concentration of approximately 2 mg/m^3. This experiment, the second in series delineating the toxicity of inhaled beryllium fluoride, and having as ultimate objective the establishment of safe limits of human exposure to this compound, has been terminated after a total of 207 calendar days.

Exposure of animals was carried out in the same chamber (216 cu. ft. capacity), using the same aspirator feed, as in previous studies of the toxicity of beryllium sulfate mist (1). The density of the beryllium fluoride solution was maintained at 1.050 throughout the experiment, since this has been found to be a critical factor in maintaining not only the water-content of the mist droplets, but also the droplet size. A highly efficient counter-current steam scrubber, packed with Raschig rings, in conjunction with a stack precipitron, removed 99.9% of the toxic material from the chamber effluent. The concentration of mist in the chamber atmosphere was controlled with reference to samples taken hourly by means of the filter paper dust sampler, aspirating for 50 minutes at a rate of 15.87 l/min. On the basis of the increase in weight of the filter

papers, the mean concentration of mist was 3.0 ± 0.35 mg/m^3. The results of spectrographic analysis of spot filter paper samples taken during the course of the experiment showed that the beryllium content was not less than 14.0 ± 1.0% (Memo: L. T. Steadman to H. E. Stokinger, 10/11/49). Assuming that the compound present in the mist droplets was BeF_2, the actual concentration of toxic agent represented not less than 73.0 ± 5.2% of the over-all mist concentration, the remainder probably being mainly water. The mean concentration of beryllium fluoride mist, therefore, was 2.2 ± 0.25 mg/m^3 (0.42 ± 0.03 mg Be^{+2}/m^3). As an additional check on the gravimetric sampling method, 18 blank filter papers and 20 beryllium fluoride mist filter paper samples were dried in vacuo over P_2O_5 for 18 hours at 100°C. The net loss in weight of beryllium fluoride mist samples, corrected for the weight loss of filter papers alone (3%), amounted to 20% of their initial weights.

Toxic Responses. In this experiment, a total of 161 animals were used, comprising four species - 5 cats, 16 dogs, 10 rabbits and 130 rats. In general, the animals were exposed in the chamber 6 hours daily, 5 da/wk. The duration of the exposure period was different, however, for individual animals as well as for different species. As stated in UR-103, beryllium fluoride mist at a concentration of 2.2 mg/m^3 proved lethal only to rats and to four dogs, although a concentration of 10 mg/m^3 caused a high incidence of mortality in cats, dogs, guinea pigs, rabbits and rats in a period of only 3 weeks. As shown in Figure 1 (Page 57), inhalation of beryllium fluoride mist in a concentration of 2.2 mg/m^3 resulted in the death of 50% of each of two groups of full grown male rats averaging 285 and 300 grams, respectively, after 9 days (54 hrs.) exposure (Ct = 7.13 x 10^3 mg min/m^3). In a third group of 40 younger male rats, initially averaging 226 grams in weight, however, only 8, or 20%, died during a period of

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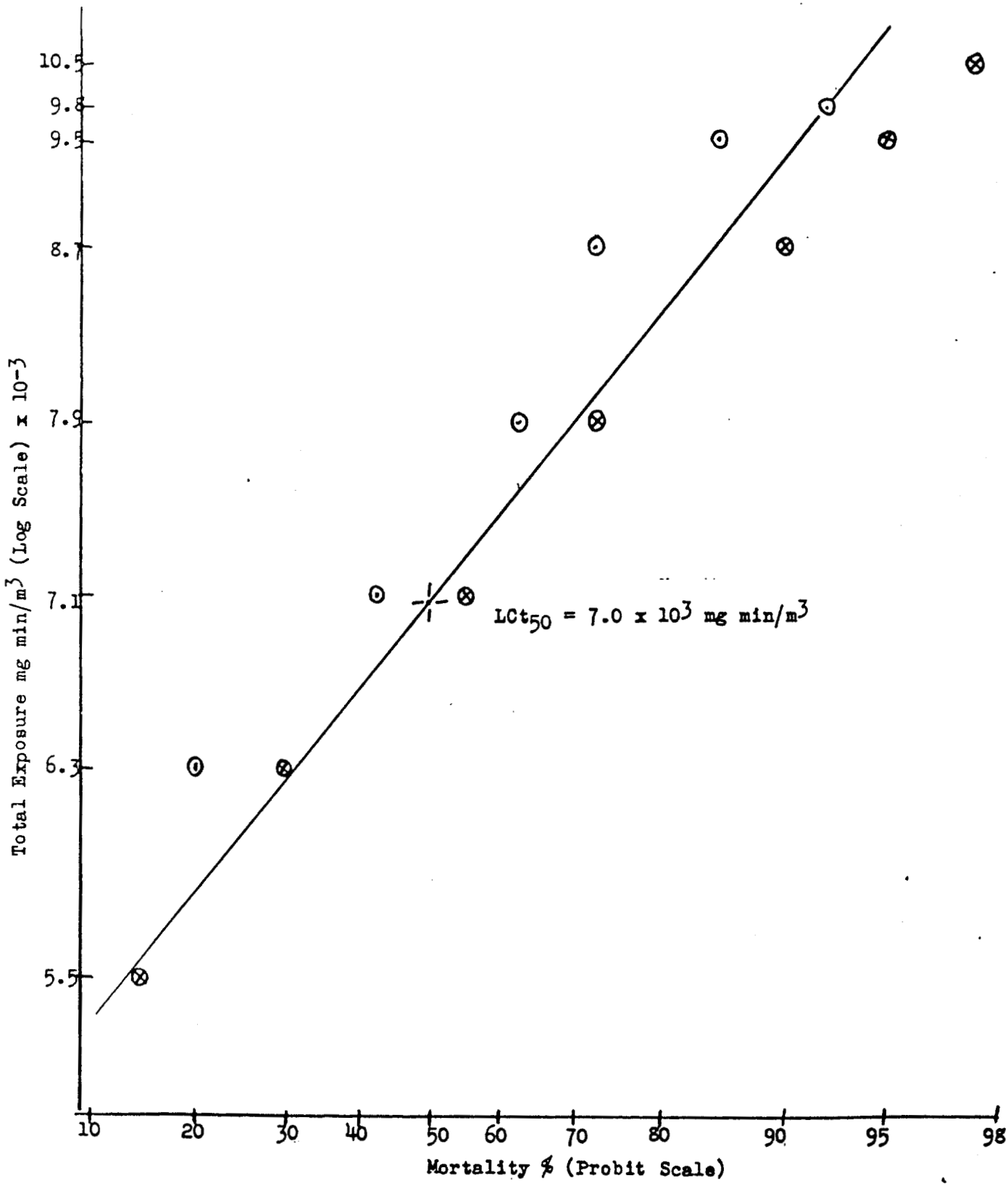
Figure 1. Dose-Mortality Curve: Fully Grown Male Rats Exposed to 2.2 mg/m^3 of Beryllium Fluoride Mist



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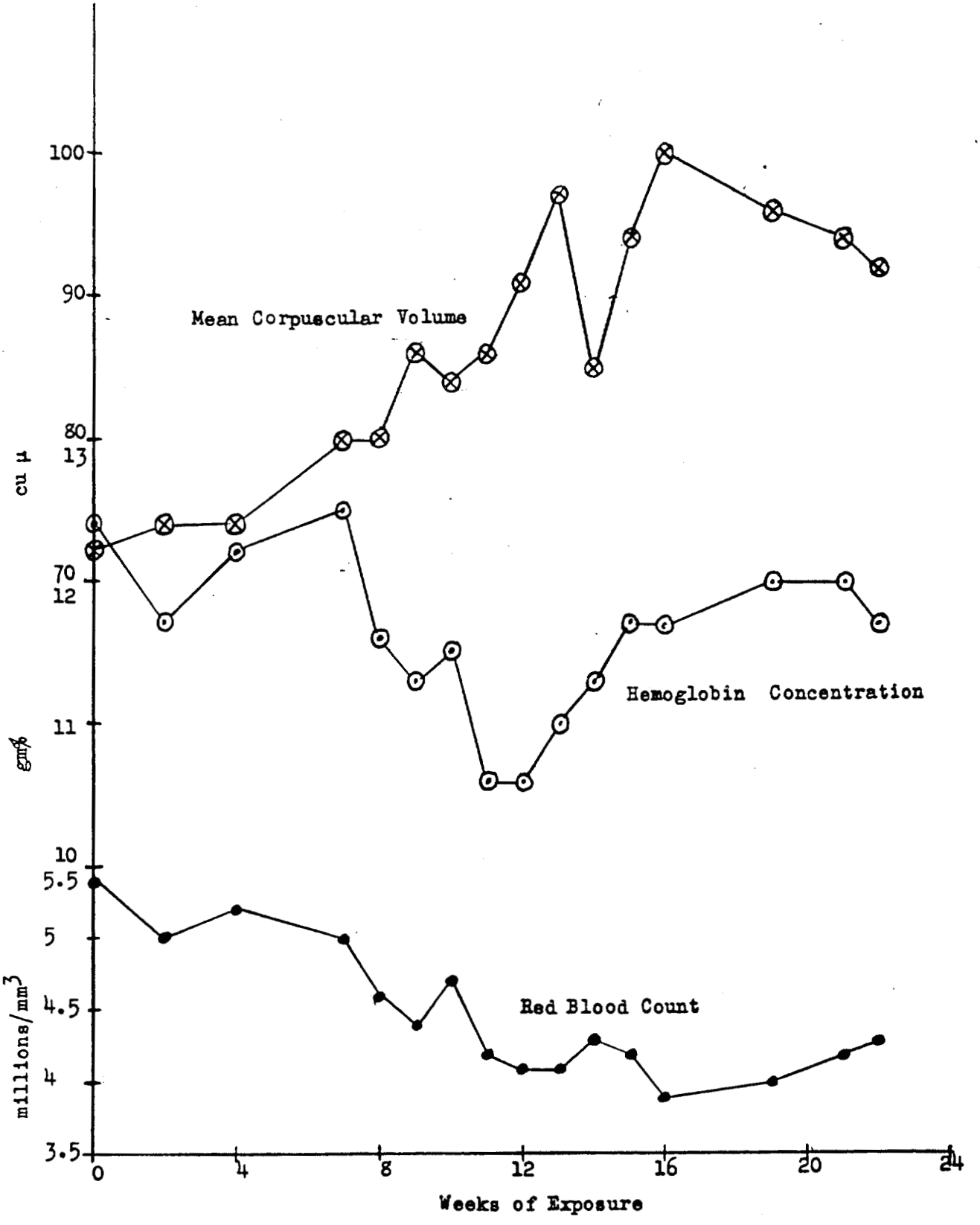
exposure of 60 hours. The weight changes and visible signs of intoxication among the animals were discussed in UR-103

Development of Anemia. Apart from the lethal effects discussed above, the outstanding result of exposure to a concentration of 2.2 mg/m^3 of beryllium fluoride mist was the progressive development of a macrocytic anemia in dogs and rabbits. The changes in the peripheral blood were more striking, although no more real, in the former than in the latter species. In the group of 3 rabbits selected for hematologic study, the mean RBC count began to decrease after approximately the fourth week (18 days) of exposure. As shown in Figure 2 (Page 59), the mean corpuscular volume increased sharply at the same time. There was a concomitant decrease in mean hemoglobin concentration, but this was followed by a sharp rise after about 12 weeks of exposure, whereas the RBC count tended to remain low and the MCV high. The mean corpuscular hemoglobin concentration tended to fluctuate approximately within the normal range of values for rabbits (33 ± 1.7 $\mu g/m\mu^3$), although a value as low as 26 $\mu g/m\mu^3$ was recorded for the rabbit (No. 346) that exhibited the most severe anemia. No trend was observed in the WBC counts of the rabbits, and there were no significant changes in their differential WBC counts.

A preliminary account of the hematologic changes in 6 dogs was given in the preceding issue of this report, where it was stated that these changes were more striking than in previous experiments with hydrated beryllium sulfate mist and beryllium oxide dust. In clarification of the latter statement, it should be pointed out that the beryllium concentration inhaled in the present experiment was greater than in the hydrated beryllium sulfate exposure at 1 mg/m^3 (0.04 mg Be^{+2}/m^3), and less than in any of the exposures to beryllium oxide dust. All six of the dogs referred to above received a dietary supplement of

Figure 2. Hematologic Changes in Rabbits During Exposure to 2.2 mg/m^3 of Beryllium Fluoride Mist



one teaspoonful of Lextron* daily for one month before exposure. Lextron was discontinued in the diet of two dogs (Nos. 1537 & 1538) at the beginning of exposure, while the other four dogs continued to receive the dietary supplement throughout the entire period of their exposure. The RBC counts and hemoglobin concentration of the blood of all of the dogs began to decrease after about the 4th week of exposure. At about the same time, the MCV began to increase. Neglecting the two dogs that died, one after 4, the other after 10 weeks exposure, for which the data are necessarily incomplete, there remained two groups of two dogs each, Nos. 1437 & 1518 that received Lextron throughout the experiment, and Nos. 1537 & 1538 that received no Lextron after the beginning of exposure. All four dogs survived the entire exposure period of 207 calendar days and were humanely sacrificed shortly thereafter. The pre-exposure values of four important hematologic indices in these dogs, together with the maximal changes in these indices are listed in Table 1 (Page 61). In Figures 3-5 (Pages 62-64) the average RBC counts, hemoglobin concentrations, and MCV's of each of the two groups of dogs are shown in relation to the number of weeks exposure. From these figures, and from the data presented in Table 1, it can be seen that the anemia was somewhat more severe in the Lextron-fed dogs than in those that received no further dietary supplement after the beginning of exposure. In both groups of dogs, the depression of the RBC count reached a maximum after 15 weeks exposure. The RBC counts of the Lextron-fed dogs remained low between the 15th and 23rd weeks of exposure, after which a slight increase occurred. The RBC counts of the control dogs (Lextron discontinued at the beginning of exposure) exhibited a secondary rise and fall between the 20th and 29th exposure weeks. The average MCV of the Lextron-fed dogs increased to a peak value during the 23rd week of

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* A liver and stomach concentrate fortified with ferrous iron.

TABLE 1

SUMMARY OF HEMATOLOGIC CHANGES IN 4 DOGS EXPOSED TO 2.2 mg/m^3 OF BERYLLIUM FLUORIDE MIST DURING 207 CALENDAR DAYS

Index	DOG NUMBERS			
	Lextron-Fed		No Lextron	
	1437	1518	1537	1538
RBC Count (million/mm^3)				
Pre-exposure	6.8 ± 0.5	6.1 ± 0.35	6.1 ± 0.6	6.3 ± 0.33
Maximal change	-3.5	-2.9	-2.7	-2.3
Hgb-concentration (gm/100 cc.)				
Pre-exposure Values	12.7 ± 0.8	15.7 ± 0.4	13.0 ± 1.5	15.4 ± 0.8
Maximal change	-3.7	-6.8	-3.6	-3.4
Mean Corpuscular Volume (cu. μ)				
Pre-exposure Values	75	71.5	72	78.5
Maximal change	+47	+46.5	+31	+37.5
Mean Corpuscular Hgb-concentration (μg/cu. mμ)				
Pre-exposure Values	29.0 ± 1.0	31.3 ± 1.3	29.8 ± 2.2	31.5 ± 2.0
Maximal change	-5.0	-5.3	-4.3	-5.5

Figure 3. Hematologic Changes in Lextron-Fed Dogs Exposed to 2.2 mg/m^3 of Beryllium Fluoride Mist

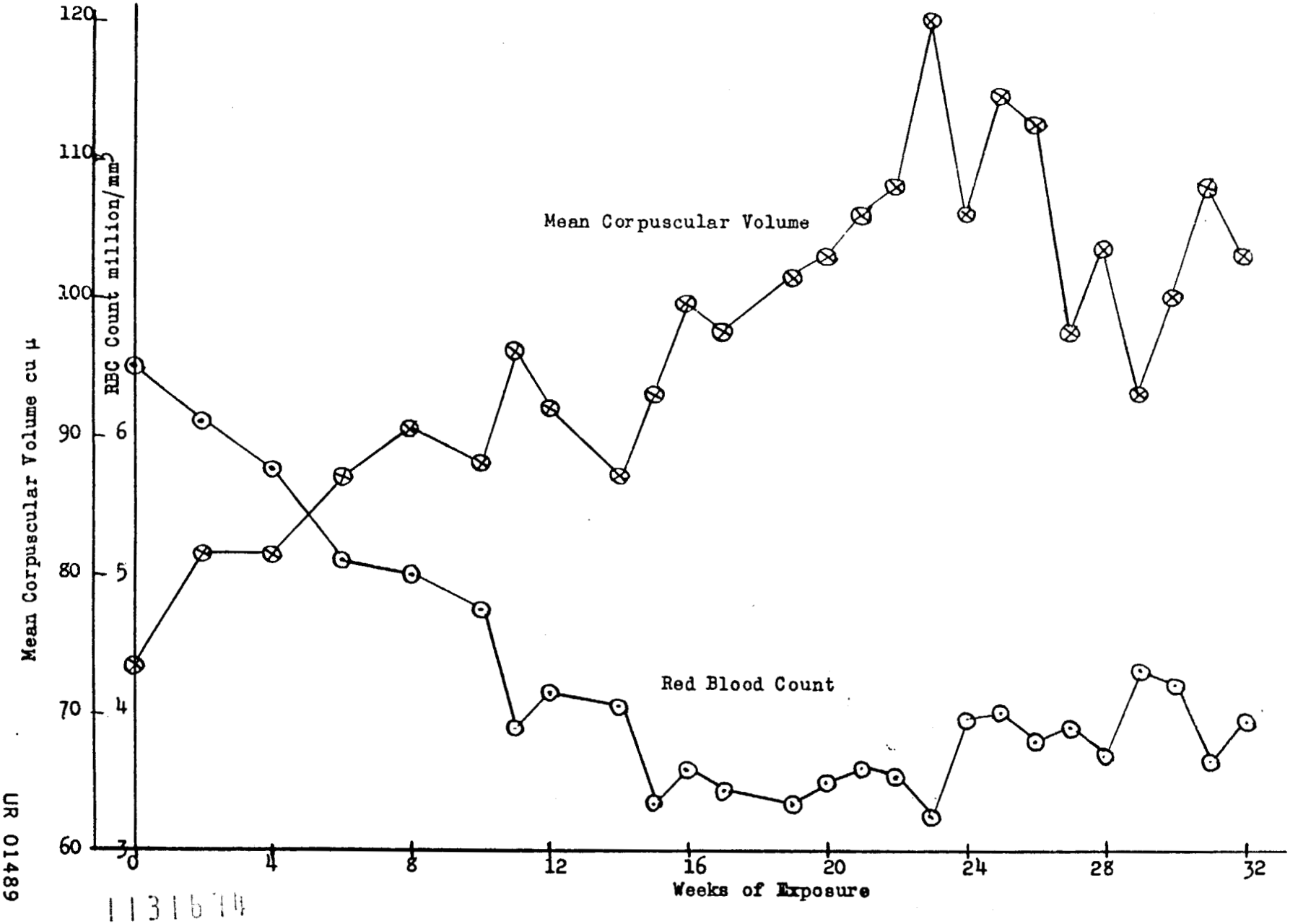


Figure 4. Hematologic Changes in Dogs not Receiving Lextron During Exposure to 2.2 mg/m^3 of Beryllium Fluoride Mist

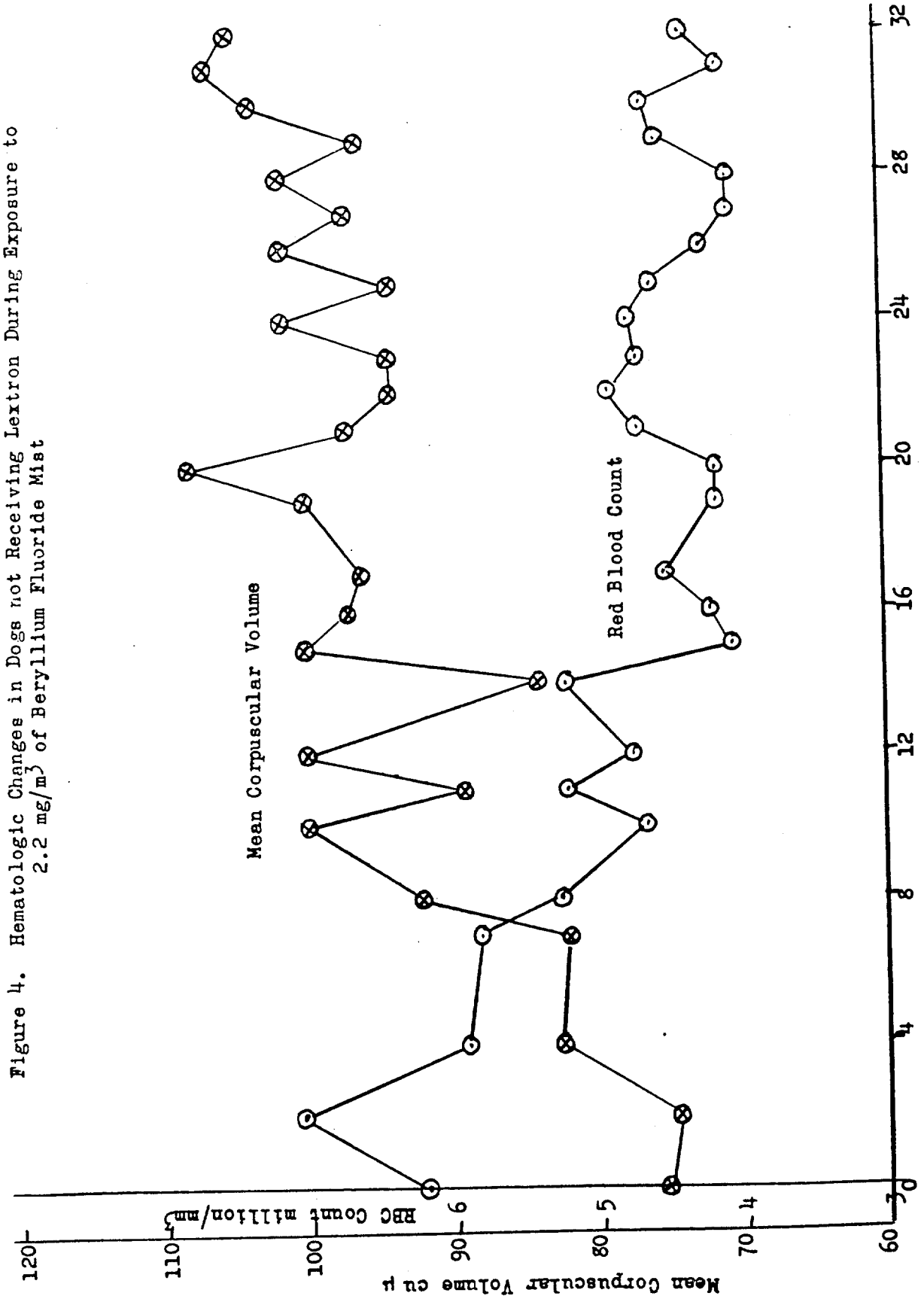
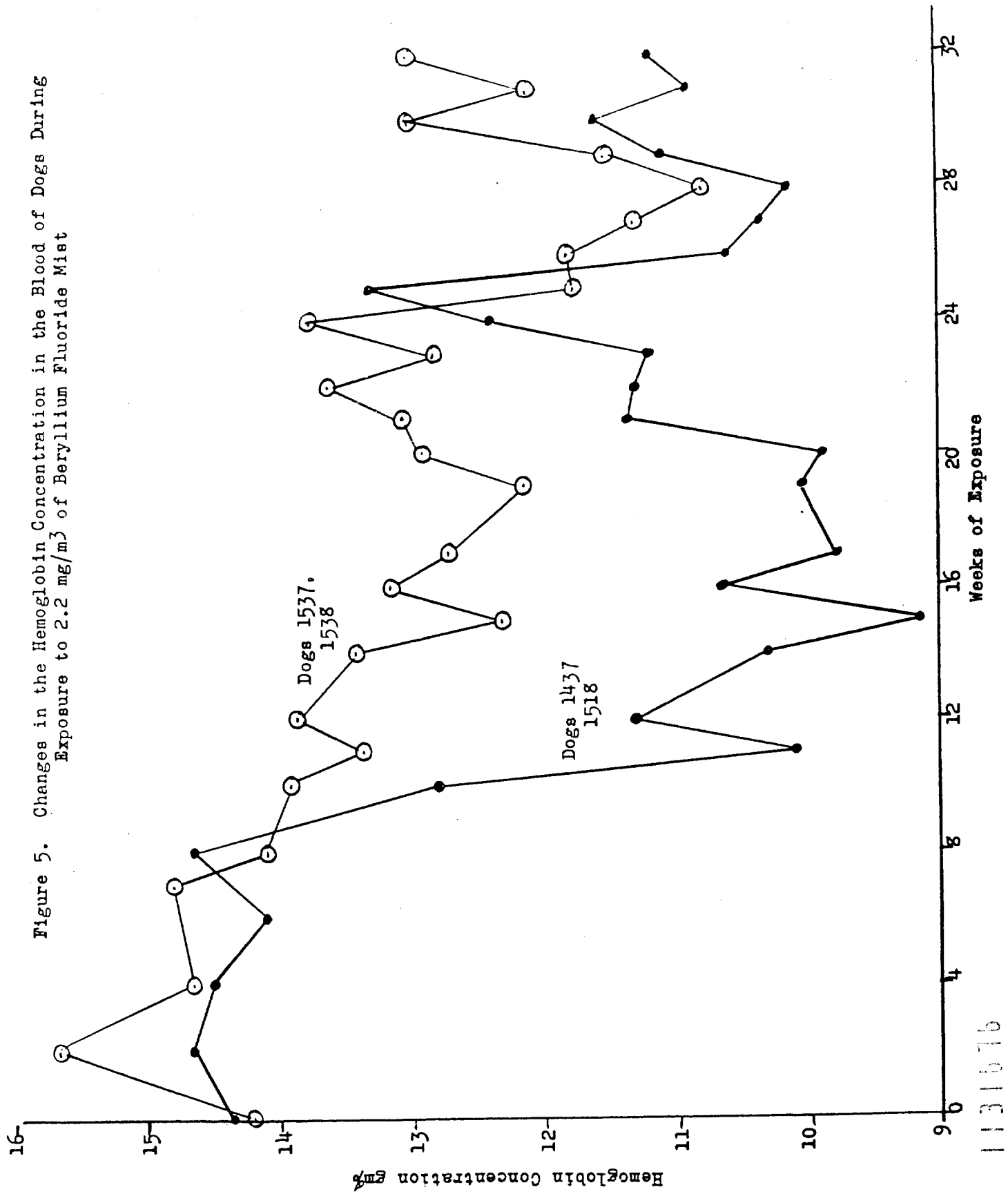


Figure 5. Changes in the Hemoglobin Concentration in the Blood of Dogs During Exposure to 2.2 mg/m3 of Beryllium Fluoride Mist

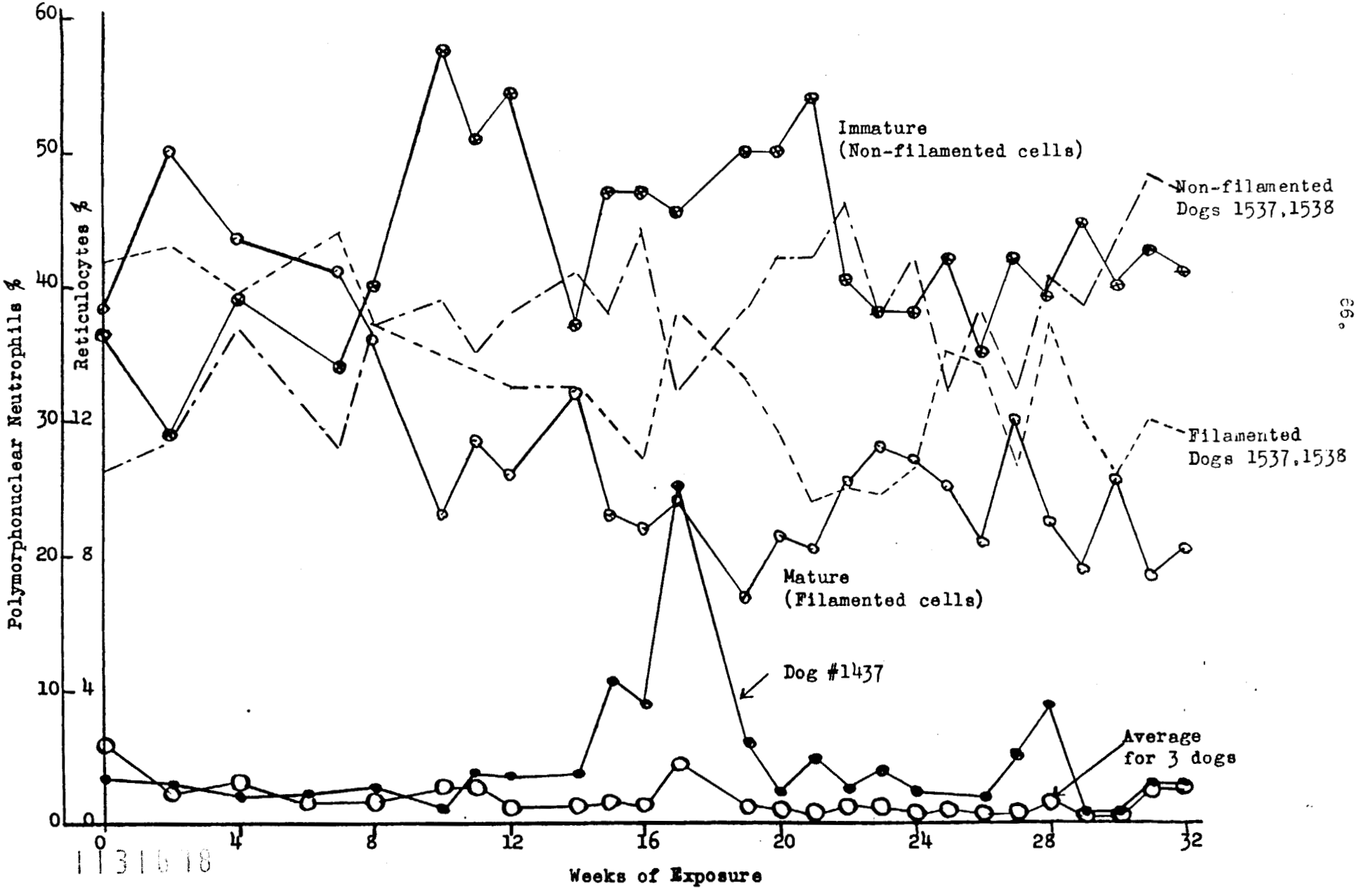


exposure, after which the value of this index tended to decrease. In the control group, the peak value of the MCV was reached during the 20th week of exposure, after which there was a small decrease and a slight secondary rise. In both groups of dogs, the hemoglobin concentration of the whole blood tended to decrease more or less steadily until a minimal value was reached during the 15th exposure week, after which a series of secondary increases and decreases occurred.

Leukocytic Changes. As shown graphically in Figure 6 (Page 66), a peculiar shift in the proportion of filamented (mature) and nonfilamented (immature) polymorphonuclear neutrophil granulocytes occurred in the differential leukocyte counts of the Lextron-fed dogs. With continued exposure to the beryllium fluoride mist, the percentage of mature neutrophils tended to decrease, while the percentage of immature forms tended to increase. About the 8th week of exposure the curves cross, and thereafter the proportion of immature cells tended to remain greater than the corresponding proportion of mature cells. A similar, but less marked shift occurred in the differential leukocyte counts of the control dogs.

Changes in Reticulocytes. The reticulocyte counts of three of the dogs steadily decreased during the first 12 weeks of exposure, and tended to remain low thereafter, as shown in Figure 6. One of the Lextron-fed dogs, No. 1437, exhibited a marked spontaneous increase of reticulocytes between the 10th and 18th weeks of exposure, the percentage of reticulocytes reaching a maximum during the 17th week, after which there was a sharp decline. Concomitantly with the increase of reticulocytes, normoblasts appeared in the peripheral blood of this dog, increasing to a peak of 27½% of the leukocyte count during the 16th week of exposure, after which they declined in frequency and disappeared.

Figure 6. Hematologic Changes in Dogs During Exposure to 2.2 mg/m^3 of Beryllium Fluoride Mist



At the same time, erythroblasts were found in the circulating blood, increasing from 1 to 4% of the leukocyte count. These cells appeared during the fourth month of exposure and persisted thereafter. This dog exhibited the most severe anemia of any of the four, but at no time did its RBC count fall below 3.3 x $10^6/mm^3$, and the lowest hemoglobin concentration reached was 8.9 grams/100 cc. whole blood.

Atypical, "foamy" lymphocytes were seen in blood smears from all four of these dogs. These cells appeared during the second month of exposure of three dogs, Nos. 1437, 1537 and 1538, but only after six months of exposure of the fourth dog, No. 1518. Their appearance in blood smears of a single animal would probably be of no significance, but since they were found in the blood of all four dogs, appearing at the same time in three of them, some importance must be assigned to them.

Biopsied specimens of bone marrow were obtained from the crest of the ileum of three of the dogs after varying period of exposure. The appearance of the bone marrow of dog #1537 was normal after 19 weeks of exposure, at which time the RBC count was 4.1 x $10^6/mm^3$, the MCV was 103 cu. μ and the hemoglobin concentration of the whole blood, 13.0 gm/100 cc. In smears prepared from the bone marrow of dogs Nos. 1437 and 1518, after 16 and 20 weeks exposure, respectivel the myeloid cells appeared normal, but there was a slight increase of megaloblasts and a definite increase of normoblasts and erythroblasts. These findings are indicative of increased erythropoiesis, which occurs in any anemia. In smears prepared from bone marrow obtained from three of the dogs at the time of sacrifice (after 30 weeks exposure), there was an increase of normoblasts and of erythroblasts exhibiting +3 or +4 hemoglobin staining, again indicative of increased red blood cell formation such as occurs in any anemia. Terminal bone marrow

smears from dog #1538 appeared normal. The terminal RBC count of this dog was 5.0 x $10^6/mm^3$, with an MCV of 110, and 15.5 gm/100 cc. hemoglobin concentration of the whole blood.

Analysis of Red Cell Stroma. (Mr. G. A. Tishkoff) Analysis of the stroma of red cells of 2 dogs, exposed for 7 months to the beryllium mist, showed normal values for all components, stroma content 9.5 and 9.1 mg/ml red cells, total mg stroma N/ml 53.5 and 59.2 and stroma lipid mg/ml, 3.8 and 3.05. Normal values for dogs of these components average respectively for stroma, stroma protein and lipid 9, 57, and 3.9 mg/ml.

Prophylactic Effect of Vitamin B_{12}. During the last 7 weeks of this experiment, 4 dogs were exposed for varying periods of time in the chamber with the object of evaluating the prophylactic effectiveness of Vitamin B-12 against the anemia that appears in experimental animals following inhalation of beryllium compounds. Two of these dogs received a dose of 3 μg of Vitamin B-12 intramuscularly three times, at 48-hour intervals, during the first week of exposure, twice weekly, with an interval of 72 hours, thereafter. The other two dogs in this group of four served as controls, receiving no Vitamin B-12. One of the control dogs, No. 1523, was exposed for a total of 132 hours at 2.2 mg/m^3 of beryllium fluoride mist. The other three dogs were exposed for a total of 150 hours at the same mist concentration. All of these dogs exhibited macrocytosis with rather low RBC counts (averaging 5.3 $\pm$ 0.3 x $10^6/mm^3$) but normal hemoglobin concentrations (14.3 $\pm$ 1.0 gm/100 cc.) before exposure. Their RBC counts decrease during exposure, as did also the hemoglobin concentrations of the whole blood. There was little change in the mean corpuscular volumes. The average depression of the RBC counts was slightly less (-0.75 x $10^6/mm^3$) in the treated dogs than in the controls (-1.3 x $10^6/mm^3$). There was no significant difference between the

treated and control groups with respect to the decrease in hemoglobin concentration of the whole blood. No trend was observed in the leukocytic counts of these dogs and the differential counts revealed no abnormal changes. From these results it appears that Vitamin B-12 alone is of little prophylactic value in the prevention of the macrocytic anemia induced by exposure to beryllium compounds by inhalation.

Therapeutic Effect of Vitamin B-12. One of the Lextron-fed dogs, #1437, and one of the controls (not receiving Lextron) was given 12 µg each of Vitamin B-12 intramuscularly in four divided doses at 72-hour intervals beginning on Monday of the 28th week of exposure. Both dogs exhibited a slight to moderate reticulocytosis followed by an increase in the RBC count of one million cells/ mm^3. The increased RBC count was not sustained, however, and it was concluded that either the course of treatment was insufficient, or Vitamin B-12 alone is not effective in the therapy of Be-induced anemia. A similar response to B-12 had been noted in dogs made anemic to beryllium sulfate mist.

Effect of Folic Acid. All four dogs were subsequently given 15 mg of folic acid per os in divided doses of 5 mg on three successive days at the conclusion of the exposure period. There was a slight to moderate reticulocytic response between the 6th and 15th days after treatment, but the dogs were sacrificed before a significant increase in their RBC counts had occurred, so that this portion of the experiment was inconclusive.

Clinical Chemical Criteria of Toxicity. Clinical chemical tests have been performed upon blood samples obtained periodically from each of the six dogs that were exposed from the beginning of the experiment. One of the dogs died on the 29th calendar day. In the brief period that this animal was exposed, there was no significant alteration in the serum protein concentrations. However,

a sharp increase in plasma fibrinogen concentration occurred, the level rising from a pre-exposure mean of 0.3 gms% to 0.63 gms% on the 17th calendar day, followed by a precipitous drop to 0.32 gms% on the 21st calendar day. A second dog died on the 71st calendar day of exposure. This animal exhibited a progressive increase of plasma fibrinogen concentration from an initial 0.5 g% to 0.73 g% on the 51st calendar day, the level returning to 0.52 g% on the 63rd calendar day. There were no significant changes in the serum alkaline phosphatase, calcium or phosphorus concentrations in the blood of any of these six dogs. No significant changes occurred in the serum protein concentrations of the four dogs that were exposed for a total of 207 calendar days. There was a very slight increase in serum albumin concentration, and a corresponding slight decrease of serum globulin, with the result that the A/G ratio increased somewhat. In all four dogs, the plasma fibrinogen concentration increased significantly between the 9th and 17th calendar days. This was followed by a gradual return to normal levels, with a second rise and fall between the 117th calendar day and the termination of the experiment. All four dogs exhibited a progressive increase in serum dilution turbidity during the first 135 calendar days. Changes in serum dilution turbidity are thought to be associated with changes in serum globulin, but the significance of the observed increase is unexplained since appreciable alterations were not seen in the serum globulin concentrations of these dogs. The serum protein concentrations in the blood of three rabbits were determined periodically during exposure for 120 calendar days. These rabbits were the same ones upon which hematologic observations were made. All of the rabbits exhibited an increase in total protein concentration between the 20th and 66th calendar days. In two of the rabbits the serum albumin concentration increased after the 66th calendar day, while the third animal exhibited a

decrease at about the same time, the decrease being followed by a rapid increase to nearly 5 gms% for the remainder of the period of study. The serum globulin level remained reasonably constant at 2-3 gms% in the blood of all three rabbits throughout the experiment. In two rabbits the A/G ratio increased very slightly, while in the third animal this ratio was nearly constant.

Electrophoretic Studies on Serum Proteins. Electrophoretic patterns of two dogs, #1518 and #1538, exposed for 6.5 months to 2 mg/m^3 beryllium fluoride mist daily showed no significant variation from normal in any of the components according to Dr. Alling, under whose direction the experiments were performed. Albumin peaks, however, were somewhat lower than those obtained normally for dogs but were still within the normal range; the globulin patterns were about normal as were the fibrinogens. The gamma globulin peaks on dog #1518, however, were fairly low. The A/G ratio of both dogs were in the low range, being 0.7 and 0.8 respectively.

Pathologic Changes in Lungs. Five cats and three rats were humanely sacrificed after 102 calendar days exposure to 2.2 mg/m^3 beryllium fluoride mist. Microscopic examination of tissue sections by Dr. J. K. Scott from each of the five cats revealed no lesions attributable to beryllium in any organs other than the lungs. With respect to lung pathology, two of the cats exhibited no lesions (Nos. 2 and 604), and two showed only mild lesions quite similar to those observed in cats exposed to beryllium sulfate mist at a concentration of 1 mg/m^3. The degree of damage in the lungs of the fifth cat, although more extensive than any of the others, was considered to be only minimal or moderate as compared with the lesions observed following exposure to beryllium sulfate mist at the higher concentrations.

Beryllium and Fluoride Content of Tissues. Tissue samples from each of

the five cats and three rats were analyzed spectrographically by Dr. L. T. Steadman for their beryllium content. The results of the analyses are shown in Tables 2 and 3 (Pages 73 and 74). In both species nearly 80% of the total beryllium recovered was found in the skeleton. The percentage recovered from the lungs of the rats was nearly double that found in the same organ of the cat, while the percentage recovery from the liver of the cats was nearly double that found in the liver of the rats. The agreement in the skeletal deposition in the two species is noteworthy. Fluoride analyses were carried out by Dr. F. A. Smith on tissue samples from these same animals. In general, there was no relation between the fluoride and beryllium contents of the tissues on a molal basis. This is difficult to interpret, especially in the case of the lung, which is presumably the source of both the beryllium and the fluoride found in the other tissues.

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TABLE 2

DISTRIBUTION OF Be IN RAT EXPOSED FOR 102 DAYS TO Ca. 2 mg BeF_2/m^3

	Animal Weight	% Wt. of Organ in Body	Organ Wt.	Be Content	Total Be in Organs
	g		g	µg/g	µg
BONE	326	10.9	35.6	0.46	16.4
	246	"	26.8	0.56	19.9
	326	"	35.6	0.50	17.8
				Average	18.0
LUNG	326	0.53	1.7	3.9	6.6
	246	"	1.3	2.4	4.1
	326	"	1.7	3.7	6.3
				Average	5.3
LIVER	326	4.17	13.6	0.007	0.09
	246	"	10.3	0.006	0.06
	326	"	13.6	0.006	0.08
				Average	0.08
KIDNEY	326	0.78	2.52	0.049	0.12
	246	"	1.92	0.062	0.12
	326	"	2.52	0.050	0.13
				Average	0.12

Total Distribution

	µg Be	%
Skeleton	18	78
Lung	5.3	22.5
Kidney	0.12	0.5
Liver	0.08	0.34

TABLE 3

DISTRIBUTION OF Be IN CAT EXPOSED FOR 102 DAYS TO Ca 2 mg BeF_2/m^3

	Animal Weight	% Wt. of Organ in Body	Organ Weight	Be Content	Total Be in Organs
	kg		g	µg/g	µg
BONE	3.6	12.7	457	1.2	550
	3.3	"	420	2.64	1,110
	2.9	"	368	2.2	810
	3.0	"	382	2.2	840
	2.6	"	330	2.2	728
				Average	810
LUNG	3.6	0.51	18.3	7.1	130
	3.3	"	16.8	5.1	86
	2.9	"	14.8	5.3	79
	3.0	"	15.3	17.6	270
	2.6	"	13.3	6.8	91
				Average	131
LIVER	3.6	2.96	105.7	0.66	70
	3.3	"	97.5	1.83	178
	2.9	"	86	0.45	39
	3.0	"	89	0.65	58
	2.6	"	77	0.57	44
				Average	78
KIDNEY	3.6	0.81	29	0.05	1.5
	3.3	"	27	0.02	0.5
	2.9	"	24	0.04	1.0
	3.0	"	24	0.04	1.0
	2.6	"	21	0.05	1.0
				Average	1

Total Distribution

	µg Be	%
Skeleton	810	79
Lung	130	12.7
Liver	80	7.8
Kidney	1	0.1

Total 1021

Other tissues such as spleen, heart, blood, pancreas, thyroid, adrenals, pituitary are considered to contain negligible amounts.

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Problem Code: Be.4 (Fate)

Section Code: 3210

Authors: H. E. Stokinger, R. E. Root and L. T. Steadman

Retention of Beryllium in Tissues Following Inhalation. II. Effect of Diet on Deposition and Retention in Rats.

In UR-103 a number of findings on the rates of intake and elimination among which was the prominent fact that the rate of elimination of beryllium from the tissues of different animal species varied widely were summarized. For example, it was found that 50% of the beryllium deposited in the lungs of rabbits exposed for 40 days to 3.6 mg/m^3 beryllium sulfate mist was eliminated within 23 days, whereas a similar reduction in the beryllium content of the lung of the hamster required a calculated 112 days following a 67-day exposure to 1 mg of the same beryllium aerosol. The conclusion that species differences were responsible for the marked difference in elimination rate was based on the assumption that beryllium is deposited in the same fashion in different species, i.e., that beryllium mist penetrated into the same recesses of the alveoli and that the difference in exposure times did not materially affect the processes of elimination.

In view of these differences in beryllium elimination rates apparently caused by species differences, a study was made to determine whether the rate of elimination (and/or deposition) might be altered by a modification in diet. This study was performed in rats.

Sixty male, albino rats were divided into 3 groups of 20 rats each, weighing at the start of the experiment 220-240 g. One group was given a regular foxchow meal ad libitum; this group consumed on the average 18-20 g of this diet per day per rat. The second group was restricted to 10 g of the same

foxchow meal daily per rat. The third group was likewise restricted to 10 g of the meal per day per rat but supplemented with 2% sulfasuxidine (for a purpose unrelated to the present experiment). The animals were maintained on this regimen for 7 days prior to and during daily 6-hour exposure to approximately 2 mg/m^3 BeF_2 for 35 days. The rats surviving this exposure* were killed in groups of from 24 rats from each of the dietary groups terminally, and 16 and 35 days later. During the post-exposure period, the animals were similarly maintained on the dietary regimen.

From the data obtained on the three different groups shown in Table 1 (Page 77), the following conclusions seem warranted from simple statistical tests (Dr. S. Lee Crump). At the termination of the exposure, similar amounts were found in the lungs of all animals irrespective of the diet; during the post-exposure periods, however, elimination of beryllium appeared to occur at a faster rate in the animals on the restricted diet than in those eating the same diet at will (38-56% remaining at the end of 5 weeks as against essentially no change in the rats on unrestricted intake). Consistently less (14-45%) was found in the femur of rats on a restricted diet, more appearing in the kidney and liver than in animals on an unrestricted intake, indicating an increased rate of elimination from the lung. The concentration of beryllium in the femur of all rat groups was approximately one-tenth of that in the lung irrespective of dietary intake.

It is possible also to demonstrate a greater absolute loss of Be from the body of the rats on the restricted diet than from those on the unrestricted

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* This dosage of beryllium fluoride was lethal to from 30-35% of the rats. There was no significant difference in mortality of the rat groups on the restricted and nonrestricted diets.

TABLE 1

BERYLLIUM CONTENT OF TISSUES OF RATS EXPOSED BY INHALATION TO BERYLLIUM FLUORIDE AT 2 mg/m^3 FOR 35 DAYS WHEN ON VARIOUS DIETS

Results expressed in micrograms Be per gram fresh tissue.
Average of from 2 to 4 rats.

	REGULAR DIET			RESTRICTED DIET			RESTRICTED DIET & SULFASUXIDINE		
	Mean	Range	Rat Wt.	Mean	Range	Rat Wt.	Mean	Range	Rat Wt.
			g			g			g
					TERMINAL				
LUNG	3.3	2.4-3.9	299 (246-326)	4.8	3.8-6.2	230 (210-240)	3.7	2.4-5.0	207 (202-216)
FEMUR	0.51	0.46-0.56		0.4	0.4-0.44		0.44	0.41-0.48	
KIDNEY	0.054	0.049-0.062		0.09	0.07-0.10		0.14	0.11-0.17	
LIVER	0.006	0.006-0.007		0.02	0.02		0.02	0.01-0.03	
					16 DAYS POST-EXPOSURE				
LUNG	3.8	2.8-4.7	325 (280-360)	2.5	2.1-2.8	232 (220-244)	3.5	2.3-4.7	240 (220-244)
FEMUR	0.38	0.30-0.42		0.22	0.21-0.23		0.21	0.20-0.21	
KIDNEY	0.05	0.039-0.078		0.08	0.07-0.08		0.05	0.01-0.08	
LIVER	0.009	0.0073-0.013		0.010	0.007-0.013		0.009	0.007-0.01	
					5 WEEKS POST-EXPOSURE				
LUNG	3.0	2.0-4.0	326 (320-330)	2.1	1.6-2.4	221 (202-232)	2.3	1.8-2.7	230 (216-244)
FEMUR	0.39	0.35-0.45		0.27	0.26-0.29		0.23	0.20-0.25	
KIDNEY	0.051	0.039-0.062		0.07	0.06-0.08		0.04	0.08-0.10	
LIVER	0.010	0.004-0.012		0.014	0.012-0.017		0.014	0.010-0.017	

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intake. Reference to Table 2 (below) shows the computations of total body beryllium* in the two groups at the start and conclusion of the analytic diet as compared with 5.9 μg on a restricted diet; on a percentage basis, Be loss amounted to 17.4 and 37.5% respectively. This difference appeared to be contributed chiefly by the lung.

TABLE 2

TOTAL Be IN UNEXPOSED AND EXPOSED RATS

	Unexposed 7 mo. of age	Exposed			
		UNRESTRICTED DIET		RESTRICTED DIET	
		Terminal	5 weeks post exposure	Terminal	5 weeks post exposure
		μg	μg	μg	μg
Skeleton	0.6	18	14	10	6.8
Lung	0.15	5.3	5.1	5.3	2.7
Liver	0.03	0.08	0.14	0.2	0.14
Kidney	0.024	0.12	0.12	0.16	0.12
TOTAL Be	0.8	23.5	19.4	15.7	9.8

Loss on unrestricted diet - 4.1 μg Be in 5 weeks
Loss on restricted diet - 5.9 μg Be in 5 weeks

If the possibility is considered that the differences found among the groups might arise from the differences in beryllium intake from the diet (analyzed beryllium content of foxchow meal, 0.1 μg/g), reference to Table 2 will show that this cannot be a factor. 0.8 μg Be was the average total amount in the body in each of 10 rats consuming the regular diet of foxchow meal but

* It has been determined that the four tissues listed in Table 2 account for essentially all of the beryllium taken into the body via the respiratory tract.

not exposed to beryllium compounds. The age of these rats was 7 months as opposed to approximately 4 months for those in the present study. It is obvious, therefore, that a restricted food intake could not be responsible for the difference in total body beryllium content.

It would appear, accordingly, from these results that it is possible to modify substantially the rate of elimination of beryllium in the lung and its deposition in the femur of animals by modifications of dietary intake alone.

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PROGRAM S.M.

SPECIAL MATERIALS

Problem Code: S.M.3 (Toxic Limits)

Section Code: 3210

Authors: Robert H. Wilson and Sidney Laskin

Improved Design for an Animal Inhalation Exposure Unit.

Experience (of this group) with inhalation exposure studies has indicated that the interpretation of the complex biologic variables in such studies can not afford to be handicapped by physical difficulties associated with faulty chamber design. These difficulties include such items as equipment, maintenance and variability of concentration and particle size of the toxic agent (contaminant) in the atmosphere. Coping with such problems in the past with poorly constructed units has often proved costly in terms of labor and time. Frequently these difficulties led to inadequate foundation for the interpretation of the biological data obtained. With these concepts in mind, work has been in progress toward the development of improved inhalation exposure equipment, aerosol feeds and better methods for the characterization of atmospheric impurities. The numerous phases of this program have been discussed in previous quarterly reports. This report is concerned with the latest developments in inhalation equipment representing attempts to approach ideal requirements. Such requirements may be summarized as follows: (1) adequate volume and air turnover for the desired animal capacity; (2) ease of loading and handling animals; (3) simplified means of decontaminating exposure-unit cages and exhaust air from the exposure unit; (4) wide latitude of concentration and particle-size levels; (5) maintenance of concentration and particle-size distributions within the limits of other experimental errors; (6) uniform distribution of concentration

and particle size within the exposure unit; (7) ease of operation and simplicity of mechanical maintenance; (8) simplified methods for the characterization of the atmospheric contaminants.

Although a cubic unit is simplest from a construction standpoint and can be easily designed to meet the animal requirements, it has been found by this laboratory that such a unit falls short of several of the above ideals. Concentration and particle-size distributions within the exposure unit often showed wide variations depending upon positions selected for sampling; maintenance of concentration at any given point frequently proved difficult and subject to large variation.

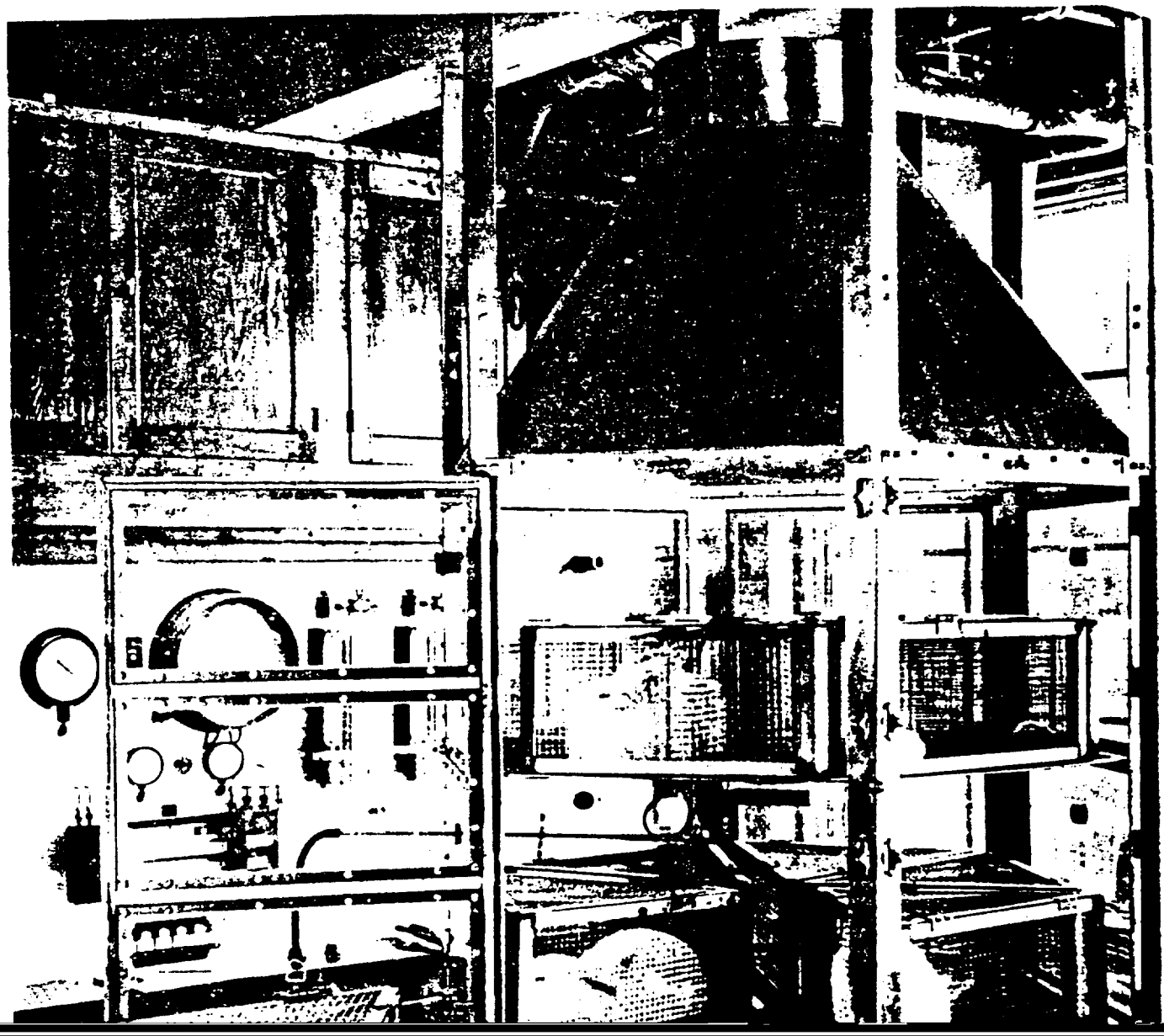
With these shortcomings in mind, corrective measures consisted in the elimination of dead spots or stagnant areas within a cubic unit by blocking off the corners (1). Excessive turbulence tending to remove larger particles by impingement against cages and walls was eliminated by the removal of fans and the substitution of low-velocity directing nozzles for the inlet air. This resulted in a more gentle motion of the air stream with the suspended particles and vastly improved distribution of the dispersing material and simplified concentration maintenance.

In the course of certain investigations performed in this laboratory, a chamber was designed that permitted sampling at any point with a high degree of reliability. This unit has been designated as "the experimental standard chamber" and is described in a previous report (2). The primary purpose of the unit was the study of aerosols and the maintenance of reference atmospheres of known composition for the comparative testing and calibration of feed and sampling devices. Because no animal requirements were involved, a radical departure in chamber design was made in order to approach the ideal based on

aerodynamic principles of a wind tunnel and modified by the practical limitations of space. The construction was in the form of a vertical cylinder with inlet and outlet regions modified to obtain minimal turbulence and maximal uniformity of concentration and particle size at any point within the body section. The design proved highly satisfactory yielding results that were uniform within the limits of experimental errors (3%). Excellent distribution of concentration and particle size have been reported for several types of atmospheres.

The opportunity to apply the experience gained from studies on the experimental standard chamber to an animal inhalation unit was furnished by the needs of a course in Industrial Hygiene and Toxicology (see page 95 this report). A major portion of the laboratory schedule dealt with animal inhalation experiments conducted by students. Utilization of the techniques for this type of experiment by inexperienced investigators emphasized the need for a unit approaching the ideal described above.

A new design of an inhalation exposure unit was developed and constructed in the new Industrial Hygiene and Toxicology teaching laboratory. Exact duplication of the experimental standard unit was not desirable because of animal requirements. As a compromise, a plane-sided approximation of the standard chamber was developed. This permitted large loading doors and air-tight closure more readily than could be obtained with a cylindrical section. The final design, illustrated in Figure 1 (Page 83), is in the form of a hexagonal prism 4 feet across the flats and 3 2/3 feet long. The end sections are frustums of hexagonal pyramids approximately 2 feet high. Although additional sides would have been preferable from an aerodynamic point of view, because of the resultant decrease in door width and the requirement of small animal cages, it was not considered practical. (Space limitations prevented the construction



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of a larger unit with added sides which would have more closely approached the ideal cylinder.) Construction of the unit was of stainless steel and plexiglass throughout. These materials were chosen for their ability to withstand the effects of corrosive salts and repeated washings.

As illustrated in Figure 1 (Page 83), air is admitted tangently to a short cylindrical section at the top. This serves as a mixing chamber in which dusts or other aerosols are distributed uniformly in the inlet air as a result of a mild cyclonic motion. A contaminant inlet is also located in the mixing chamber entering tangently 90° ahead of the supplied air inlet. Exhaust air is drawn axially from the bottom of the exposure unit. Air supply and exhaust lines to the exposure unit are 2-inch, electrical, thin-walled conduit chosen for its streamline construction. Conditioning air is supplied by an I.L.G. b-12 blower and contaminated air from the unit is exhausted through a Rexair vacuum cleaner. The latter was chosen because of its operation as a high capacity centrifugal wet impinger yielding satisfactory scrubbing efficiency.

Control and sampling apparatus mounted on a panel board, located 3 feet behind the unit in order to permit easy access to all sampling ports, provides simplicity of operation. Conventional air, refrigerator and vacuum equipment is mounted above and behind the panel board. Wet and dry bulb temperatures are obtained from a Foxboro, long-distance recording psychrometer. Temperature is controlled by a 1/2 hp Kelvinator unit. Provision is also made for as many as 3 simultaneous medium-rate (0-30 liters/min) samplers from the unit.

Both the supply and the exhaust lines are controlled by gate valves to allow adjustment of air flow from 0-40 cfm. Chamber static pressures can be maintained from +0.1" of water to -0.5" water with respect to normal ambient pressures. These values are determined from a 2-tube draft gauge one tap of which is connected to a pitot tube measuring the velocity head in the exhaust

line, from which the volume of air can be calculated. The second tube directly measures the exposure unit static pressure. Normal operation is maintained at 35 cfm with a static pressure of -0.02 to -0.04" of water. The static pressure is kept negative to prevent external contamination in the event of leakage.

The unit has a total air volume of approximately 75 cubic feet with an animal capacity of 20 rabbits and 40 rats. These animals are exposed in cages as illustrated in Figure 1 (Page 83). The cages are equilateral triangles 2 feet along the side and 10 inches deep, divided into two sections for rabbits and four sections for rats. Racks are provided at two levels for placement of the cages. Assuming adult animal size, animal volume to chamber volume ratios of 5% are obtained. Ventilation requirements are met by an air turnover of one change every two minutes.

The exposure unit is adaptable to almost any type of contaminant feed. The one currently installed is a submerged aerosol unit of the type previously reported (3). Preliminary studies of the operational characteristics of this exposure unit indicate very satisfactory maintenance of concentration and particle size distribution. The unit is currently in use by a group of students engaged in a 3-week study of a uranyl nitrate hexahydrate mist atmosphere at a concentration of 15 mg/m^3. Typical results of operation for a 4 1/2-hour run show a mean concentration obtained of 15.8 mg/m^3 with a standard deviation of 1.0. Seventeen concentration determinations were made with the filter paper dust sampler and although seven different sampling sites were used, the entire range of concentrations obtained were from 13.5 to 17.2 mg/m^3. More complete data on the operating characteristics of this unit will be available at a later date.

The unit was found to be easily operated and decontamination following exposure was facilitated by the exhaust design and the presence of a floor

drain located below the outlet.

With respect to future design of such units for animal inhalation studies, one serious difficulty must be noted. The present design has a comparatively small animal exposure unit yet its height requirements are over 9 feet. Any increase in body dimensions for increased animal capacity will necessitate increasing over-all height beyond the limitations of the average laboratory room. Although no information has been developed as yet on the limitations of the top and bottom sections in terms of decreasing their size, it is surmized that probably an apex angle of 90° would be maximum for satisfactory results.

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1. Wilson, H. B., et al, The Relation of Particle Size of Uranium Dioxide Dust to Toxicity Following Inhalation by Animals, J. Ind. Hyg. and Tox., 30, 6 (1948)

2. Laskin, S., Wilson, R., Frank, P., Experimental Standard Chamber, Univ. of Rochester, A.E.C. Project, Quarterly Report, UR-45, Sept. 1948 pp. 77-90

3. Laskin, S., Submerged Aerosol Unit, Univ. of Rochester, A.E.C. Project, Quarterly Report, UR-38, June 1948

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PROGRAM I.N.

INSTRUMENTATION (SPECTROSCOPY, ELECTRON MICROSCOPY, X-RAY AND NUCLEAR RADIATION DETECTORS, X-RAY DIFFRACTION, ELECTRONICS)

Problem Code: I.N.1 (Research and Development)

Section Code: 3160

Authors: M. L. Watson, B. L. Jacobson, D. C. Scott

Preliminary Report on the Preparation of Tissue Sections for the Electron Microscope

The electron microscope is able, under the best conditions, to provide a resolution of about 20 Å. Good light microscopy can provide a resolution of the order of 1000 Å. It is clear then that the electron microscope can resolve detail some fifty times smaller than the light microscope.

It is of considerable interest to apply the electron microscope to the examination of biological materials. There is, however, an important point which must not be overlooked if good resolution of preparations is to be obtained. The material through which the beam passes must be extremely thin. Electron scattering in a paraffin film of 0.1 μ thickness will result in a resolution of about 120 Å in the standard RCA type EMU microscope. Tissue culture methods, smears, macerated preparations, replicas, and other techniques have been applied to prepare tissue for the electron microscope. With these methods, while fine scale detail has been retained, very often gross large scale "artifact" is introduced - e.g. the artifact of tissue culture cells compared to the same cells grown in the living organism.

A certain amount of work has been done towards the production of tissue sections suitable for the electron microscope. However, no really satisfactory routine method appears to have been published, and it is toward this end that

we are working.

The major problems are the cutting of thin enough sections - ca. 0.1 μ -, fixation without artifact on the 50 Å level, and the removal of the embedding material without the introduction of appreciable further artifact. We will take these up in order, insofar as we have solved them.

Cutting Thin Sections. Under this heading we may consider embedding materials and knife sharpening.

We have in this laboratory tried three embedding mediums, straight 55° - 60° paraffin, paraffin-celloidin, and butylmethacrylate. Of these the butylmethacrylate has proved to be the easiest to cut in a reliable way, so nearly all our work has been done with this. Rather than a mixture of 25% methylmethacrylate and 75% butylmethacrylate with 1% catalyst suggested by Newman et al (1) we find that the softer preparation of pure butylmethacrylate with 2-3% catalyst is easier to cut. However, Newman reported on preparations of rather dense tissue, liver and kidney, and we have worked mostly with lung which is 80% free of tissue. It might be worth mentioning that lung is a convenient tissue to work with since its porosity allows easier and more uniform fixation and embedding than denser tissues. We have also been using the carbon dioxide feed Newman suggested with satisfactory though somewhat unreproducible results.

A most critical step in the cutting of thin sections is the sharpening of the knife. We have developed a method which, though it may not produce the ultimate keenness, does produce a reliable edge.

The knife is sharpened by hand on a plate glass surface. It is supported in a special holder shown in Figure 1 (Page 89). Since the back rests on only one point, pressure along the blade is equalized.

The tilt of the blade is adjustable by the position of the wheel in the

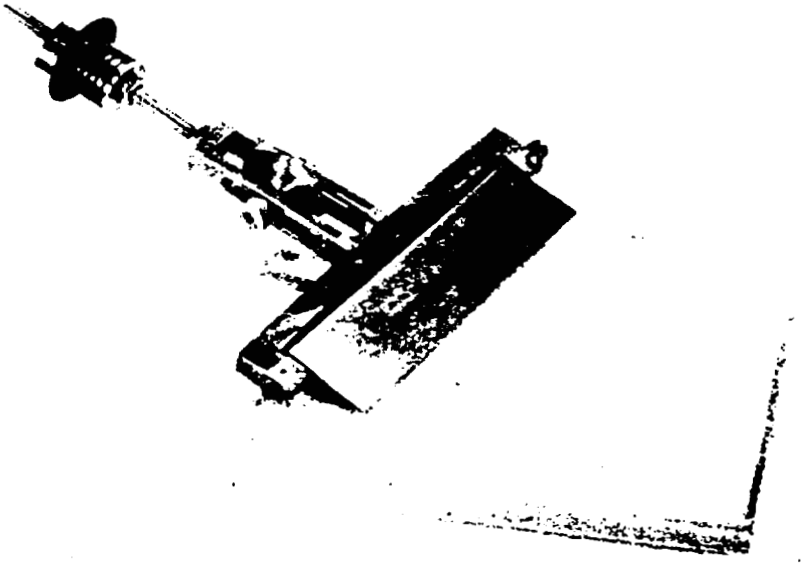


Figure 1. Knife sharpening holder shown in operating position on a glass plate

holder. Our blades have been ground so that the angle between the faces is about 22 degrees instead of the customary 30 degrees. Presumably the harder the tissue blocks, the greater this angle should be.

The surface of a piece of plate glass is ground evenly all over with #2200 emery so that the ground surface is just as flat as the original polished surface. The knife is then mounted firmly in the holder, tilted to 1-2 degrees less than the final desired tilt. It is polished by sliding normal to the edge with the blade in contact with the glass only when the direction of motion is such that the edge leads. In this initial stage a mixture of #8 and #9 Linde Powder is used with ethyl alcohol as dispersant. This operation is continued until all nicks are removed and, under the microscope, the polished bevel has the same appearance across its width. The blade holder is then adjusted to increase the angle of the blade with respect to the glass polishing surface by 1 or 2 degrees. The final polishing is carried out on a polished plate glass surface using alcohol alone with no grinding compound. Care must be taken to select a glass surface which matches the ground surface used for the initial polishing sufficiently well so that the final polishing takes place evenly along the length of the blade. Surfaces may be checked by actually trying them. If the polish comes up only at the ends of the blade, the surface is unsatisfactory. If the polish comes up in the center first, it may be possible to use the surface provided it is not too convex with respect to the blade. Considerable care must be used to scrub all glass working surfaces with alcohol before using them. The Linde alcohol mixture and the alcohol polishing lubricant should be changed every two or three hundred strokes. Final grinding or polishing spells are tapered off from fifty strokes on each side down to one stroke in graded steps. Rather light pressure is used on the blade throughout the polishing operations.

Fixation. We have worked only with three fixatives - Bouin's, Formalin, and Osmic acid.

The Bouin's and Formalin have proved very unsatisfactory, introducing serious artifacts. Some of these artifacts may be due to the period of some 12 hours during which the butylmethacrylate must be heated to 50°C for polymerization. Osmic acid, however, has proved very satisfactory indeed, introducing artifact on less than a 100 Å scale for densely fixing regions such as cell walls. Loosely fixing material such as plasma shows more artifact apparently in the form of scattered particulate chains. However, not much can be said about this at present, since there is little basis for comparison.

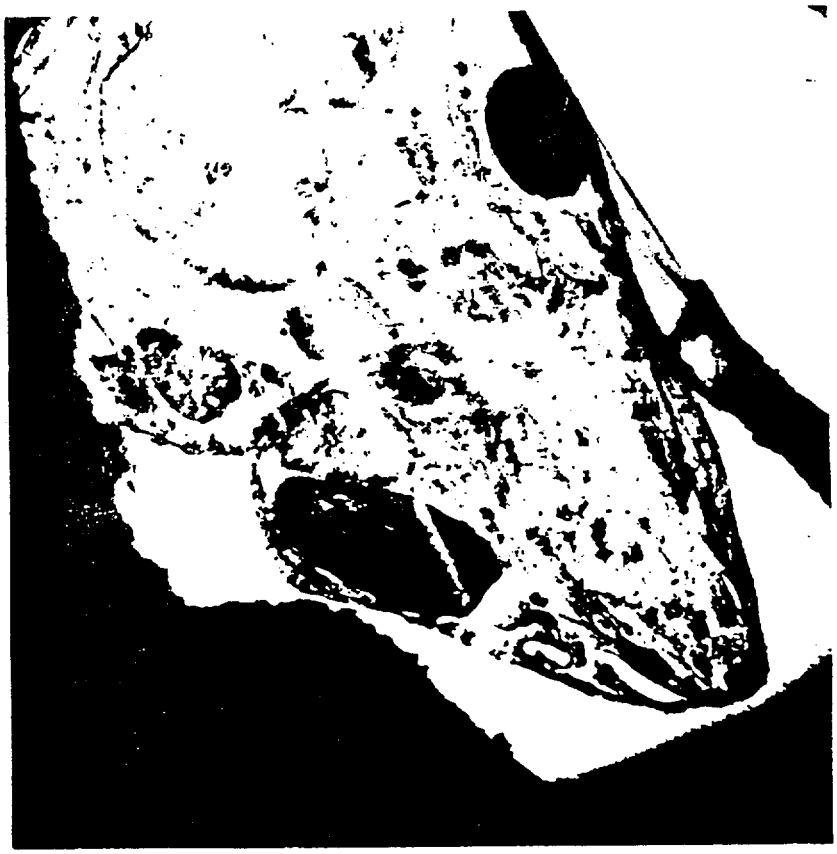
Some work has been done on the problem of removing the embedding material with suitable solvents such as acetone or amyl acetate with or without the addition of a little collodion. More work will be done on this and will be reported on later. The problem is to remove the embedding material from the tissue without introducing tissue shrinkage on evaporation of the solvent. It is possible that a balance in favor of the extracted preparation can be made between fine scale shrinkage artifact and electron scattering "artifact" due to the presence of the embedding matrix.

Following are electron micrographs of tissue sections prepared as described in this report (see Figures 2 and 3, Pages 92 and 93).

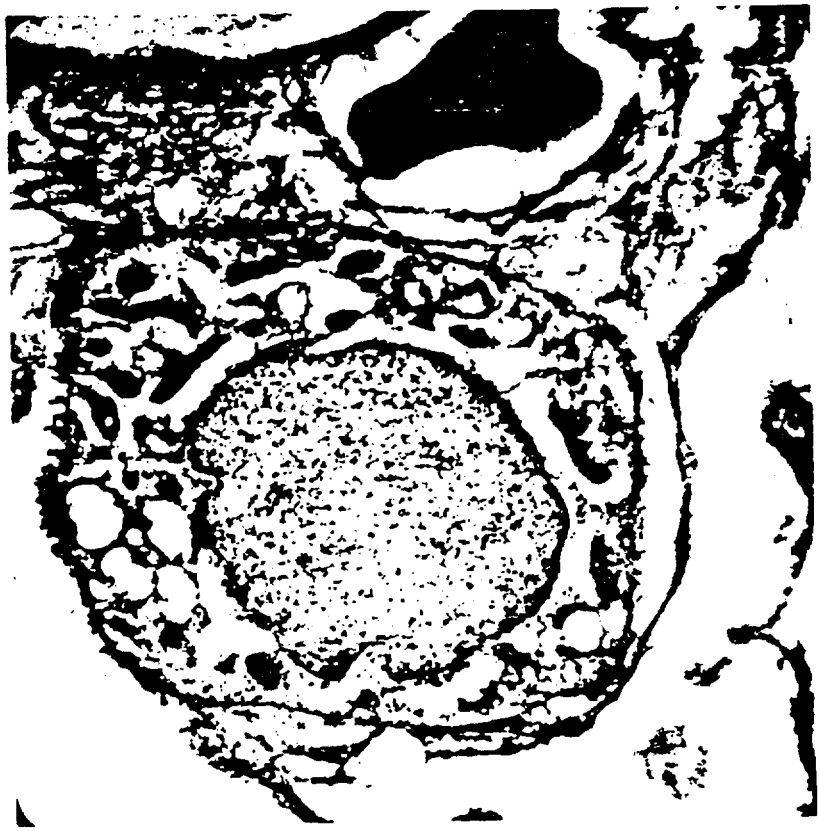
Bibliography

1. Newman, Sanford B., Borysko, Emil, and Swerdlow, Max, Science, 110, 66-68 (1949)

Figure 2. Normal rat lung fixed 24 hours in 1% Osmic acid. Magnification about 3700x.



Magnification 5600x



Magnification 8700x

Figure 3. Normal rat lung fixed 24 hours in 1% Osmic acid.

Problem Code: I.N.2 (Service)

Section Code: 3150

Author: L. T. Steadman

1. 137 chamber air samples were analyzed for beryllium.
2. 20 chamber air samples were analyzed for zirconium.
3. 15 air dust samples were analyzed for zirconium.
4. 256 animal tissues were analyzed for beryllium.
5. 3 samples were analyzed for phosphorus.
6. 2 samples were analyzed for copper.
7. 1 standard zirconium sample was analyzed for impurities.

EDUCATIONAL PROGRAM

Problem Code: None

Section Code: 3480

Authors: J. N. Stannard and S. Laskin

Industrial Hygiene and Toxicology (Sidney Laskin). A course in Industrial Hygiene and Toxicology, given by the Division of Pharmacology, has been current during this quarter as part of the general program in Radiological Physics described in earlier reports. The total course of 10 weeks is outlined in the lecture and laboratory schedule (Pages 98-101). Emphasis is placed on the basic principles of toxicology, characterization of toxic atmospheres and specific toxic agents and the biological application of statistical methods. The field of industrial hygiene and toxicology is considered both from the protective aspects and the evaluations of hazards in terms of animal experimentation and industrial surveys. Special emphasis is placed on problems relating to inhalation toxicity, dust particle size and materials of interest to the Atomic Energy Commission.

Laboratory problems are designed to give the student experience in the use of various devices and procedures for the evaluation of toxic atmospheres and in the use of experimental animals for the evaluation of toxic agents.

Work has been in progress on the completion of a new laboratory for this course in the educational wing. Installation of special equipment, including several pilot inhalation units, a full-scale inhalation exposure chamber and special dust analysis apparatus was completed in time to permit its use in the current course. Other facilities include fume hoods, animal storage space and a laboratory preparation room. Laboratory space is provided for a group of 20

students. The class this semester comprises the ten A.E.C. Fellows in Radiologica Physics and two graduate students enrolled at the University.

In addition to Project personnel aiding in the teaching of this course, full advantage was taken of the generous aid of Dr. James Sterner*. Dr. Sterner lectured on practical health problems particularly those relating to the halogenated hydrocarbons and the role of the industrial hygienist in plant health programs. We are also indebted to Dr. D. Fassett of Dr. Sterner's staff for arranging a tour of the Eastman Kodak Company at Kodak Park to demonstrate in practice many of the principles discussed in the course.

Civil Defense Training (J. N. Stannard). A course for physicians concerned with the problems of civil defense against atomic warfare was given during the week of March 27th. This course was one of several sponsored by the Atomic Energy Commission at the request of the National Security Resources Board. It was intended primarily for physicians who would occupy key positions in developing medical preparedness programs in their own geographical areas and would take the lead in similar courses organized by state or city public health or similar cognizant authorities. The states of Maine, New Hampshire, Vermont, Massachusetts Rhode Island, and New York were represented, with, in addition, observers from Great Britain and Canada. Other areas of the country were or will be represented at similar courses at other centers. Only unclassified information was discussed.

All lectures and discussions will be reproduced from wire recordings and circulated as a project report (UR-112).

Most of the topics were discussed by personnel from the Atomic Energy Project and Medical School except that the lecture on genetic effects of radiation was presented by Dr. Donald R. Charles, Professor of Biology, College of Arts

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* Associate Medical Director, Eastman Kodak Company, Rochester, New York

and Sciences, and the final discussions covering psychological factors in major disasters were led by Dr. Alexander Leighton, New York School of Industrial and Labor Relations, Cornell University, Ithaca, New York, and Dr. Ralph Low, Trumansburg, New York.

For information the following specific items are appended:

1. Outline of Course for Physicians (P. 102)
2. Topical Outline (P. 103-106)
3. Films (P. 107)
4. Literature Distributed (supplied by A.E.C., Washington) (P. 107-108)
5. List of Physicians Attending (P. 108-109)

INDUSTRIAL HYGIENE AND TOXICOLOGY

LECTURE AND CONFERENCE SCHEDULE

Monday and Friday - 10:30 A.M., Tuesday and Thursday 9:00 A.M.

February 20 to April 29, 1950

	Title	Instructor
1.	Introduction - Nature of Problem	Hodge
	Principles of Toxicology	
2.	Introduction to Pharmacology	Stokinger
3.	Factors Modifying Action of Toxic Agents -A	Stokinger
4.	Factors Modifying Action of Toxic Agents -B	Stokinger
5.	Assaying Damage in Exposure	Spiegl
6.	Biological Variations	Rothstein
	Conference	Staff
	Experimental and Practical Toxicology	
7.	Contact Exposures	Maynard
8.	Ingestion and Injection Studies	Maynard
9.	The Respiratory Route - Animal Experimentation for the Determination of MAC Values	Stokinger
10.	Plant Conditions and Health Program Organization	Sterner
	Conference	Staff
	Characterization of the Atmosphere	
11.	Sampling and Analytical Methods for Gases	Laskin
12.	Aerosols - Physical and Chemical Properties	Laskin
13.	The Particle Size Problem I	Laskin
14.	The Particle Size Problem II	Laskin
	Conference	Staff

	Title	Instructor
15.	Indirect Aerosol Methods	Laskin
16.	Sampling for Concentration and Particle Size	Laskin
17.	Thermal and Electrical Precipitators	Laskin
	Conference	Staff
18.	The Cascade Impactor I	Laskin
19.	The Cascade Impactor II	Laskin
20.	Surface Area and Other Physical Measurements	Lauterbach
	Conference	Staff
	Material Constituting Hazards	
21.	Lead, Mercury	Salomon
22.	Uranium, Arsenic	Neuman
23.	Beryllium, Silica and Pneumoconiosis Producing Agents	Scott
24.	Fumes, Mists and Smogs	LaBelle
25.	Noxious Gases and Vapors I	Smith
26.	Noxious Gases and Vapors II	Smith
27.	War Gases and Supertoxics	Hodge
	The Industrial Problem	
28.	Medico-Legal Aspects of Toxicology	Miller
29.	Protective Engineering	Wilson
30.	The Field Survey	Laskin
	Round Table on Field Problems	Spiegl, Wils Laskin
	Conference	
	Final Examination	

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INDUSTRIAL HYGIENE AND TOXICOLOGY

LABORATORY SCHEDULE

February 21 to April 27, 1950

	Title	Instructor
1.	Acute Toxicity I	Maynard
	A. Comparative study of route of administration	
	1. Demonstrations	
	2. Arsenic in rats	LaBelle
	B. Experimental measurement of LD_{50}	
	1. I.P. toxicity of soluble uranium in mice	Berke
2.	Acute Toxicity II	Maynard
	B. 2. BAL - mercury poisoning in rats	LaBelle
	3. Acute oral toxicity of soluble uranium in rats	
	4. I.P. toxicity of NaF in g. pigs	Berke
3.	Acute Toxicity III	
	B. 5. Vapor exposure with carbon tetrachloride	Spiegl, Frank Wilson
4.	Biological Effects of Aerosols	
	Histamine-antihistamine demonstration	Laskin
5.	Biochemistry	
	A. Analysis of hair and tissues for arsenic	Smith
	B. Determination of sugar in urine	Spiegl
	C. Nonprotein nitrogen	Berke
	D. Protein	
6.	Inhalation Toxicity	
	A. Physical operation of chamber	Wilson
	Demonstration of dust feeds	
	B. Uranium analysis (ferrocyanide)	Smith
	C. Chamber operation	Frank
	Sampling for concentration	Laskin
7.	Inhalation Toxicity	
	D. Planning session for inhalation experiment	Staff
	E. Report writing	
	F. Special problem	
	G. Preparation and care of animals	

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	Title	Instructor
8.	Inhalation Exposure	Laskin
	A. Preparation of animals	Wilson
	B. Collection of samples	Smith
9.	Operation of Chamber	Frank
	A. Concentration sampling	Smith
	B. Analysis of samples	
10.	Particle Size Measurement	Laskin, Lauterbach
11.	Sampling for Concentration	Smith
	A. Concentration analysis	Frank
	B. Biochemistry	Berke
	C. Dust analysis	Lauterbach
	D. Particle size	Laskin
12.	The Cascade Impactor	Laskin
	A. Sampling and analysis of results	
13.	The Thermal Precipitator	Laskin, Lauterbach
14.	Electronmicroscopy	Watson
15.	Gross Autopsy	Scott
16.	Analysis of Acute Toxicity Results	Maynard LaBelle
17.	Pathology	Scott
	Library Research	
	Student Seminars	Staff
	Field Survey - Round Table	Laskin Spiegl Wilson

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OUTLINE FOR A COURSE OF ONE WEEK'S DURATION FOR PHYSICIANS CONCERNED WITH CIVIL DEFENSE AGAINST ATOMIC WARFARE

March 27 - March 31, 1950

Monday	Registration	
	Statement of Problem	Blair
	Radiation Syndrome in Man	Howland
	Discussion	
	Elementary Radiation Physics, Properties of Radiation and Dosage Units	Steadman
	Discussion and Film	
	Operating Plan for Atomic Disasters	Dahl
Tuesday	Handling of Food and Water Supplies	Bale
	Discussion and Film	
	Elementary Nuclear Physics	Steadman
	Atomic explosions and fission reactions	Hursh
	Radiation Detection and Measurement - Lecture	Mermagen
	Radiation Detection and Measurement - Demonstration	Mermagen, Dahl
Wednesday	Biological effects of radiation	Stannard
	Film	
	Genetic effects of radiation	Charles
	Human radiation pathology	Scott
	Hematology	Ingram
	General radiation pathology	Casarett
Thursday	Internal radiation hazards, general problems	Hursh
	Detection and handling of radioactive materials - Demonstration	
	Internal radiation hazards, specific substances	Stannard
	Mechanical and thermal injury	Kingsley
	Film, demonstration of burns	
	Mechanical and thermal injury	Kingsley
Friday	Medical aspects of Atomic Disaster Control - General	Howland
	Medical aspects of Atomic Disaster Control - Therapy	Howland
	Medical aspects of Atomic Disaster Control - Organizational Problems	Payne
	Psychological factors in major disasters	Leighton
	Experiences with organization of local study groups in the atomic energy field	Low
	Review and Recapitulation	Staff

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CIVIL DEFENSE COURSE - Topical Outline

The Radiation Syndrome in Man - Clinical

General comment
- Nature of the physical agents
- Types of injury produced

Clinical manifestations following ionizing radiations
- Local exposure
- Whole body exposure

Elementary Atomic and Nuclear Physics

Elementary particles and their properties
Fission
Chain reaction
Fission products
Pile and bomb fission reactions

Atomic Explosions and Fission Reactions

Physical aspects of the explosion
The destructive effects of the air explosion
Comparison of underwater and air explosions
Evaluation of the importance of the different destructive agents
Physical means of protection
Time warning factor in effectiveness of precautionary measures

Radiation Detecting Devices

Ionization monitoring devices
- Theory of operation of ionization chambers
- Electrostatic type of electrometers
- Vacuum tube electrometers
- Calibration of ionization chamber type portable instruments

GM counter monitoring devices
- Theory of GM counters
- Basic circuits for GM counters
- Rate meters versus scalers
- Calibrations of portable monitoring GM counters

Photographic monitoring devices

Demonstration of Use of Instruments

Laboratory experiments
- Use and operation of ionization type survey meters
- Use and operation of GM counter survey meters

Biological Effects of Radiation

Effects on cells
- Specific ionization, biological effectiveness ratios
- Types of effect - structural, biochemical, functional
- Possible mechanisms

Effects on tissues
- Concept of tissue sensitivity
- Types of changes (killing, growth inhibition, abnormal growth, carcinogenesis)

Effects on intact organism
- Summation of tissue changes
- Species and age effects
- Influence of dosage rate
- Direct vs. indirect effects

Genetic Effects of Radiation

Introduction; human chromosomes; the internal architecture of chromosomes; genes, their nature and role in development

Types of mutation and their consequences in individuals
- Chromosome mutations, fragmentation and translocation
- Gene mutations; dominant, recessive, sex-linked

Effects of mutation on the genetic structure of a population
- Spontaneous mutation rates in man
- Persistence of mutant genes from generation to generation
- Number of individuals affected by each single mutation, and total harm done

Radiation-induced mutation rates in mice and other experimental organisms; implications for human populations and individuals at various exposure levels

Human Radiation Pathology

Anatomical lesion resulting from radiation of normal organs and tissues

Variations in sensitivity of tissues to ionizing radiation

Brief statement on radiation therapy for malignant tumors

Tumors induced by ionizing radiation

Pathological anatomy of Hiroshima cases
- General discussion of findings
- Case reports

Pathology and Laboratory Demonstration

Autopsy of irradiated animals

Study of gross specimens

Study of microscopic changes (projections)

Internal Radiation Hazards, General Problems

Radioactive decay series, types of disintegration, types of radiation, specific activity vs. half-life

Factors modifying biological effect
- Absorption, distribution, excretion, effective half-life

Effects produced

Dosage problems, estimation of body content

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Internal Radiation Hazards, Specific Substances

Fission products
Radioactive elements produced by neutron bombardment of common substances
α - emitters
Special problems in dealing with mixtures of radioactive materials

Mechanical and Thermal Injury

Components of explosion
Range of effects based on Japanese experience
Importance of trauma and burns as compared with ionizing radiation
Mechanical trauma - fractures, penetrating wounds
Thermal trauma - primary, secondary burns, late effects
Therapy
    Can burns be prevented?
    Personnel and material requirements for treatment of large numbers of casualties
Importance and course of thermal research
Demonstration of research equipment and present results

Medical Aspects of Atomic Explosions

Nature and extent of the radiation emitted in an atomic bomb explosion
Potential type of injury from bomb explosions - general
    Direct - gamma rays, neutrons
    Induced - neutrons
    Absorption of deposited fission products
Type of casualties from air burst
Type of casualties from land burst
Type of casualties from underwater burst
Review of the medical findings in severe, moderate and mild cases with demonstrations (slides) of general nature of each type
Factors complicating the picture of radiation sickness
    Mechanical trauma
    Burns
    Infection and general aspects
    Nutrition
    Age, sex
General comment on medical planning and care of casualties

Preliminary Preparation

Disposal preparation - map of city and satelites

Warning

    Hospitals
    Physicians
    Civilian groups

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Definition of damaged and involved areas

- Fire and blast
- Radioactivity
- Extent of damage and/or contamination
  - Water system
  - Power
  - Communications - Telephone and radio
  - Transportation - Rail - Road

Rescue attempts

- Aid station concept
  - Team of physicians, aid men, surveyors
- Evacuation

Treatment centers

- Shelter and triage
- Rx of minor casualties with return to duty
- Further evacuation
- Hospitals
  - Existing facilities - 10 miles - away
  - Evacuation of existing patients

Community dangers

- Prevention of and/or warning of attack
- Definition and isolation of field
- Rescue measures
  - Mechanisms available
  - "Safety" facilities
    - Planning of shelters
    - Planning of food and water
    - Medical planning
      - First Aid
      - Definition

Salvage and rehabilitation

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CIVIL DEFENSE COURSE - Films

Monday A.M.	Operation Crossroads
P.M.	PMF 5148 Atomic Medical Cases, Part II (Radiation Syndrome)
Tuesday A.M.	Atomic Physics, Part IV (British)
P.M.	PMF 5058 Atomic Medical Cases, Part I (Nuclear Fission)
Wednesday A.M.	Atomic Physics, Part V (British)
Thursday P.M.	Atomic Medical Cases, Hiroshima and Nagasaki, abridged L-306
Friday A.M.	PMF 5149 Atomic Medical Cases, Part III (Organization and Planning

CIVIL DEFENSE COURSE - Literature Distributed

"What you should know about the Atomic Bomb". A message from the Surgeon General U. S. Army Medical Department

"The Acute Radiation Syndrome in Man", by Shields Warren, M.D., and John Z. Bowers, M.D., Washington, D. C. Annals of Internal Medicine, 32:No. 2, February, 1950 (Presented at the Thirtieth Annual Session of the American College of Physicians, New York, N.Y., April 1, 1949)

"The Metabolism of the Radioactive Elements created by Nuclear Fission", by Joseph G. Hamilton p. 863, The New England Journal of Medicine, 240:No. 22, June 194

"The Radiation Syndrome", by Elizabeth E. Painter and Austin M. Brues p. 871, The New England Journal of Medicine, 240:No. 22, June 2, 1949

"The Acute Radiation Syndrome", by L. H. Hempelman, AECU-379, Supplement #2, Nucle Science Abstracts, 3:No. 4, Atomic Energy Biophysics, Biology, and Medicine edition

"Report on the Medical Studies of the Effects of the Atomic Bomb", by Dr. Masao Tsuzuki, Prof. of the Tokyo Imperial University. This report appeared originally as Appendix No. 9 of the General Report, Atomic Bomb Casualty Commission, January, 1947. National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

"The Medical Sequelae of the Atomic Bomb Explosion", by George V. LeRoy, M.D., J. Amer. Medical Assoc., Aug. 1947, 34:No. 14, p. 1143-1148

"Medical Aspects of Atomic Weapons". Prepared for the National Security Resources Board by the Department of Defense and the U. S. Atomic Energy Commission

"Pathology of Atomic Bomb Casualties", by Averill A. Liebow, M.D., Shields Warren, M.D., and Elbert deCoursey, Col. M.C., U.S.A. (from the Army Institute of Pathology, A.E.C., etc.) Reprinted from the Amer. J. Path., 1949, 25:No. 5, p. 853-1027

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"Gross Autopsy Observations in the Animals Exposed at Bikini". A Preliminary Report, by John L. Tullis and Shields Warren. J. Amer. Medical Assoc., August 2, 1947, 134: pp. 1155-1158

"Medical Progress: Mechanical and Thermal Injury from the Atomic Bomb", by Herman E. Pearse and J. Thomas Payne. The New England J. of Medicine, 241: No. 17, p. 647, October 27, 1949

"Atomic Bomb Explosions - Effects on an American City", by R. E. Lapp (revised) Bulletin of the Atomic Scientists, 4: No. 1, January 1948 (AEC Oak Ridge)

"Atomic Scientists News" (British) III: No. 1, July 21, 1949

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Dr. Franklyn B. Amos
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New York Central Surgeon
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Dr. John M. Benny
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Ed. J. Meyer Memorial Hospital
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TECHNICAL REPORTS ISSUED FOR DISTRIBUTION

January 1, 1950 thru March 31, 1950

Report No.	Title	Authors	Subject Category
UR-92	"Clinical, Pathological and Hematological Effects of Chronic Neutron Radiation" (RESTRICTED) Issued: January 25, 1950	Ely, Ross et al	Health and Biology
UR-99	"Hemolytic Effect of Radiation" (UNCLASSIFIED) Issued: January 12, 1950	Young, Davis Dole, Izzo	Health and Biology
UR-100	"Experiments with Paper Chromatography of the Animal Phospholipids" (UNCLASSIFIED) Issued: January 12, 1950	O'Leary Neuman	Health and Biology
UR-101	"Metabolic and Cardio-Respiratory Studies on Patients with Beryllium Granulomatosis" (UNCLASSIFIED) Issued: January 12, 1950	Waterhouse Keutmann Howland et al	Health and Biology
UR-105	"Physico-Chemical Studies of Beryllium Complexes. III. A Quantitative Investigation of the Beryllium Citrate Complex in Basic Media" (UNCLASSIFIED) Issued: March 7, 1950	Danley Feldman Neuman	Chemistry General
UR-106	"Potentiometric Titrations of Beryllium" (UNCLASSIFIED) Issued: February 24, 1950	Kosel Neuman	Health and Biology