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Health and Biology

THE UNIVERSITY OF ROCHESTER
Atomic Energy Project
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* * *

SUMMARY OF RESEARCH AND SERVICE PROGRAMS

January 1, 1948 thru December 31, 1948

Submitted by: Henry A. Blair,
Director

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Health and Biology

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INTRODUCTION

This report is unlike the usual Quarterly Technical Reports issued by The University of Rochester Atomic Energy Project in that it reviews all of the research and service work done during the entire calendar year of 1948.

The 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 author or 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 Pages 12 and 13 what administrative officer can be approached for information about particular work. This does not imply either authorship, or scientific credit which will appear only in final reports issued from this Project.

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 program, in general, indicate broad fields of investigative or service activities while the problems indicate divisions of these fields. Although no consistent method of division into problems was possible, an attempt was made to achieve a natural division in the sense that each problem would encampass a subject normally written up and generally considered as a unit. The program on 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 report of our research and service activities, all material being assembled according to the program and problem codes. The contribution of each section 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, survival 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 AND 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. 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

IX. I.S. ISOTOPES

I.S.1 Tracer Chemistry

I.S.2 Radioautography

I.S.3 Therapy

X. O.S. OUTSIDE SERVICES

XI. P.H. PROJECT HEALTH

XII. H.P. HEALTH PHYSICS

H.P.1 Research and Development

H.P.2 Service

XIII. C.S. SPECIAL CLINICAL SERVICE

XIV. 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

ORGANIZATION

I. DIVISION OF RADIOLOGY AND BIOPHYSICS (3100): William F. Bale

Section Code	Section	Administrative Head
3110	Instrumentation	John B. Hursh
3120	Tracer Chemistry	Leon L. Miller
3130	Radiation Physiology	Thomas R. Noonan
3133	Radiation Animals	Thomas R. Noonan
3140	Radiation Chemistry	Kurt Salomon
3150	Spectroscopy	Luville T. Steadman
3160	Radiation Mechanics	Francis W. Bishop
3161	Electron Microscope	Francis W. Bishop
3170	Radiation Toxicology	J. Newell Stannard
3171	Autoradiography	George A. Boyd

II. DIVISION OF PHARMACOLOGY AND TOXICOLOGY (3200): Harold C. Hodge

Section Code	Section	Administrative Head
3210	Industrial Hygiene	Herbert E. Stokinger
3220	Biochemistry	William F. Neuman
3230	Ingestion Toxicity	Elliott Maynard
3250	Pathology	James K. Scott
3260	Physiology	Aser Rothstein

III. DIVISION OF MEDICAL SERVICES (3300): Joe W. Howland

Section Head	Section	Administrative Head
3310	Industrial Services	J. Russell Hayes

Section Code	Section	Administrative Head
3312	Clinical Problems	Joe W. Howland
3320	Health Physics	Herbert E. Mermagen
3330	Project Medical Service	Joe W. Howland
3340	Medical Research	Joe W. Howland
3350	Radiation Therapy	Leslie R. Bennett
3351	Hematology	Marylou B. Ingram
3390	Photographic Service	Robert L. Hay

IV. DIVISION OF DIVERSIFIED PROBLEMS (3400): Henry A. Blair

Section Code	Section	Administrative Head
3410	Mouse Genetics	Donald R. Charles
3420	Hematology	Lawrence E. Young
3440	Protein Metabolism	G. Burroughs Mider
3441	Embryology	Karl E. Mason James G. Wilson
3442	Immunity	William L. Bradford
3450	Flash Burns	Herman E. Pearse
3460	Theoretical Problems	W. Burkett Mason

PROGRAM X.R.

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

Problem Code: X.R.1 (Tolerance Studies)

Section Code: 3130, 3133

Effect of Acute Whole Body X-irradiation Upon the Life Span of Albino Rats:

Background: Studies on effects of whole body radiation on animals, in general, have been limited to observations on immediate effects. Data from the experiments done here by Boche and Dowdy indicate, however, that a definite shortening of life may be produced in rats by repeated daily exposures in which the total accumulated dose is of the order of 600 r (1 r per day for 2 years). It seemed of interest, therefore, to study the effect of a single acute exposure to x-irradiation upon the life span of the rat.

Work done during the calendar year 1948: The experiment has been set up in two units, each unit consisting of 96 rats divided into four equal groups. Unit 1 was composed exclusively of female, unit 2 of male rats. In each unit, one group of 24 has served as a control, while three groups have received respectively 150 r, 300 r, and 600 r of whole body x-irradiation from a 250 kv. source. The animals used were secured directly from the animal colony of the Wistar Institute, were of uniform age (all born within a period of 20 days) at time of receipt, and were radiated at the age of approximately 5 months. The unit composed of females was radiated in January 1948, and the other unit in April 1948.

On December 1, 1948, the data in Tables I and II (Page 15) were obtained.

It is not possible to evaluate the present data without statistical analysis. While at first sight, it might appear that females are more susceptible than males, this difference may be only to random variations in sensitivity between the two groups. It is planned to carry on this study until all of the animals have died and to make a careful statistical analysis of the life span and tumor incidence as related to exposure to radiation.

Proposals for future work: Further extensions worthy of consideration are:

1. Repetition of above experiment to provide better data for statistical analysis.

2. Exposure of animals to daily doses of 20-100 r until a total dose of 1000-2000 r has been accumulated; then determining the life span of such animals.

Table I

Unit 1, Females, 10 1/2 Months Post-Radiation

	Dead	Tumors noted in survivors
Control	3 (1 with tumor)	0
150 r	4	3
300 r	4 (1 with tumor)	3
600 r	13*	2

*8 of deaths within 40 days of radiation

Table II

Unit 2, Males, 7 1/2 Months Post-Radiation

	Dead	Tumors noted in survivors
Control	1	0
150 r	1	0
300 r	0	0
600 r	2	0

Effect of Daily and Weekly Exposure of Growing Rats to X-irradiation Upon General Body Growth and Hematopoiesis:

Background: The effect of x-irradiation upon growth has been studied in tissue cultures and in the long bones of mammals. The effect of whole body radiation upon growth of mammals, while expected, had never been definitely established. It seemed to be of interest, therefore, to expose groups of weanling (21-day-old) albino rats to daily and to weekly doses of whole body roentgen rays to learn the approximate dose required to produce significant inhibition of growth. A correlation between the growth-retarding effect and the production of alterations in the numbers of blood cells by x-rays was also sought. An experiment designed to investigate these phenomena was begun late in 1947, and completed during the past year.

Work done during the calendar year 1948: Albino rats were secured from the animal colony immediately after weaning and divided into four groups, each consisting of nine males and nine females. The animals were weighed and the hemoglobin level, white blood cell count, and differential white cell counts were obtained on all animals. When the animals were 25 days old, radiation was begun (using a 250 kv. source) according to the following pattern:

Group I	10 r/day, 5 days per week
Group II	20 r/day, 5 days per week
Group III	100 r/day, 1 day per week
Group IV	Controls

The radiation was continued for 30 weeks for all animals and for the majority of animals for 35 weeks. The animals were weighed once a week and blood counts were made every two weeks for the first three months of exposure. After this, the weighings and counts were made at two and four week intervals, respectively.

The mean body weights of animals receiving 100 r per week, either in a single dose or divided into five equal doses, were lower than the controls three weeks after the beginning of radiation. The reduction was noted in both sexes and was highly significant by standard statistical tests. The body weights of males receiving 10 r per day were significantly reduced from the control males four weeks after beginning radiation, but there was no difference in growth between the female rats receiving 50 r per week and the controls during the entire period of study. The magnitudes of the effects can be judged from Table III.

No significant differences in the levels of hemoglobin or absolute neutrophils were found. The level of absolute lymphocytes, however, was significantly depressed in animals receiving 100 r per week, either in single or divided doses, the effect being noted three weeks after the start of radiation. Animals exposed to 10 r per day showed a reduction in absolute

Table III

Mean Body Weights (in grams) of Animals

after Four Weeks of Irradiation

	10 r 5 times/wk	20 r 5 times/wk	100 r 1 time/wk	Control
Males	107.1	113.3	96.9	139.6
Females	118.8	98.0	98.0	114.7

lymphocytes which was significant only after nine weeks of exposure, although quite apparent after seven weeks. None of the hematological effects could be shown to be dependent upon sex.

The data obtained from this experiment seem to justify the conclusion that general body growth is relatively sensitive to whole body radiation, since a significant reduction in rate of growth may be detected practically as soon as a significant reduction in the absolute lymphocyte count, which is generally considered to be a very sensitive indication of radiation damage. No information is available concerning the specific organs and tissues which show inhibition of growth nor has this particular experiment provided any information concerning the mechanisms by which radiation affects growth.

Proposal for future work: Future extension of this work may develop along two lines. The first, of most direct interest to the Atomic Energy Commission, would be to study the effects of daily exposure at lower dose rates (down to the tolerance level) upon growth. It is improbable, however, that any definite effect could be established at low levels without the use of extremely large numbers of animals. The second development along this line is of less direct interest but may offer a much better overall method of securing useful information concerning problems in radiation biology. This approach would involve the use of whole body radiation as a tool for the study of growth phenomena. Such a line of attack would require a clearer understanding of the mechanism of radiation effects upon growth. As examples of possible experimental work along these lines, carbon 14-labeled amino acids could be employed to study protein synthesis in animals whose growth had been inhibited and the effects of growth hormones could be studied with irradiated animals.

Proposal for future work:

Effects of X-radiation on Spermatogenesis in Dogs:

From previous experiments initiated under the direction of Dr. Andrew H. Dowdy and Dr. Robert Boche, it is known that chronic x-radiation at dosage levels of 1.0 r or greater per day produce a condition of aspermia in dogs within one year. A dose of 0.5 r per day produces a lowered sperm concentration in dog semen, with complete aspermia in some cases. Sperm counts have been made on dogs exposed to 0.1 r per day of x-radiation and according to Drs. S. Lee Crump and Donald Charles, a definite, statistically significant depression in sperm concentration occurred.

The sperm counts which supplied this information were not begun until eight months after the start of chronic x-radiation, and pre-exposure counts were not obtained. Evidence of recovery was found in some of the dogs in each of these three dosage groups in the post-radiation period. These final recovery studies were only recently completed.

If the results of such radiation studies can be transferred to man, the significance in setting a permanent permissible human exposure level of radiation is apparent.

We, therefore, propose to initiate and carry out a chronic study on dogs in an endeavor to discover the lowest dosage level at which significant changes in sperm concentration can be induced. This program will be in charge of George W. Casarett as a part of the research program of this Section. It is proposed to use for such experiments young adult males, and to continue such exposures over the reproductive lifetime of these animals or until significant results have been obtained at the lowest radiation levels. Therefore, this experiment will extend over a period of several years.

The work of several investigators has indicated that hyaluronidase plays an important part in mammalian fertility by virtue of its effect of dispersing the follicular cells surrounding theova when present in sufficiently high concentrations. It has been shown that the amount of hyaluronidase in semen of normal humans and other animals is proportional to the concentration of spermatozoa. Semen specimens with excellent concentrations of sperm may lack hyaluronidase, however, and such specimens are considered infertile. Excessively high concentrations of hyaluronidase in semen are thought to affect fertility adversely because of the destructive action on the ovum itself. It would be of interest to determine the relationship between hyaluronidase and sperm concentrations in the semen of dogs and to ascertain the effect, if any, of chronic x-radiation on this relationship, in addition to the study of other sperm factors which may influence fertility.

The dosage levels proposed for this more extensive study of dog sperm and semen are 0.5 r per day, 0.1 r per day, and 0.05 r per day.

Problem Code: X.R.1 (Tolerance Studies)

Section Code: 3390

Fingerprint Analysis:

In the dermatoglyphics activities, a new technique for the procurement of suitable fingerprints for analysis has been developed and in preliminary testing has proved to superior to the original method in which dental molding compound was used. It is planned in the future to repeat all existing fingerprint monitoring by this new technique. In addition, exposures using the old compounds have been analyzed and questionable ones referred to Dr. Roger Harvey (University of Illinois College of Medicine) who is retained as consultant. It is planned that all pathological changes will be photographed and returned together with the interpretation to the originating installation, thus providing them with a permanent record. Any request concerning this activity should be referred directly to this installation.

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

Section Code: 3120

I. Early Functional Changes Secondary to Minimal Radiation Injury. 1. Serum Proteolytic Enzymes Derived From Leukocytes. The Relation of the Serum Enzyme Level to Minimal Radiation Injury:

Background: It has long been known that leukocytes contain a group of powerful proteolytic enzymes classed as peptidases because they are capable of catalysing the chemical splitting of peptides. It is also known that inflammatory responses associated with leukocytosis are accompanied by increased serum peptidase activity above levels observed in normal animals and humans. It is not clearly established whether the enzymes are released solely as a result of leukocyte destruction, or whether developing leukocytes release the enzymes. In recent years the lymphocyte and polymorphonuclear leukocyte have been separately studied for their peptidase components, and it is possible to distinguish the two in some measure on the basis of their enzyme content.

Because of the outstanding sensitivity of the leukopoietic system to ionizing radiations, we believe an exploratory study of the effects of low dosage whole body radiation may reveal significant changes in peptidase activity without significant changes in the circulating leukocyte count.

Work done during the calendar year 1948: Work has been underway to set up accepted methods for measuring the activity of enzymes which can split the peptides leucylglycylglycine, glycylglycine, and benzoylarginineamide.

Proposal for future work: It is proposed to expose dogs and rabbits to single doses of whole body radiation which are known not to cause significant changes in the leukocyte counts of the peripheral blood. The animals will be bled small amounts (2 to 4 ml) and the serum enzyme activities measured every 48 hours over an interval of at least two to three weeks after exposure.

I. Early Functional Changes Secondary to Minimal Radiation Injury. 2. Hepatic Functional Changes Secondary to Radiation Injury:

Background: It is believed that the liver is comparatively insensitive to damaging effect of ionizing radiations. It is not entirely clear whether this is an intrinsic property of the organ *per se*, or whether its "resistance" is referable to its tremendous capacity for regeneration after injury.

The proposed tests for liver functional impairment are numerous. A few of the common tests (e.g. bromosulfonphthalein clearance) have been applied in studies of liver function without positive findings. The ability of an

animal, such as the dog, to remove bilirubin, a normal hepatic excretory product, from the blood can be measured by injecting bilirubin into the blood and measuring its removal rate. This is among the most sensitive of liver function tests, but it is not widely used because of the expense of the bilirubin, and because the injection solution must be freshly prepared for use.

It is also known that the liver can respond to minimal noxious stimuli by pouring out into the blood greatly increased amounts of fibrinogen. This may be the sole manifestation of irritation, if not injury, to the liver and may be seen under conditions where no other known test of liver function will reveal any abnormality.

The laboratory methods have been set up and active experimentation will be started within the first two weeks of January 1949.

Proposal for future work: It is believed that these two "tests" of liver function should be studied first under conditions where minimal x-ray dosage is used (e.g. 15-25 r); later it may be worth studying the effects of higher single dosage levels. The former will be studied in conjunction with the effects of low x-ray dosage on serum proteolytic enzymes in the same dogs.

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

Section Code: 3140

Background: The fundamental biochemical nature of the effects of radiation on living organisms and living tissues is at present largely unknown. It seems, therefore, worthwhile to study the metabolism of organisms and tissues employing a variety of techniques in order to further our knowledge concerning the effects of radiation on metabolic processes.

I. Studies with Glycine Labeled with C^{14} in Its Alpha-carbon Atom:

Compounds labeled with isotopes have proved to be valuable tools in the study of anabolism and catabolism of cellular constituents. In accordance with our present concepts of the biochemical events in cellular metabolism, the tissue components are considered to exist in a state of flux being constantly broken down and resynthesized. As a consequence of this dynamic state of the tissue constituents, it is possible to introduce isotopically labeled building stones into various tissues. This technique permits the study of the rate of synthesis and degradation of various tissue components, as well as the isolation of such labeled metabolites. Since it seemed probable that radiation would affect the ratesof snythesis and degradation of tissue components, a study along these lines was initiated.

The use of glycine (amino acetic acid) for this purpose was prompted by the fact that glycine would be incorporated in proteins, thus permitting the

study of protein metabolism with respect to radiation. In addition the nitrogen atom of glycine has been shown to be incorporated in hemin, and for this reason it was thought that the alpha-carbon atom of glycine would also serve as a specific precursor of the hemin molecule. This would then provide an approach to the study of hemoglobin synthesis as affected by radiation. It has also been shown that the nitrogen and carboxyl-carbon atom of glycine serve as specific precursors in the biological synthesis of purines, such as uric acid and the purines which are present in nucleic acids, and thus alpha-carbon labeled glycine should also be useful as a tool for the study of purine (e.g. nucleic acid) metabolism. Because of the metabolic versatility of glycine, this two-carbon amino acid appeared to be a good choice in studying the problems suggested above.

A. Total Body Radiation (Rats)

Total body radiations were performed with roentgen rays in dosages of 300 and 600 r. Rats were used as experimental animals in all studies pertaining to total body radiation. In order to interpret any changes produced by radiation, it was essential to investigate first the metabolic behavior of alpha-carbon atom of glycine in untreated animals. Such preliminary studies involved distribution studies in the various organs, studies of hemoglobin metabolism, and studies of various tissue components as isolated from different organs.

1. Effect on the incorporation of the alpha-carbon atom of glycine into tissues:

a. Preliminary studies with untreated animals: In the untreated animals the highest isotope concentrations were found in liver, kidney, and gastro-intestinal tract, whereas brain and muscle showed the lowest isotope concentrations on the basis of C^{14} per gram of dried tissue.

Since it is known that radiation produces decreased appetite and also causes decreased absorption from the intestinal tract, it seemed of interest to investigate the effect of variations in food intake on glycine metabolism. In order to study extreme conditions the animals were starved for a period of five and seven days prior to the administration of glycine. It was found that after five-day starvation more of the alpha-carbon atom of glycine was incorporated in visceral tissues, such as liver, gastro-intestinal tract, and kidneys of the starved rats than in the controls. The incorporation in muscle of the starved animals was not significantly changed.

b. Radiation effects: Since it is known that various organs respond to radiation at different time intervals after exposure, experiments were designed in such a way that one microcurie of glycine was injected intravenously immediately, five hours, 48 hours, of 8 days after exposure to x-rays. The results showed that the incorporation of the alpha-carbon atom of glycine into muscle was significantly depressed when the labeled amino acid was injected immediately or eight days after radiation. When glycine was administered 48 hours after radiation, the C^{14} activity of brain, kidney,

pancreas, and gastro-intestinal tract was higher than that found in the controls. A considerable lowering of C^{14} activity in bone marrow was observed when glycine was administered immediately after radiation and the animal sacrificed five hours later.

2. Effects on hemoglobin synthesis:

a. Preliminary studies with untreated animals: Shemin and Rittenberg have presented evidence based on studies with N^{15} labeled glycine that the amino nitrogen of glycine is a specific precursor for hemin nitrogen. We have found, using methylene-labeled glycine, that the alpha-carbon atom of this amino acid is also incorporated in hemin to a relatively high degree. Considerable amounts of C^{14} are also incorporated in globin but hemin contains eight times more C^{14} on a gram to gram basis. This finding formed the basis for further experiments intended to throw light upon the effect of radiation on the synthesis of hemin and globin.

b. Radiation effects: When glycine was administered intraperitoneally six days after exposure to 600 r roentgen irradiation and the animal sacrificed 24 hours later, a substantial depression of hemin synthesis was observed. Globin synthesis under identical conditions appeared only slightly depressed. As set forth under subheading (1) starvation experiments were conducted in the manner described above. The ratio of C^{14} activity of hemin to globin in the control rats ranged from 4.2 to 12. In the five-day starved rats this ratio was 65, and in seven-day starved animals, 1.2. After 300 r radiation the ratio was 34 and 38. When glycine was administered to rats radiated with 600 r, immediately after radiation the values for the ratio ranged from 30 to 36. In one animal irradiated with 600 r and injected with glycine after six days, the ratio was found to be 2.3. It should be pointed out here that both in radiated and starved animals of the type described above, the ratio of C^{14} activity of hemin to that of globin, which normally is of the order of 7.0, deviates from normal. This might be interpreted to mean that under the conditions mentioned hemin and globin syntheses are not necessarily coupled.

3. Effects on nucleoproteins:

a. Preliminary studies with untreated animals: As mentioned above, glycine serves as a specific precursor for certain definitive carbon and nitrogen atoms in the purine ring. We, therefore, have fractionated liver, muscle, and intestinal tract into protein, desoxyribonucleic acids and ribonucleic acids. Considerable overall activity has been found for the desoxyribonucleic acid and the ribonucleic acid fractions of the liver. The data indicate changes of the ratio of the C^{14} activity of ribonucleic acid to desoxyribonucleic acid.

b. Radiation effects: Experiments of this type applied to the study of the effect of radiation on nucleic acid synthesis are now in progress but not sufficient data are at present available to be reported here.

4. Effects on brain phospholipids:

a. Preliminary studies with untreated animals: Since approximately 30 per cent of the brain consists of phospholipids, and since in all probability phospholipids play an important role in the physiology of the

central nervous system, it seemed of importance to investigate the C^{14} content of the phospholipid fraction of the brain. The data indicate that changes of C^{14} activity occur in thephospholipid fraction of the brain of the rat after radiation with 600 r.

B. In Vitro Studies on Bone Marrow (Rabbit):

Because of the occurrence of secondary anemia as the result of radiation, a study of certain aspects of bone marrow metabolism was undertaken. These studies were carried out in vitro with bone marrow homogenates prepared from rabbit bone marrow with the use of the Waring Blender.

1. Hemin synthesis:

Since it is well known that bone marrow is able to synthesize hemin in the intact animal, the ability of bone marrow homogenates to incorporate the methylene carbon atom of glycine in the hemin molecule was investigated. Appropriate amounts of glycine were added to bone marrow homogenates in the presence of sodium acetate as a metabolizable substrate and incubated for certain periods of time. Appreciable C^{14} activity was found in the protoporphyrindimethylester (derived from hemin of bone marrow cells) after an incubation period of at least one and a half hours. Thirty minutes of incubation did not yield any significant incorporation of C^{14} in protoporphyrindimethylester. These experiments laid the ground work for experiments to be conducted with irradiated bone marrow and with bone marrow of irradiated animals.

2. Protein synthesis:

Bone marrow preparations such as described above are also capable of synthesizing protein. These protein preparations probably represent globin to a large extent, although chemical identification has not yet been satisfactorily established. Appreciable amounts of C^{14} are incorporated within half an hour at 37° C with glycine labeled in the alpha-carbon atom have shown that appreciable amounts of the initial C^{14} activity are incorporated in the bone marrow fats. In these experiments bone marrow fats were extracted and fractionated into the saturated and unsaturated fatty acid fractions. It was found that within half an hour appreciable amounts of C^{14} were incorporated in both fractions, the saturated fatty acids containing on the average about four times the activity of the unsaturated fatty acid fraction. This system also will be investigated with respect to radiation effects.

C. Diversified Problems:

1. Studies on chlorophyll synthesis (chlorella vulgaris):

Since we have shown that the alpha-carbon atom of glycine is a specific precursor for hemin, it appears likely that the alpha-carbon atom of glycine will assume a similar function in chlorophyll, a pigment which is also chemically related to prophyrin. To this end chlorella vulgaris has

been grown in culture media containing glycine labeled in its alpha-carbon atom. After a certain period of culturing the chlorella cells were harvested and lyophylized. Because of the limited quantity of chlorella cells obtained, it was necessary to develop micromethods for the isolation of pheophorbide a and b, the crystalline split products of chlorophyll a and b. The development of these methods is now underway.

II. In Vitro Studies of the Effect of Radiated Media on Tissue Metabolism:

Recent reports seem to indicate that micro-organisms grown in media previously irradiated with ultraviolet light undergo genetic changes with the production of mutants. It appeared of interest, therefore, to study the influence of irradiated media on the metabolism of a variety of mammalian tissues. To this end, dog serum was irradiated with 10,000 r and slices as well as homogenates of various tissues were incubated in this medium. Their oxygen uptake as well as aerobic and anaerobic glycolysis were measured with the Warburg Micromanometric technique.

Significant changes were observed in all tissues investigated, but the direction and order of magnitude of the changes observed differed with respect to the tissue employed. For example, bone marrow showed a great depression in anaerobic glycolysis; the most striking changes observed so far were found in brain homogenates. Gel formation was observed as soon as brain homogenates were added to radiated serum a fact which may account for the great depression of all metabolic activities under those conditions. In general, a depression of all metabolic activities of the tissues investigated as measured by means of oxygen consumption and CO_2 production was noted, with the possible exception of kidney tissue, where an increase in aerobic glycolysis has been observed. We assume that this phenomenon must be produced by biochemical changes in the serum due to radiation. The nature and significance of these findings is now under investigation.

III. Studies on Ferritin:

The role of ferritin, an iron-protein molecule, in iron absorption and metabolism is under study.

It has been postulated by S. Granick that iron is absorbed from the intestine in the reduced (ferrous) form and that the presence of iron in the intestinal mucosa serves as an impetus for ferritin synthesis. The presence of ferritin thus serves as a mucosal regulator of iron absorption. It is of interest to investigate these points further in normal and irradiated animals (guinea pigs) since the gastro-intestinal tract is very sensitive to x-radiation. Gillman and Ivy have studied iron absorption and have found an increase in the numbers of iron-containing phagocytes through the mesenteric lymph gland. From preliminary experiments in this laboratory, we believe that this iron is present partially or wholly as ferritin. The role of the lymphatics in iron absorption will be studied.

Experiments on the isolation and purification of ferritin from horse spleen, one of the best sources, have been undertaken. A slight degree of success in increasing the purity of ferritin has been obtained thus far. Uniformly crystalline ferritin was obtained without any amorphous precipitate when viewed in the microscope. These experiments were essential in order to secure a pure compound with which to carry out antigen-antibody reactions which will serve as a basis for a quantitative method for the determination of ferritin. The details of thismethod are being worked on now. Preliminary experiments with a new iron reagent (Tiron) for the separation of iron from the protein portion of ferritin have been carried out, and the iron part seems to be removed quickly, easily, and quantitatively from the protein component (apoferritin).

Other experiments planned include the in vitro synthesis of ferritin using tissue homogenates and slices, and a study of the amino acid composition of the protein moiety of ferritin with the use of paper chromatography.

Proposal for future work:

1. Effect of Radiation on the Incorporation of the Alpha-Carbon Atom of Glycine in Hemin and Globin:

The ratio of C^{14} activity of hemin to that of globin, which in normal rats is of the order of seven, has been found to deviate in radiated rats. Because of the importance of this observation, it seems desirable to establish the occurrence of this phenomenon unequivocally. This will require a repetition of the experiment with an appropriate number of animals. It is also important to extend the experiment temporally in order to establish the time of occurrence and the duration of this phenomenon. A wide range of dosages will be used for the purpose of establishing the relationship between the magnitudes of dosage and effect.

Since bone marrow is presumably the site of hemoglobin formation in the body, investigation of the isotope distribution in this tissue should be of value in elucidating the mechanism of this radiation effect. For this type of investigation the quantities of bone marrow which can be obtained from rats are insufficient. It is, therefore, planned to use the rabbit as the experimental animal in this investigation. In order to gain information concerning the mechanism of radiation effects, it is necessary to investigate the incorporation of C^{14} in hemin and globin in rabbit bone marrow irradiated in vitro, as well as in bone marrow taken from normal and irradiated rabbits.

2. Studies Concerning the Effect of Radiation on Nucleic Acid and Protein Synthesis in Tumors:

Nucleoproteins play an important role in cellular proliferation and have been shown to be coupled with protein synthesis. Their role in tumor growth has been studied using P^{32} as a labelling agent. Although some investigations have been carried out with respect to metabolic changes in tumors following radiation, our knowledge of biochemical responses of tumors to x-rays

is meager. As mentioned in this report, the alpha-carbon atom of glycine is incorporated in proteins and nucleic acids. It seems, therefore, promising to study nucleic acid synthesis in untreated and irradiated experimental tumors in rats. It is planned to investigate the rate of incorporation of the alpha-carbon atom of glycine in the purine and pyrimidine components of the nucleic acids of these tumors and to relate nucleic acid synthesis to the rate of incorporation of the alpha-carbon atom of glycine in proteins. Such a study should yield information concerning the effect of x-rays on an important growth regulating system, the nucleic acids, and the relation of this regulatory system to protein synthesis.

It is planned to carry out these proposals (1 and 2) in collaboration with the Section of Radiation Physiology.

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

Section Code: 3150

Study by Absorption Spectroscopy Methods of the Effects of X-radiation on Plasma Proteins:

Background: The syndrome of radiation sickness as it usually occurs in man may include nausea, vomiting, hemorrhagic manifestations and a symptoms group related to infection. Although the primary causes behind this complex set of symptoms are incompletely understood, it is reasonable to regard damage to the hemopoietic system as one of the principle underlying causes. The changes produced in the relative and absolute number of the formed elements of the blood as a result of irradiation have been widely investigated and are commonly used as a measure of the extent of radiation damage produced in humans and in experimental animals. The amount of established information about the irradiation changes produced in the composition of the plasma matrix as opposed to the formed elements in exceptionally scanty.

The Spectroscopy Section planned a series of experiments in the hope that the relatively unexplored use of absorption spectra measurements in the ultra-violet, visible and infrared regions would reveal quantitative and qualitative changes in the protein fractions of blood plasma from irradiated animals. The identification of such changes in the plasma might serve two ends. In the first place, the discovery of specific changes in the plasma proteins might throw light on the underlying processes contributing to the complex syndrome of radiation sickness. In the second place, study of plasma protein by the spectroscopic method might reveal a more sensitive index of radiation damage than is at present available.

Work done during the calendar year 1948: It was proposed to first conduct some exploratory experiments using a rather high level of radiation in order to accentuate any changes that might be produced. Rabbits

were chosen as the experimental animal. A number of sublethal doses of x-radiation (1000 r/dose at 100 kv.) were administered to the same animal with recovery periods between each dose.

One of the problems encountered was the development of a satisfactory method for isolating the four main protein groups from blood plasma. It was necessary that the fractions be salt-free and the proteins be unmodified by denaturation or other changes. It was desirable that the method employ small samples and that the precipitating reagent be such as not to interfere with subsequent nitrogen determination. A method was developed fulfilling all of these requirements. This method employs the buffered phosphate reagent of Butler and Montgomery and a dialysis technique modified from McMeekin. The procedure divides the plasma protein into four fractions. Fraction I contains fibrinogen and some of the globulin of lower solubility. Fraction II consists if englobulin and some pseudoglobulin. Fraction III consists of pseudoglobulin and some englobulin. Fraction IV contains the albumin and some pseudoglobulin. Recoveries as checked by Kjeldahl determinations have been of the order of ninety per cent.

Since our attention was focused on early changes, the first blood sample was usually taken within the first hour or in some cases within the first few days after irradiation. Subsequent samples were taken at varying times up to 53 days.

After irradiation of the rabbit with 1000 r at 100 kv., transitory changes in amounts of the various plasma protein fractions were found. The maximum effect could be demonstrated in the samples collected two days post-irradiation. The changes consisted in a significant increase in the fibrinogen, a significant decrease in the albumin and smaller increases in the globulins.

Protein determinations were made both by chemical nitrogen determination methods and by absorption spectrum measurement in the ultra-violet.

Out plans for study of the qualitative changes in the proteins call for the use of infrared absorption spectrum measurements. This technique, for proteins, is almost entirely unexplored and requires a great deal of method development. Some considerable progress has been made in working out procedures, and we now have completed some experiments in which differences have been found in the character of the infrared absorption spectrum between plasma samples taken from normal and irradiated animals. Further work is in progress to verify these observations and to identify the nature of these changes.

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

Section Code: 3160

Construction of Condenser-Type X-ray Machine:

Background: Since the biological effects of x-radiation are known to be

affected by the rate at which the dose is given, it was proposed to construct a condenser-type x-ray machine which could deliver a large dose in a very short time.

Work done during the calendar year 1948: A condenser-type x-ray machine was constructed and preliminary experiments have been carried out. We have been able to obtain between 400 and 500 r with a single discharge. This large energy is somewhat destructive to the x-ray tube and few animals can be treated at a time. The results are, therefore, slow in materializing. It is hoped that the problem can be successfully solved during the coming months.

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

Section Code: 3350

Work in the radiation therapy group has been divided into a number of studies which may be listed as follows:

1. The exchange of radio-sodium in the acute irradiated rat.
2. The absorption and distribution of Vitamin A following acute irradiation.
3. The effect on mortality of various substances following acute irradiation.
4. The vascular mechanisms following acute irradiation.

In rats during the first four days post-radiation a marked retention of radio-sodium occurs with shift of sodium into the intestinal tract in amounts as high as 2 per cent of the administered dose. Shifts into the kidneys, spleen and liver are also noted and elevation in the serum occurs with total serum sodiums occasionally rising 5-8 Meq. Urinary excretion may be reduced to one-half of control values. This information agrees with previously reported work on increased KSCN space.

Studies on Vitamin A show a tendency toward increased absorption in the irradiated rat in the period from 24 hours to 5 days after irradiation. Following administration of Vitamin A to an animal 3 days after midlethal x-radiation, it was noted that in a 10-hour test lesser amounts could be recovered than in the controls, suggesting an increased utilization of the material. Distribution studies showed that in contrast to the normal, liver values were low and values elsewhere higher than the controls. No specific sites of deposition could be found. Diarrhea occurring in such rats did not show excessive amounts of fat. Investigation of the utilization and distribution is continuing with emphasis on the comparative absorption of Vitamin A both in alcohol and ester forms.

Carefully controlled studies of the effect of various physical agents and substances on the mortality following midlethal irradiation have been carried out with particular emphasis upon the possible value of metabolic stress as a protective mechanism. Influences of pre-radiation cold given in measured doses, adrenal cortical extract given pre- and post-irradiation, and metabolic stresses, such as phloridzin-propylene glycol and propylene glycol alone have been studied. Some preliminary studies have been reported in previous Quarterly Reports on which the following interpretation can be made -- that stress either from the physical or general agents may produce an influence on mortality of a favorable type in certain instances. Results are not sufficiently well in hand for detailed interpretation.

Methods for the estimation of hemorrhagic phenomenon in animals of varying species have been perfected up to the point in which duplicable results can be obtained on the same animals in day-to-day studies. With such a tool, findings of interest are as follows: a definite species sensitivity as regards bleeding has been noted with the rabbit and rat, showing marked resistance to capillary changes; the dog, guinea pig, and man representing definite sensitivity. Increased heparin levels in the blood do not increase capillary fragility to any measurable extent. The same may be said for concentrations of hyaluronidase even above biological levels. Various substances previously thought to be beneficial in control of hemorrhage are being investigated and evaluated by the above biological test procedure. Methods of the possibility of flow rate and local pooling is also being studied by means of iodine labeled proteins.

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

Section Code: 3441

Histochemical Study of Changes Induced in the Germinal Epithelium of the Testis of Rats by Irradiation, and the Influence of Nutritional States Upon the Susceptibility to Injury:

The effects of irradiation upon the testis and other organs of animals have been studied extensively with the use of routine histologic and cytologic methods. These procedures involve the application of a variety of chemical agents for the purposes of fixation, dehydration, imbedding and staining. They register certain morphologic alterations in cellular components of the tissues without providing much insight into those biochemical and physiological changes which precede, or are associated with them.

In one tissue, namely blood, the normal and abnormal morphology of individual cell types have been studied in great detail. This is largely due to the fact that the cells can be readily obtained and studied in the form of fresh and unstained smears, as supravitally stained smears in which reactions of certain cells to supravital dyes can serve as a sensitive indicator

of their physiological activities, and as dried smears stained selectively but with a negligible exposure to chemical agents. The testis of mammals is also admirable suited for study by fresh and stained smears, such as have been applied to blood; yet, in the many classic studies on the cytology and histopathology of the testis, stained sections of fixed tissues have been almost universally used.

The initial phase of the present study has been to demonstrate that testis histology, and cytology of germ cells in particular, can be evaluated by the study of living cells in supravital smears and by examination of smears subjected to fixation and staining, the latter being especially adapted to current procedures in histochemistry for the demonstration of lipids, nucleoproteins and mucopolysaccharides in cells and tissues. The present report details some of the progress in this direction.

With the development of adequate methods, subsequent phases of the study will deal with (a) the cytological and cytochemical alterations demonstrable in irradiated testes, (b) comparisons between these and the changes associated with dietary deficiency of vitamin A, vitamin E, and general inanition, and (c) influence of such dietary states upon the sensitivity of the testis to irradiation. The present report can deal only with one preliminary series of observations related to the first of these approaches.

Development of Methods:

1. Supravital Smears: Many modifications of procedures used for blood cells were tried, using such dyes as neutral red, janus green, pinacyanole and brilliant cresyl blue. Best results were obtained by tapping a small bit of testis in a drop of saline on slides coated by a thin film of dye deposited thereon by evaporation of a very dilute absolute-alcohol solution of known strength, ringing the coverslip with vaseline to prevent evaporation, and microscopic examination in a warm box or at room temperature. Separate smears prepared with neutral red and with pinacyanole proved most satisfactory. With experience it was possible to readily differentiate different stages of germ cell maturation using such criteria as cell size, nucleo-cytoplasmic ratio, distribution of mitochondria, location of the Golgi apparatus, and certain nuclear characteristics.

Considerable difficulty was encountered in differentiating Leydig cells from macrophages and other connective tissue elements of the interstitial tissue. Information derived from certain fixed and stained smears, and from supravital smears of testes from rats stained intravitally with Trypan blue, eventually provided adequate criteria for differentiation; they also revealed certain undescribed and interesting features of the Leydig cell which are being explored as opportunity permits.

2. Fixed and Stained Smears: Wet and dried smears of fresh rat testes were exposed to a great variety of fixing fluids followed by a much greater variety of stains, singly and in combination. Particular attention was given to methods that might demonstrate lipids of various types, mitochondria, Golgi substance, nucleoproteins and mucopolysaccharides and, at the same time, permit good differentiation of cell types. For the most part, procedures were carried out in aqueous media so as to avoid undesired action of lipid solvents.

After innumerable trials certain methods were selected as being most suitable for the particular purposes of the study. Briefly stated, they are as follows:

Fixation	Stain	Suited for Study of:
Formalin	Sudan black Sudan IV	Total lipids Neutral fats
Zenker-acetic or Zenker-formol	Carbol fuchsin-hematoxylin	Certain lipoproteins
	Eosin-methylene blue	Ribonucleoproteins
Picric acid-alcohol-acetic acid	McManus Pinacyanole	Glycogen, and other mucopolysaccharides Metachromasia

3. Frozen Sections: Frozen sections of formalin fixed testes and stained according to many of the methods used for fixed smears, by virtue of maintaining the spatial interrelationship of cells, have served as a check on interpretations made from such smears, especially in identifying and establishing criteria for the various cell types encountered. They have proved especially useful in the study of lipids after treatment with sudan IV, sudan black, nile blue sulphate, and osmic acid.

4. Paraffin Sections: Testis tissue fixed and, after dehydration and paraffin imbedding and sectioning, stained according to the methods applied to fixed smears have been used also as a check on identification of cell types in smears. Certain methods will be adopted for routine study of normal and damaged testes.

Fixation	Stain	To Demonstrate
Zenker	Eosin-methylene blue	Ribonucleoproteins
	Feulgen reaction	Desoxyribonucleoprotein
	Carbol fuchsin-hematoxylin	Certain lipoproteins
Picric acid-alcohol-acetic acid	McManus	Glycogen, and other mucopolysaccharides

Preparation of Standard Reference Material: To secure data and material on normal testes for later reference and comparison, covering both the prepubertal and postpubertal stages of the testis, a series of normal male rats were sacrificed at ages of 20, 25, 30, 35, 40, 45, 50, 55, 60 days. A study of supravital smears was carried out and, in addition, a series of fixed and stained smear preparations and stained paraffin sections of fixed testes and epididymides were prepared according to methods selected as most applicable to later studies on damaged testes.

3. Preliminary Observations on Irradiated Testes:

1. Material: A group of 12 adult male rats was subjected to local

radiation of the left testis, a total dosage of 500 r being given at the rate of approximately 50 r per minute. Animals were anesthetized with nembutal, fastened to a wooden board approximately 17 cm from the tube, and the right testis pushed into the abdomen and shielded by a 1/4 inch lead plate. The left testis was irradiated directly through the anterior scrotal wall, with a 1/4 inch, hollow lead cylinder interposed between the tube and the scrotum.

On the basis of results reported by other investigators, who have observed rather profound testis damage after slightly larger dosage, the level of 500 r was selected as one that might provide moderate or moderately severe damage.

The rats were sacrificed at intervals of 3 to 4 days. The only gross change noted at necropsy was a purplish discoloration due largely to vascular engorgement. This was usually apparent in the left testis but never in the right testis.

2. Histologic Findings:

(a) Supravital smears: Examination of supravital smears of the left testes, using neutral red and pinacyanole separately, revealed considerable injury to certain of the germ cell types, as outlined below. In some instances the right testis, when examined in the same manner, showed changes similar to but usually less intense than those noted in the left testis of the same animal.

3-6 days post-irradiation

Spermatogonia shrunken, with nucleus distorted. Primary spermatocytes undergoing most marked changes, with nuclear swelling and chromatin distortion, fragmentation or loss of chromatoid body (marking region of Golgi apparatus). Numerous multinucleate giant cells. Tendency for cytoplasm of many germ cells to acquire hyaline-like appearance and give a faint and diffuse stain with

and sometimes in the nucleus of many germ cells were interpreted as evidence of mild cellular damage. Sudan black staining after formalin fixation proved especially valuable for following abnormal changes in the chromatoid body.

(c) Stained paraffin sections: On the basis of results obtained from study of smears, as described above, it was anticipated that sections stained with hematoxylin and eosin or other stained customarily applied of the testes would show at least moderate degrees of histologic damage. It was rather disconcerting to find that the testes were consistently within the range that would be considered normal. This was further confirmed by histologic examination of the epididymides. In all instances the ducts of the caput, corpus, and cauda epididymis contained a normal complement of spermatozoa and were entirely devoid of sloughed germ cells such as always accompanies testis damage.

From other standpoints, on the other hand, these findings seem to have valuable significance. They indicate that the methods developed for study of the testis by means of smears, especially the supravital smears, may permit recognition of varying degrees of cellular damage not recognizable in conventional histologic sections of the testis. If this interpretation is verified, such procedures should aid in attaining one of the major objectives of this study; viz., a better understanding of the precise manner in which radiation interferes with normal germ cell functions and thereby induces the structural alterations generally recognized as radiation damage.

Problem Code: X.R.4 (Hematology)

Section Code: 3351

Activities in the Hematology Section have included the (1) establishment of a portion of the health program for the cyclotrons of The University of Rochester Physics Department, (2) the editing of research of the former Hematology Section which is included in the National Nuclear Energy Series, (3) cooperation with the Project health in the investigation of the local beryllium exposures, (4) the carrying out of the routine hematology on Project personnel, and (5) research on the life span and functional activities of leukocytes, using the technique of the marked leukocyte.

All individuals engaged in cyclotron activities at the University have been examined, appropriate blood counts made and recommendations for future program correlated with the health physics activities.

Routine laboratory procedures during the 6 months of operation are:

224 complete blood counts
182 partial counts
113 sedimentation rates
65 urinalysis

Preliminary experiments have been carried out on the possible means and mechanisms for the marking of white cells in the normal animals using saccharated iron and similar materials. Attempts are being made to mark the leukocytes in vitro using the animals own cells as a source. Methods of sampling have been developed which appear promising.

Problem Code: X.R.4 (Hematology)

Section Code: 3420

Hemolytic Effect of Total Body Radiation in Dogs:

Two renal bile fistula dogs were given total body radiation after suitable base-line observations had been made. A dose of 150 r produced no demonstrable effect on the erythrocytes in one dog. The dog receiving 250 r, however, showed substantial increases in bilirubin excretion during the first and fourth weeks after radiation despite the fact that significant increases in serum bilirubin and in osmotic and mechanical fragilities could not be demonstrated. The total red blood cell count fell from 5.5 to 4.3 millions during the first 50 days after radiation, but there was no significant reticulocytosis.

Pre-radiation observations have been made on two additional renal bile fistula dogs which will be given 250 r total body radiation. In addition to hematologic studies, liver function will be investigated and liver biopsies taken in order to detect possible hepatic damage due to the fistulae or to radiation.

Effect of Total Body Radiation on Excretion of Urinary Coproporphyrin in Dogs:

There was no significant change in excretion of urinary coproporphyrin in the renal bile fistula dog that was given 150 r. The dog that received 250 r showed a substantial increase in coproporphyrin excretion within 24 hours and a peak of 10 times the base-line figure was reached at one month after radiation. Measurements of urinary coproporphyrin are being carried out on the two additional fistula dogs which are scheduled to receive 250 r.

Use of Iso-immune Systems in Dogs in Quantitative Studies of Hemolytic Phenomena:

Individual differences among dog blood have been explored in collaboration with an adjacent laboratory operating under a contract with the Office of Naval Research. Four different isohemagglutinins have been demonstrated in sera of dogs immunized by transfusions of dog erythrocytes containing factors lacking in their own cells. Factors thus far identified serologically in tests of

approximately 350 dogs may conveniently be called "canine A, B, C, and D" and the corresponding antibodies "canine anti-A, -B, -C, and -D". Anti-A is most consistently potent, most easily produced by isoimmunization and is capable of causing hemolytic transfusion reactions and hemolytic disease of newborn pups.

Immune anti-A plasma has been transfused into two normal and two renal bile fistula dogs whose cells contained the A factor. The object of these experiments was to chart as accurately as possible the course of events following injection of isoantibodies. These studies were carried out with the hope that they might aid in elucidating the hemolytic mechanisms operating in human beings after transfusion of incompatible plasma or of blood from dangerous universal donors. Attachment of transfused canine A antibody to recipient dogs' cells was demonstrated for approximately three weeks by employing anti-dog-serum rabbit serum in "Coombs" tests. Spherocytosis and increased osmotic fragility of recipients' cells were demonstrated for similar periods, but increased bilirubin excretion persisted for only 4 to 7 days, and hemoglobinemia for 1 to 3 days.

Studies employing the technique of differential agglutination of dog erythrocytes showed that transfused normal cells lacking the canine A factor survived for at least 3 months in the circulation of a normal recipient dog whose cells contained the A factor. An effort is being made to improve the technique of differential agglutination of dog cells in order that further studies on erythrocyte survival may be made. It will be of particular importance to measure the periods of survival of radiated cells in normal recipients of normal cells in radiated recipients.

Miscellaneous Hematologic Investigations:

A six-year old child with severe megaloblastic anemia was given vitamin B_{12} intramuscularly with excellent hematologic response. She had previously responded to injections of refined liver extract at age two and to administration of folic acid at age four but had relapsed each time after treatment was discontinued.

Problem Code: X.R.5 (Genetics)

Section Code: 3410

Investigation of Semi-Steriles:

This problem is designed to determine the time of death of mouse embryos in semi-sterile, sex-linked lethal lines and to evaluate the death time in terms of human embryology. The report is based on a study of six semi-sterile lines and three lethal-bearing lines isolated by Dr. D. R. Charles in determining the mutation rate under chronic exposure to 250 KVP x-radiation.

Materials: Tested semi-sterile females were mated with non-related normal males of the CFCW strain, or the reciprocal cross was made. At stated intervals after the appearance of a mating plug, an autopsy was made and the numbers of live young, dead or retarded embryos, and placentae in the uterus were recorded. As embryos die, they are rather rapidly resorbed, but the placenta marking the site of an implantation is retained in place until parturition. A count of the corpora lutea gave the total possible young for that pregnancy, and the difference between the corpora and the contents of the uterus was assumed to have died before implantation.

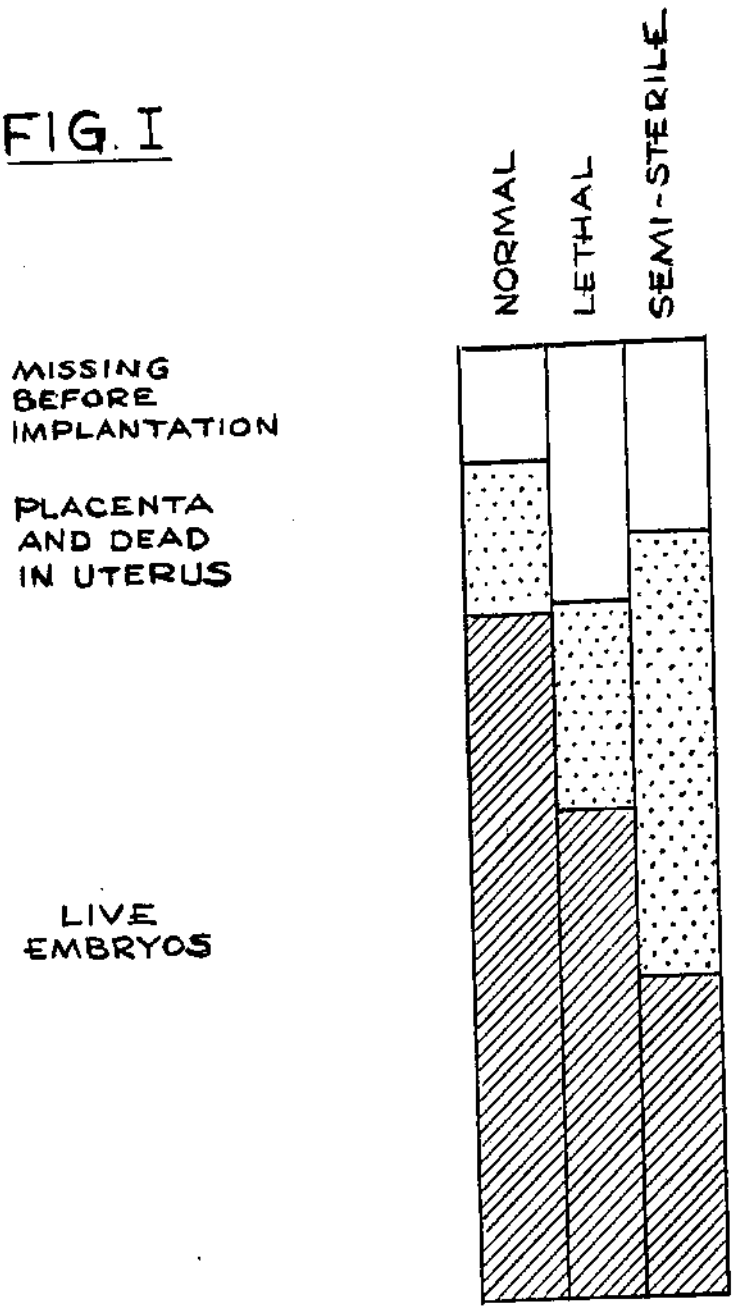
For construction of mouse embryological time tables, normal siblings of mice known to be semi-sterile were mated to the CFCW strain and autopsied from 9-16 days later. Mating and autopsy times were recorded to the nearest half hour. Serial sections were made of some or all embryos in a uterus, depending on the gestation age at autopsy. Since variations in the development between embryos in the same uterus seem to decrease with age, fewer preparations were made of older embryos. References for human structural development were derived from many sources; however, the most comprehensive source of information on human timing in the critical period of 21-40 days was G. L. Streeter's series of papers, Developmental Horizons in Human Embryology.

Results: In all of the semi-sterile lines studied so far, embryo deaths have occurred before the 16th day of gestation. At that time, the mean per cent of live embryos in the semi-sterile uterus is 33.9 per cent, which is to be compared with 73.0 per cent for all normal uteri at the 16th day. The figure is in good agreement with what is to be expected since semi-sterile litter size is approximately half normal liter size.

The fate of all zygotes at 16 days, expressed as per cent of corpora lutea, is recorded in Fig. I (Page 38). This table includes in the second column, information gathered from two sex-linked lethal lines. The mean per cent of live embryos recovered from known lethal bearing females was 52.3 per cent. Since half of the male young of such females die, litters would be expected to be three-fourths the size of normal litters, or in this instance to average about 55 per cent of live embryos. In the semi-sterile lines, a significantly greater proportion of zygotes die in the uterus rather than before implantation.

Fig. II (Page 39) shows the per cent of live embryos on successive days, from three semi-sterile lines for which information is almost complete. The solid line at the top of the figure is the mean per cent of live embryos in all normal uteri for the period 12-16 days, 70.99 per cent. The dotted lines lie one standard deviation above and below the mean. It may be noted that at 9 days of gestation, semi-sterile line 7642 slightly overlaps the normal range for embryos still alive. At 12 days of gestation, the per cent of live embryos for the same line lies wholly within the range typical of the translocation uterus at 16 days; the mean, 33.9 per cent, and deviations for the latter are indicated by the solid and dotted lines respectively at the left of the graph. This indicates that developmental failure begins on or after 9 days of gestation and is complete by 12 days. Reference to the comparative mouse-human time table (Fig. III - Page 40) shows that this is most nearly equivalent to the fourth week of human pregnancy.

FIG. I



FATE OF ZYGOTES
AT 16TH DAY OF PREGNANCY

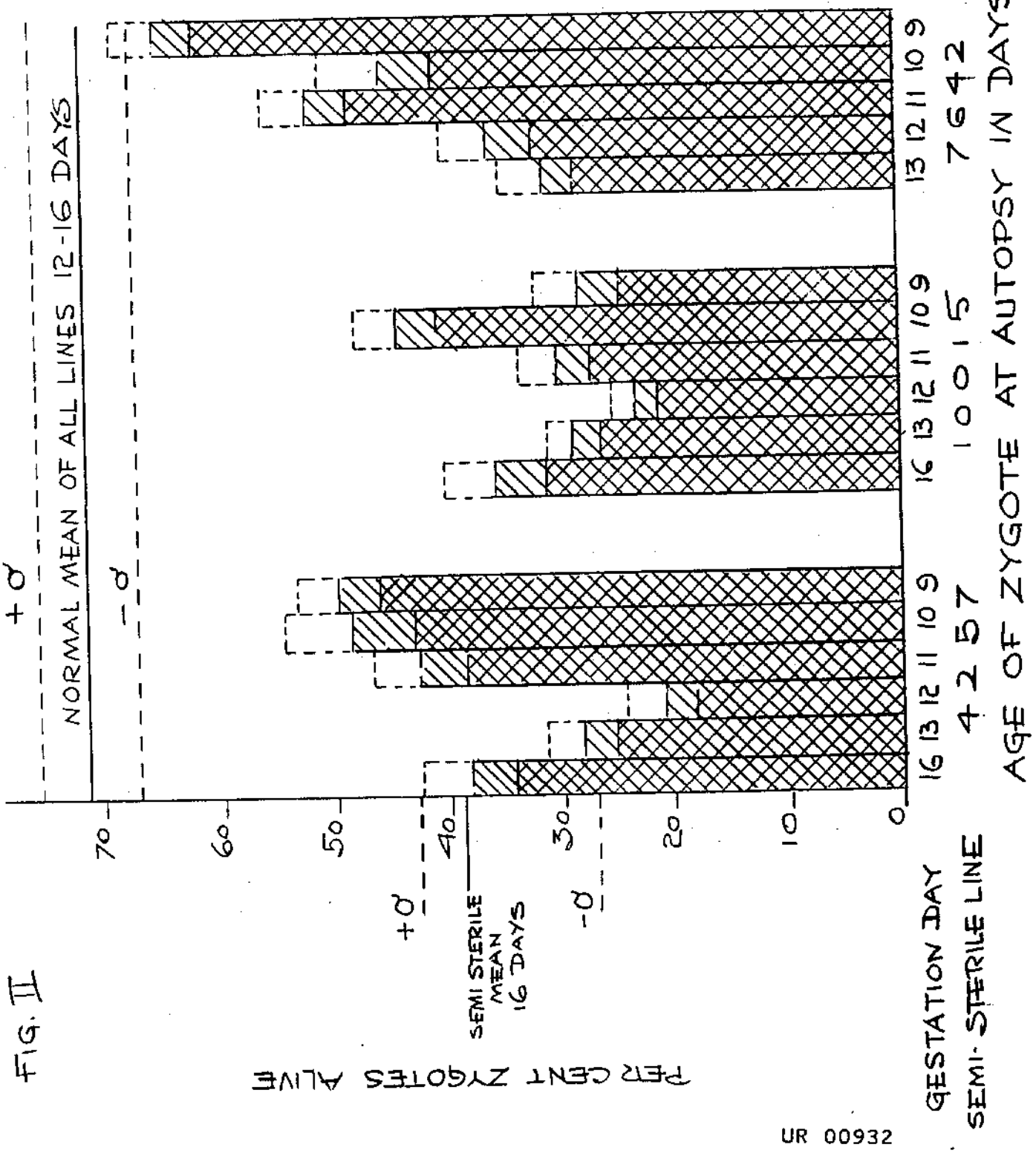


FIG. II

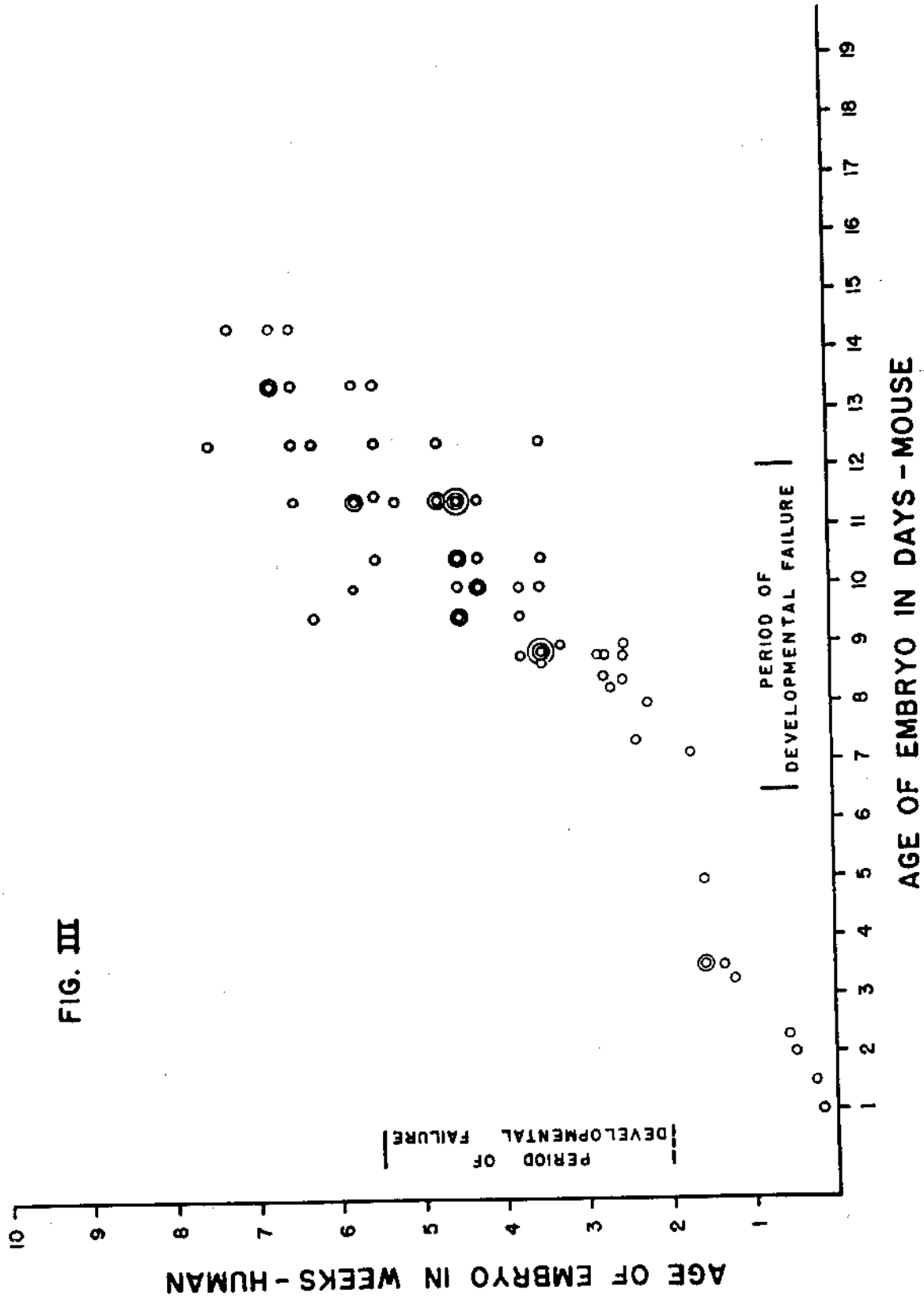


FIG. III

By contrast, semi-sterile line 10015 even as early as nine days of gestation lies within the range characteristic of all semi-steriles at 16 days. Since there are still to be accounted for 44.5 per cent dead after implantation as evidenced by the presence of placental remains, the inference is that death occurs at some time between 5 days (when the developing egg settles into a uterine crypt) and 9 days. The period can be further narrowed by the fact that the placental remains are those of the typical allantoic placenta established between the 6th and 7th day of pregnancy. This is roughly equivalent to the third week of human pregnancy. No attempt has yet been made to determine failure in this very early period since the distinction between live and dead embryos and placental remains cannot be made by simple inspection under a dissection microscope. Serial slides must be studied to obtain this information.

Semi-sterile line 4257, after the tenth day of gestation, has no more live embryos in the uterus than at 16 days; however, at 9 and 10 days, about 11 per cent more zygotes are still living. So developmental failure begins prior to day 9 and is complete at 11 days. Around 20 per cent of placental remains are still to be accounted for at 9 days and indicate still earlier deaths. The time period for failure in this line is equivalent to the late third and the fourth week of human pregnancy.

In three additional semi-sterile lines for which the information is less complete in the earlier stages of pregnancy, all deaths have certainly occurred before the 13th day of gestation or before the equivalent 6th week of human pregnancy. The period of developmental failure will almost certainly be lower when earlier pregnancies are more fully explored.

The equivalency table, Fig. III, was composed in such a way that each point on the graph represents the time of appearance or differentiation of some organ in mouse and in man. For example, the lens placode is present in all mouse embryos at 10.0 days (but not at 9.5 days) and in human embryos at the end of the fourth week according to the literature. Hence, a lens placode point is plotted on the graph at the intersection of 10.0 days and 3.7 weeks. It will be obvious that the time values are subject to error, perhaps a few days for the human embryos, and at most, eleven hours in the mouse.

No attempt to express human embryological time as a mathematical function of mouse embryological time has been made in this report since additional organ systems remain to be entered on the graph; however, three systems are now there and in reasonably good agreement with each other. It is anticipated that the points will not lie unreasonably far off some smooth and relatively simple curve.

Plans: In the immediate future, determination will be made of the time of developmental failure in the three semi-sterile lines for which we have inadequate data below the 13th day of gestation. Two additional semi-sterile lines are now available for study, one of which behaves irregularly: some pregnancies from tested individuals of this line result in large litters. A possible explanation is that the genetic deficiency is sufficiently small so that some individuals which would be expected to die during development survive until birth and after. Here, developmental failure may occur through the entire gestation period. It seems important to investigate this possibility,

and, because of the aberrant normal-type litters, many more autopsies must be made at each day of gestation than are needed for the typical semi-sterile.

Four additional systems will be added to the mouse-human embryological equivalency table. Points based on tissue differentiation times are needed in the late period of gestation to correspond to the period from the tenth to the fortieth week of human pregnancy. With these added, it should be possible, within reasonable limits of accuracy, to translate mouse developmental time into human equivalents.

Slides of abnormal embryos have been prepared. It is proposed to study these -- and to make others of embryos in the period just before developmental failure becomes apparent -- to determine, if possible, the causes of failure.

Since the primary purpose of this work is to assess the danger of radiation-induced semi-sterilities in man, it would seem wise to base our judgment on a sample larger than the eight studied so far. Suppose, for example, that among all possible semi-sterilities, about 35 per cent produced embryonic deaths <u>after</u> 16 days; there would be an appreciable chance that eight lines chosen at random would all show deaths <u>before</u> 16 days. The conclusion might be drawn that all deaths are early in semi-sterilities, but the truth would still be that deaths are late in 35 per cent of cases. On statistical grounds, it can be shown that fairly adequate information about semi-sterilities could be obtained by investigating a sample of 20 or 30 semi-sterile lines. This would mean obtaining 15-25 new semi-steriles, which could be done economically by acute exposures.

Problem Code: X.R.6 (Embryology)

Section Code: 3441

<u>Effects of Roentgen Irradiation on Embryonic Development</u>:

Developing embryos have been subjected to roentgen irradiation by several other investigators; but in all previous experiments the pregnant mothers were exposed to whole-body irradiation, and the effects of such exposure upon the embryos was not observed until delivery at full-term, 6 to 12 days later. The experiments summarized herein were planned specifically: (1) to avoid exposure of the mother in order to eliminate the likelihood that the maternal reaction to irradiation might itself alter embryonic development; (2) to reduce variations in the quantity of irradiation reaching the embryos, resulting from passage of the rays through variable thicknesses of maternal tissues and intestinal contents; (3) to observe the early reactions of the embryo to the irradiation and trace the effects of these reactions upon the overall process of development.

Methods: Twenty-eight female rats of the Wistar strain were mated and certain of the offspring of each exposed to roentgen irradiation on the 10th day of gestation. Pregnancy was carefully timed in reference to the onset of estrous behavior, since the time of ovulation (and presumably fertilization) in the rat is known to bear a more constant relationship to the beginning of behavioral estrus than to any other apparent event in the reproductive cycle. By this means the duration of pregnancy at the time of irradiation was reckoned in terms of hours, plus or minus 2 hours.

Ten days after the approximate hour of ovulation the pregnant females were anesthetized with nembutal and the ventral abdominal wall opened by a midline incision about 3.5 cm in length. With a minimum of handling, one entire uterine horn was brought to the surface of the incision. A small lead shield was then placed underneath 2 to 5 of the implantation sites of this horn; and the shield, as well as the overlying implantation sites, were secured in place by means of gauze packs soaked in physiologic saline, precautions being taken to avoid occluding any uterine blood vessels. A larger lead shield (10 x 10 cm), containing a port near its center of sufficient size to fit loosely around the 2 to 5 implantation sites selected, was then placed over the ventral surface of the abdomen, leaving only the selected implantation sites uncovered by lead shielding. A check was always made to make sure that the small, underlying shield was so arranged that it coincided with the port of the larger shield and overlying the smaller shield, it was possible to irradiate the 2 to 5 embryos contained in these sites without irradiating any of the remaining embryos or any maternal tissues except the segment of one uterine horn containing the exposed embryos. After irradiation of the unshielded implantation sites, they were restored to their usual position in the abdominal cavity. The second uterine horn was then brought to the surface of the incision, a similar number of implantation sites selected, and the same manipulations carried out as before except that the latter group of implantation sites wasnot irradiated. The sham exposure of the second uterine horn provided non-irradiated controls. Before this horn was also restored to the abdominal cavity and the incision closed, the total number and arrangement of all implantation sites in both uterine horns was noted in a diagram to facilitate later identification of the irradiated and non-irradiated embryos.

The dosage of irradiation administered to all unshielded embryos of each litter was either 50, 100, 200, or 400 roentgen units. Table I (Page 44) shows the number of embryos exposed to each dosage.

At 24, 48, 72, 96, or 120 hours after irradiation of the embryos, the mothers were killed by decapitation and both uterine horns inspected in situ for resorbed implantation sites. The total number of implantation sites was checked with the diagram made at the time of irradiation and the exposed and control embryos identified and tagged. The entire uterus was then removed, hardened in Bouin's fixative for 15 minutes, partially opened to allow better penetration of fixative to the embryos, and then allow to remain in fixative for 24 to 48 hours. After being passed through successive grades of alcohol to 70 per cent, the embryos were removed from the uterus, measured and weighed. All irradiated embryos together with 1 or 2 of the controls were prepared in the usual way as continuous serial sections for microscopic study.

Table I

Rate of Growth and Survival of Irradiated as Compared with Non-Irradiated Embryos

Dosage (r)	IRRADIATED EMBRYOS: Number Studied	% Dead or Resorbed	% Small* but Alive	Mean Wt.† of Survivors(mg)	NON-IRRADIATED EMBRYOS: Number Studied	% Dead or Resorbed	% Small* but Alive	Mean Wt.† of Survivors(mg)
24 hours after exposure on the 10th day:								
100	5	0	60	2.4	9	11	0	3.8
200	4	0	100	< 2.0	6	33	50	3.0
400	9	100	---	---	15	7	7	4.3
48 hours after exposure on the 10th day:								
50	4	0	0	18.7	8	0	0	18.7
100	13	23	15	18.0	21	19	5	18.9
200	15	13	80	10.8	18	11	22	16.3
400	10	100	--	----	13	8	0	22.2
72 hours after exposure on the 10th day:								
50	7	29	0	60.3	13	31	8	56.1
100	13	38	31	42.1	17	19	35	43.6
200	15	46	20	42.9	19	26	5	55.4
96 hours after exposure on the 10th day:								
50	8	13	0	135.4	12	0	0	134.7
100	10	30	20	108.9	13	16	8	119.5
200	4	50	50	74.0	7	0	0	115.0
120 hours after exposure on the 10th day:								
50	2	0	0	243.5	8	38	0	202.8
100	5	0	0	170.0	6	17	0	186.0

* Embryos were classified as "small" if their weight was less than 75 per cent of the mean weight of controls of the same age.

† The means include the embryos classified as "small".

Four females, some of whose embryos were exposed to 50 or 100 r on the 10th day, were allowed to go to full-term. When it was apparent that labor was in progress, these mothers were killed and their litters observed *in utero* in order that the offspring might be identified while in position in the uterus. The young were then removed; and, after ligation of the umbilical cord, were weighed and examined for gross abnormalities. All surviving newborns that were previously irradiated, together with an equal number of non-irradiated, littermate controls, were given to foster mothers to be raised. Thereafter they were weighed and examined externally at two-weekly intervals until sexual maturity was attained, at which time they were allowed to mate.

Results: Survival and Rate of Growth (Table I):

Twenty-four hours after exposure: Dosage of 200 roentgen units or less did not cause death or resorption during the first 24 hours after exposure. A dosage of 400 r, however, resulted in intra-uterine death in all of 9 embryos; and several of these had been dead for a number of hours when removed and preserved, judging by the degree of autolysis apparent in the histologic sections. Although not fatal, dosages of 200 r were generally no more than one-half the weight of their controls.

Forty-eight hours after exposure: Dosages of 50, 100, and 200 roentgen units did not significantly increase the rate of intra-uterine death within 48 hours after exposure. As with the shorter interval above, 400 r were invariably fatal to embryos exposed to this dosage. The rate of growth, as reflected by weight, was not altered by 50 r and was not appreciably changed by 100 r. The apparent retardation of growth observed at 24 hours after irradiation with 100 r may indicate only a temporary slowing of growth rate which has been obscured at the 48th hour due to resumption of a near-normal rate. To establish this point a larger number of embryos must be examined at the end of the 24-hour interval. Growth was unquestionably retarded by 200 r after a 48-hour post-irradiation period. The mean weight of all surviving embryos that received this dose was considerably less than the mean weight of their siblings. Eighty per cent were classified as "small" since their weights fell outside an arbitrarily established range for controls of this age.

Seventy-two hours after exposure: Survival during this post-irradiation interval was not altered by a dosage of 50 roentgen units. A dosage of 100 r appears to have increased the rate of intra-uterine death after 72 hours: 38 per cent of the exposed embryos died, while only 19 per cent of the sibling controls died during the same period. Likewise, exposure to 200 r resulted in an appreciably higher death rate among irradiated embryos than among non-irradiated siblings. Growth was not affected by dosages of 50 r and 100 r, but was significantly retarded in embryos receiving 200 r. The mean weight of surviving embryos exposed to the latter dosage was 42.9 mg., as compared with 55.4 mg for their controls.

Ninety-six hours after exposure: During this post-irradiation interval, a dosage of 50 r appears to have caused a higher death rate among irradiated than non-irradiated animals; but considering the data obtained after intervals both longer and shorter than this, it seems doubtful that

the difference observed here was a real one. Treatment with 100 r was followed by intra-uterine death in 30 per cent of exposed animals, while only 16 per cent of their siblings died during the same period. Although only 4 embryos were exposed to 200 r, half of these died before the 92nd hour, while none of their unexposed siblings died. Again, the rate of growth was not altered by 50 r. It was, however, slowed somewhat by 100 r and was greatly reduced by 200 r.

120 hours after exposure: The number of embryos removed after this post-irradiation interval is too small to permit any generalizations. It may be noted, however, that the few data available are in accordance with those obtained after shorter intervals, to the extent that 50 r did not reduce the rate of growth while 100 r appears to have resulted in some reduction.

Full-term, 11 days after exposure: Five embryos in each of two mothers were exposed to 50 r and the mothers allowed to carry their entire litters to full-term. All irradiated embryos survived and did not differ from their non-irradiated littermates in size or appearance. Only 4 of these were successfully raised by foster mothers, but these sired or delivered normal offspring after reaching sexual maturity.

Four embryos in each of two mothers were exposed to 100 r and the mothers allowed to go to full-term. All irradiated embryos survived but, when examined at the time of delivery, they weighed an average of nearly 1 gm less than their non-irradiated litter-mates. One never established regular respiration and died within one hour. One other survived less than 24 hours. Five were raised by foster mothers, but it was later discovered that 3 of these were blind. All of the surviving 5 sired or delivered normal young after reaching sexual maturity.

Results: State of Development in Irradiated Embryos (Table II):

After 50 r: This dosage did not alter the course of development at any post-irradiation interval at which embryos were examined, that is, at 48, 72, or 96 hours or at full-term. Neither histologic nor embryologic differences were found when exposed animals were compared with their unexposed siblings.

After 100 r: About 60 per cent of embryos exposed to this dosage, and examined at various intervals thereafter, exhibited some type of developmental defect. The most commonly affected organ was the eye, and the most frequent ocular defect was microphthalmia, that is, abnormal smallness of the eye as determined by actual micrometer measurements. In addition to the small size, which reflected deficient growth, there often were indications that specific developmental processes concerned with formation of the eye were retarded. In one instance recognizable eyes failed to form, a condition known as anophthalmia. Five embryos possessed eyes that were microphthalmic and in addition were malformed, for example, in one instance the lens was found outside the optic cup. The frequency and severity of these ocular defects suggests that the eye was in a critical phase of its development at the time of irradiation. Indeed, on the 10th day of gestation, the primordium of the eye is just appearing as a slight evagination from the ventro-lateral aspect of the forebrain.

Table II

State of Development in Irradiated Embryos, as Observed in Histologic Serial Sections*

Hours after Exposure	Number Embryos Studied	Number with Ocular Defects: Microphthalmia	Anophthalmia	Malformed	Number Showing Retarded Development in Various Organs or Systems	Other Malformations
50 r on 10th day of gestation:						
48	4	0	0	0	none	none
72	3	0	0	0	none	none
96	7	1(?)	0	0	none	
100 r on the 10th day of gestation:						
24	4	3	1	0	brain(3), ear(2), urinary(3).	none
48	10	4	0	3	brain(1), heart(1), aortic arches(1), urinary(1).	none
72	7	7	0	2	heart(2), aortic arches(1), urinary(2).	none
96	7	2	0	0	heart(2), aortic arches(2), urinary(4)	urinary(1)
200 r on 10th day of gestation:						
24	4	3	1	0	brain(3), ear(2), urinary(3)	none
48	11	8	2	3	brain(6), ear(4), heart(7), aortic arches(7), urinary(7)	none
72	2	1	1	2	brain(2), lungs(2), urinary(1)	brain(1), urinary(1).

* Many of the irradiated embryos listed in Table I have not yet been studied, hence, only a part of the available material is represented here.

This dosage also affected the development of other structures, but to a less striking degree than the eye. The only fault in other organs was usually retardation in development, that is, the processes were proceeding in the right direction but at a slowed rate. The brain, urinary system and heart were most frequently retarded, but these were affected in somewhat less than half of the embryos. Only one instance of malformation, and this of a minor nature, was found in any other organ or system that the eye.

The cases of uncomplicated microphthalmia and of retardation in other organs were generally of such character that they might conceivably have been corrected by an acceleration of development at some time prior to birth. In fact, of the 8 embryos exposed to 100 r and allowed to go to full-term, all survived until delivered, and only one was known to be defective at the time of delivery (this one never breathed properly and died within 1 hour). Of the 5 such animals raised by foster mothers, however, 3 were later found to be permanently blind. An autopsy of the blind animals will reveal whether the cause is anophthalmia, severe microphthalmia, or some other ocular malformation.

After 200 r: This dosage did not change the general pattern of developmental defects observed after treatment with 100 r; but it did raise the frequency and increase the severity of the defects. The eyes were severely affected in all but one of these embryos: of the 17 studied, 12 had microphthalmia, 4 had anophthalmia, and 5 had some variety of malformation in addition. Other organs were in general only retarded in development, but sometimes to a considerable degree. The brain exhibited retardation in 11 instances and in several of these the state of development was far behind that of normal embryos of the same age. Certain parts of the urinary system were as frequently but not as markedly retarded as the brain. Organs occasionally affected were the heart, aortic arches, ear, and lungs. Malformations, other than ocular, were found in only two instances, one in the brain and one in the urinary tract.

The localized developmental defects commonly seen in embryos receiving 200 r were undoubtedly related to the small size of the embryo as a whole (noted in the preceeding section). Both reflect a reduction in the rate of cell division: in the case of specific defects and retardations, the mitotic rate was altered focally or locally, whereas in the case of overall smallness of size the mitotic rate throughout the embryo was suppressed. The higher embryonic death rate after this dosage may also be related to the frequent occurrence of altered developmental processes in a localized region. Such a relationship can be conceived if it is assumed that a localized retardation becomes sufficiently acute that the affected organ is no longer able to meet the functional needs of the embryo, with the result that death would sooner or later ensue depending upon the functional importance of the organ. However, no example of this can be cited at present.

After 400 r: As already noted, this dosage was universally fatal within 24 hours after exposure. The state of development in these embryos has not been critically studied, but it was apparent that the brain in all cases was arrested at a stage of development only slightly in advance of that known to exist at the time of irradiation. Specific evidence of this was seen in the fact that no true optic vesicles had formed but, instead, the eye had remained in its primordial state as a slight evagination from the forebrain.

Conclusions: A dosage of 50 r on the 10th day had no effect on embryonic development. Survival, growth rate, and the state of development of specific organs and systems did not differ in irradiated embryos and their non-irradiated siblings, when compared at 48, 72, 96, and 120 hours and at full-term.

Exposure to 100 r increased slightly the rate of intra-uterine death, reduced the rate of growth, and caused localized developmental defects, particularly in the eye, in about 60 per cent of animals. The effects of irradiation were apparent 24 hours after irradiation, but they did not become appreciably more pronounced after longer post-irradiation intervals.

Treatment with 200 r was followed by the same general reactions as the dosage of 100 r, but the effects were more intense in degree. Death rate was higher, rate of growth was lower, and localized developmental defects were more striking. After progressively longer post-irradiation intervals the death rate and the degree of developmental abnormality tended to increase.

A dosage of 400 r always caused intra-uterine death within the first 24 hours after irradiation.

After all effective dosages, the eye was the most sensitive organ to the deleterious effects of roentgen rays. With minimally effective dosages, the eye was frequently the only organ to show developmental aberrations, and with higher dosages it was the only structure consistently malformed or completely suppressed by irradiation. The brain was second in order of sensitivity to irradiation, but it was generally affected by retarded rather than aberrant development. Some parts of the urinary system were almost as frequently but rarely as severely affected as the brain.

Problem Code: X.R.7 (Bacteriology and Immunology)

Section Code: 3442

Studies on the Effect of X-ray on Experimental Murine Pertussis:

The studies, made between April 1, 1948, and December 31, 1948, were designed to observe the effect of external x-ray irradiation on mice infected with freshly isolated strains of Hemophilus pertussis.

Background: The studies were undertaken to test the theory that the protective antibody is in someway related to the lymphocyte as proposed by the work of Dougherty and White. Since experimental murine pertussis is regularly characterized by a significant increase in the total leucocyte count, of which an increase in the number of lymphocytes occurs, and since the lymphocyte is sensitive to x-ray, it occurred to us that the effect of x-ray on this experiment would be of some interest.

A. Experiment to Compare Different Methods of Inoculation: Similar groups of mice were infected with equal doses of a suspension of freshly isolated organisms by the intranasal, intracerebral, and intraperitoneal routes. When the mortality rates were compared by the 50 per cent end point method of Reed and Muench, the mortality rate (MLD) in each group was as follows:

Intracerebral	7.8 million organisms
Intranasal	15.6 million organisms
Intraperitoneal	>250 million organisms

The total white blood cell counts were increased by the infection to similar levels of approximately 45,000 in the mice inoculated by the intracerebral and intranasal routes. The total leucocyte unit of the group inoculated intraperitoneally was about 7,000 which compared favorably with that of non-infected mice.

B. Experiment to Test the Effect of X-ray on Positive Blood Cultures in Mice Surviving Intraperitoneal Infection: Mice surviving experimental inoculation by the intraperitoneal route (Exp. A) were given 200 r x-ray radiation. Two days later, they were re-inoculated with a suspension of a 24-hour culture of H. pertussis.

Tail blood cultures were made one-half hour after inoculation and the average number of colonies resulting from the growths were calculated. Results were as follows:

Dose	X-ray	Average Number of Colonies
500 mil. orgs.	yes	50
500 mil. orgs.	no	5
125 mil. orgs.	yes	20
125 mil. orgs.	no	2

Under the conditions of the experiment, x-ray treatment appeared to increase the invasion of the blood stream by organisms injected intraabdominally into mice which had survived a previous infection.

C. Experiment to Test the Effect of X-ray Irradiation on Mice Vaccinated 10 Days Previous to Intracerebral Infection: Groups of 20 mice each were treated as follows:

Group 1. Vacinated with 2 billion killed organisms; 10 days later given 200 r x-ray and 2 days after x-ray treatment, challenged by intracerebral infection.

Group 2. Same as Group 1, except they received no x-ray exposure.

Group 3. Not vacoinated, but x-rayed.

Group 4. No vaccine; no x-ray.

The mortality rate (MLD) in millions of organisms for each group was as follows:

Group 1.	Vaccine + x-ray	3.9 million
Group 2.	Vaccine; no x-ray	14.2 million
Group 3.	No vaccine + x-ray	0.65 million
Group 4.	No vaccine; no x-ray	<0.5 million

Summary: In non-vaccinated mice, the influence of x-ray exposure was only slightly favorable. In vaccinated mice, exposure to 200 r appeared to make them less resistant.

D. Experiment to Test the Effect of 200 r X-ray Irradiation on the Resistance of Mice to Infection with H. Pertussis when Given Prior to Immunization: In this experiment, mice were exposed to 200 r inoculation 2 days before a single intraabdominal injection of 2 billion organisms. They received the challenge infection intracerebrally 15 days later.

Results expressed in terms of dose required to kill 50 per cent of the mice in each group. (MLB):

Group 1.	X-ray + vaccine	125 million organisms
Group 2.	Vaccine only	93.3 million organisms
Group 3.	X-ray only	15.6 million organisms
Group 4.	Neither x-ray nor vaccine	39.0 million organisms

According to this data the protection given by vaccine injection to previously x-rayed mice (Group 1) was only 1.3 X that given by vaccine to mice not previously x-rayed. (Group 2) The effectiveness of the vaccine in non-x-rayed mice (Group 2) was only 2.4 X that in the non-vaccinated group (Group 4). However, in x-rayed animals (Groups 1 and 3), vaccine protection (Group 1) was 8 X that of animals (Group 3) that received no vaccine.

Experiments are planned to vary the dose of inoculation, to increase the period of immunization, and to introduce another antigen (sheep cells). It is planned to test the effect of x-ray on isolated lymphoid organs of immunized animals and its effect on the skin test in immunized rabbits.

E. Experiments unrelated to x-ray irradiation, performed during the period, included the testing of the effectiveness of Aureomycin against the experimental disease. These experiments revealed that this antibiotic is definitely effective.

PROGRAM I.R.

BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (INFRA-RED AND ULTRA-VIOLET)

Problem Code: I.R.1 (Flash Burns)

Section Code: 3450

Production and Histopathological Study of a Flash Burn:

Background: Thermal injury has been the subject of increasingly intense study during recent years. The burns studied were those produced by moderate temperature applied over fairly long periods of time. However, during the bombing of Pearl Harbor, a different type of burn was encountered. It was superficial in degree and was produced by a high intensity source applied over a very brief period. This was termed the "flash burn".

After the war, LeRoy, in investigating the effects of the atom bomb at Hiroshima and Nagasaki, noted that about 65-85 per cent of the casualties sustained burns and a large number of these were of the "flash type". It seemed possible that the "flash" burn might be a distinct entity with unusual healing characteristics and worth investigating.

It has been pointed out by several observers (Leach, Peters, and Rossiter in England, and Henriques and Moritz in the United States) that the severity of a burn is influenced by the relationship of time and temperature. A given degree of burn, e.g. one producing transepidermal necrosis, can be reproduced roughly by an exposure of 44° over a six-hour period or 51° applied for only two to six minutes. Thus, it can be said that "the higher the temperature of the heat source, the less time required to produce a burn". The relationship was well worked out down to the interval of one second, but from there on, the curve so produced was calculated and no experimental lesions were produced.

In the longer time zones, i.e. above one second, certain physiological factors, such as vascular response and edema, serve to modify the severity of the thermal effect. Under one second, some of these factors are not involved, but thermal conductivity of the tissues per se becomes of extreme importance.

Methods: With the relationship of time and temperature in mind, a search for a heat source of about 0.1 to 0.5 second duration and over 1000° C was made. Thermite, the exploding wire of anderson, the Xenon flash tube, burning magnesium and several other high intensity sources were tried. Magnesium seemed to be the best source and previous work in the development of protective creams bore this out.

The burning of 124 grams of magnesium flash powder produced a flash, high in ultra-violet, of about 0.335 second duration, with a color temperature of around 3700° K. This amount of magnesium when fired about 30 cms. from an animal produced a distinct, uniform burn.

The pig was selected as the experimental animal as its skin has been shown to be the nearest to that of the human in morphology as well as reaction. The animals were anesthetized with nembutal, the skin clipped, and the subject placed in a box which was so designed as to expose the clipped skin through numerous ports of 3.0 cms. in diameter. Exposures were made at various distances from the source. The lesions so produced periodically were photographed, observed in gross and biopsied at all stages of their course from immediately after to ten days.

Results: The severity of burns produced varied with the distance of the skin from the source, the amount of magnesium burned, and the anatomical location of the burn area. The mild lesions were characterized by an erythema which developed within the first few hours, deepening in color and becoming tan or brown over the next few days and finally disappearing within a week with perhaps a fine scaling.

The moderately severe and severe lesions demonstrated a peri-burn flare. This flare subsided within one hour, leaving a ring of erythema about a central gray-white area of dry, cutaneous necrosis. The erythema again deepens in color, subsiding within a week. The central area also deepens in color and becomes thickened and raised by twenty-four hours. The edema reaches its peak by forty-eight hours, then subsides, gives way to induration and is later transformed into a flat, brown crust covering the burn. The crust remains adherent to the hairs which have grown out but finally drops off in seven to ten days.

Occasionally fluid free vesicles are present in the central necrotic area immediately after the burn, or the epidermis may be off entirely, leaving a raw dermal surface exposed.

Histologically, the magnesium flash burn presents several interesting points not noted in the moderate temperature burn. The common type of burn (moderately severe, produced by 124 grams of magnesium exploded at 30 cms. from the skin) presents a shredded stratum corneum. The epidermal transition from burned to normal epithelium is abrupt and often accompanied by epidermal-dermal separation at the line of juncture. The normal epidermis is basophilic, the cells of normal architecture, and immediately adjacent the burned cells are eosinophilic, present nuclear pyknosis and cytoplasmic vacuolation. The burned cells run the gamut of types described by Leach and Rossiter. Various degrees of dermal-epidermal separation are present throughout, with attachment by elongated tonofibrils in some areas.

A similar abrupt demarcation of burned from normal epithelium is seen in the skin crypts and in the hair follicles.

The dermis reflects the depth of injury by a coagulation of the fibrils, with some fragmentation appearing later.

In the sections, the edema is manifest by a loosening of the dermal collagen fibrils and appears about six hours after burning. Polymorphonuclear leukocytic infiltration appears simultaneously. By twenty-four to forty-eight hours, both phenomena are at a maximum, fading off thereafter to disappear entirely by the fifth day.

Healing is fairly rapid in the mild and moderately severe burns. Re-epithelialization begins at forty-eight hours and generates from surviving epithelium of hair follicles, crypts and lateral margins. It is completed at five days. The thermally damaged epithelium is undercut by the growing epithelium and the former shed as a dense sequestra. This eschar remains covering the lesion several days after complete re-epithelialization has occurred. Only rarely was granulation seen.

In the more severe transcutaneous burns, demarcation is not as marked laterally and is non-existent in depth. The epidermis, dermis and underlying fat have a fixed, coagulated appearance. The characteristic leukocytic boundary and healing by granulation seen in moderate temperature burns are present in this degree of flash burn.

Discussion and Summary: While many variations in the picture of the flash burn were noted, two characteristics are outstanding and seem to be unique for this particular lesion. The abrupt demarcation and the rapid healing beneath the sequestered eschar characterize the burn. Apparently both of these characteristics are functions of radiant thermal conductivity. The brevity of the thermal assault, although intense, does not allow the tissues to come to equilibrium while it is in effect. Thus it is, that which is injured is injured beyond repair. What remains is virtually unaffected.

With the establishment of the flash burn as a lesion with certain distinct characteristics, it is now possible to amplify the studies to include physical and biological factors simultaneously. The effect of such factors as spectral changes, dietary deficiencies, infection, and ionizing radiation are to be studied eventually.

PROGRAM R. M.

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

Problem Code: R.M.1 (Polonium)

Section Code: 3170

Background: The results obtained in the earlier work on polonium toxicity depended upon a rather small number of animals in some cases, work performed for the most part on rats, although some dog experiments and rabbit experiments were performed, and paid, in general, little attention to the detailed mechanism by which the body handled polonium. It may be further commented that for the purpose of setting maximal permissible exposure levels to polonium, the presently available information must be supplemented by long-term studies at low dosage levels with large numbers of dogs.

Work done during the calendar year 1948:

Estimation of Maximal Permissible Levels of Polonium:

Since no comprehensive Project Report existed on maximal permissible levels of polonium in the body, it was decided to issue a compendium of the revelant biological data and pertinent calculations. This work appeared as Rochester Report, UR-44. This report estimated a maximum permissible body content of polonium to be 0.2 μc/70 Kg man. This level of polonium in the body is accompanied by about 400-500 d/min/24 hr. sample of urine. If polonium is taken in via the air alone, maximum permissible air concentration is about 1380 d/min/cu. m. for continuous exposure. If polonium is taken in via ingested water alone, a maximum permissible water content is calculated to be about 110/d/min/ml.

Influence of Route of Administration on the Distribution and Excretion of of Polonium by Rats:

Differences in the behavior of polonium in the body when it enters via the gut rather than by direct intravenous ingestion were noted in earlier work but required confirmation and extension. A summary of present information comparing the content of polonium (expressed as the per cent of body content)* after oral and intravenous administration is presented in Table I (Page 56).

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*Body content refers only to polonium absorbed from the gut in oral administration rats and does not include the large fraction of fed polonium unabsorbed and excreted as feces.

Table I

Distribution and Excretion of Polonium After Oral or Intravenous Administration to Young Rats (Approx. 20 μc/kg initial body content)

DISTRIBUTION

Tissue	Average Per Cent of Body Content per Gram of Tissue at 10 Days	
	Oral (5 rats)	Intravenous (4 rats)
Plasma	0.2	0.04
Blood Cells	12.8	1.2
Kidney	1.9	4.0
Liver	0.7	2.8
Spleen	4.0	9.0
Lymph Nodes	0.8	2.4
Bone	0.7	0.4
Bone Marrow	0.9	2.3
Lung	0.9	0.7
Testis	0.1	0.2
Adrenal	0.5	0.6
Heart	0.8	0.3
Muscle	0.06	0.1
Skin	0.1	0.2
Intestine	0.4	0.4
Residual Carcass	0.2	0.5

EXCRETION

	Average Per Cent of Body Content per Day for 10 Days	
Urine	3.22	0.14
Feces	18.94	2.09

All tissues except the plasma and blood cells are seen to contain a considerably lower proportion of the body content of polonium ten days after oral administration. On the other hand, the formed elements of the blood contain a much higher fraction of the body content after oral administration. Probably related to the higher blood content, is a more rapid initial urinary excretion rate after ingestion of this material.

Both the lower organ contents and the higher initial excretion rate of polonium administered orally versus intravenously would be reflected as an increase in the estimation of the maximum permissible oral intake of polonium since present calculations are based on distribution and excretion data from intravenous experiments. The error, if any, is therefore in the conservative direction and tends to increase the safety factor.

Proposal for future work: A serial study of distribution and excretion of polonium-bearing dusts or aerosols is planned. Improved methods not employed in earlier work must be employed. Six months is the estimated time for this work.

Pathology of Chronic Polonium Poisoning:

A recent Rochester Report, UR-42, on the pathological effects of polonium on tissues describes a kidney lesion in rats which is marked at a dosage level of 10 μc/kg but not so marked at 20 μc/kg. The lesion may be inhibited by the higher concentrations since the primary effect appears to involve proliferation of arterioler endothelial cells. This has been and is being investigated at dosage levels of 1.0, 5.0, and 10 μc/kg. These levels may be considered in the "chronic" range in that the life span of rats is not greatly shortened. Results to date confirm the production of this kidney lesion at 10 μc/kg, show that it definitely occurs at 5 μc/kg within about 90 days, and indicate that it may not be of importance at the 1 μc/kg level. These investigations are a part of a general study of pathological effects of polonium. Hematological investigations carried on concurrently show that the critical level for effects on total white cell counts lies somewhere between 1 and 5 μc/kg in the rat. (Differential blood cell counts have not yet been completely assembled.)

The renal lesion is of interest intrinsically because it apparently involves proliferative changes in arteriolar endothelium and because of its limitation to dosage levels in the "chronic" range. It is likely also to be supporting evidence for the choice of kidney as the most vulnerable organ in chronic polonium poisoning.

Proposal for future work: The present experiment is scheduled to continue until Summer 1949. It is not anticipated that investigation of tissue pathology in rats at lower dosage levels will be necessary unless the renal lesion appears this Spring at the 1 μc/kg level or unexpected changes in other organs appear. However, extension to other animal species should be considered, and the application of organ function tests is essential.

Distribution of Polonium to Tissues at Low Dosage Levels:

All animals from the "chronic" experiment described above are utilized for both pathology and determination of tissue polonium concentrations. It was desired first to determine whether or not distribution was qualitatively the same at these low dosages as at the higher dosages reported earlier. Also the trend of tissue contents with time could be determined since this experiment is scheduled for one and one-half years, much longer than previous ones.

Table II (Page 59) presents average tissue contents expressed as per cent of the body content up to 250 days after administration of a single dose. There is reasonably clear evidence of shifts with time in the relative amounts of polonium in various tissues. At longer sacrifice times after injection, a larger percentage of the remaining polonium is found in the blood cells, whereas correspondingly the organs contain a smaller percentage. This indicates a significant redistribution of the material after or because of the initial attempts of the body to eliminate it by phagocytosis and excretion.

These shifts were evident in earlier work at higher dose levels. It can be concluded that the dosage level per se does not greatly influence the relative distribution of polonium to the tissues. Since tolerance calculations were based on distributions as found for higher dose levels, this conclusion supports the validity of the calculation in this respect.

Proposals for future work: It is planned to continue the present experiment and to extend it to other animal species if circumstances permit. The mechanism of the polonium shift to blood cells requires further investigation.

Therapeutic Measures for Polonium Poisoning:

Previous work in this laboratory has shown that BAL (British Anti-Lewisite, a simple sulfhydryl compound) injected intramuscularly in rats about doubles the excretion of intravenously administered polonium during the first ten days. A minor extension of this work was performed during 1948, in that the action of BAL glucoside was tested in rats and was found to be less effective than BAL in promoting excretion of polonium.

Proposal for future work:

(1) Influence of phlorizin and alloxan diabetis, diuresis, etc. in hastening excretion.

(2) Effects of beet pectin, apple pectin and related substances which have been demonstrated to be of use in mobilizing selenium and other metals related to polonium.

Table II

Polonium Content of Rat Tissues at Vatious Times

After Intravenous Administration of 1-10 μc/Kg*

Tissue	Average Content as Per Cent of Body Content per Gram of Tissue						
	Time after Injection						
	21 days	42 days	59 days	90 days	160 days	220 days	250 days
Plasma	0.09	0.01	0.03	0.16	0.15	0.07	0.12
Blood Cells	1.20	2.41	3.07	7.00	7.69	12.22	7.75
Kidney	8.36	5.74	5.31	5.11	2.05	1.32	1.58
Liver	2.65	0.76	0.52	0.80	0.62	0.38	0.45
Spleen	12.53	8.16	6.87	5.26	2.18	2.57	3.27
Lymph Nodes	2.97	2.58	2.34	2.09	1.01	0.12	0.67
Bone	--	0.50	0.24	0.28	0.49	0.18	0.21
Bone Marrow	1.60	1.35		0.92	0.92	1.15	0.50
Lung	1.46	1.00	1.38	0.92	0.90	0.73	0.79
Testis	--	0.46	0.94	0.82	0.52	0.55	0.72
Adrenal	(0.90)	0.84	0.76	0.91	0.69	0.82	0.72
Heart	0.68	0.36	0.51	0.52	0.68	0.82	0.80
Muscle	0.20	0.17	0.10	0.18	0.62	0.10	0.11
Ovary	(1.26)	--	--	0.71	0.33	--	--
Intestine	4.71	0.65	0.51	0.63	1.06	0.20	0.25
Residual Carcass	0.32	0.24	0.22	0.30	0.20	0.16	0.20

* Some variations of distribution between dosage levels were seen, but these have been neglected here for presentation of the overall picture.

Problem Code: R.M.1 (Polonium)

Section Code: 3171

The Presence of Polonium Aggregates in the Blood:

Background: In devising therapeutic measures, as well as in calculations of maximal permissible dose, the distribution of a toxic material (specifically of polonium in this case) among the body tissues is of utmost importance. Preliminary experiments suggested that the physical form as well as the chemical properties of polonium might be concerned with determining this distribution. Autoradiographic studies were made to investigate this possibility.

Work done during calendar year 1948: The autoradiographs showed that large polonium aggregates (hundreds of thousands of atoms) may be found in body tissues. After intravenous injection of 16 microcuries of polonium per kilogram body weight to a rat, the polonium aggregates in the blood were detected up to 16 hours. No aggregates were discovered in the samples taken at 24 and 32 hours. Aggregates were again found in the 48 hour sample. There were no aggregates found in the feces.

If 500 μc/kilogram were given by stomach tube*, no aggregates were found in the blood but did appear in the feces.

This data suggests that in the preliminary intravenous experiment, the aggregates are at least in part removed from the blood by the phagocytic cells since some aggregates are found in the reticuloendothelial organs. Since it is not known where the aggregates originate, in the original solution of polonium chloride or immediately after injection, a technique has been developed for observing aggregates in solution. However, we have not yet proved that the technique does not produce the solution aggregates observed.

Proposals for future work: Further experiments are proposed to substantiate the preliminary findings reported above.

These experiments will be done by counting the tracks in each of a large number of aggregates in each of a large number of blood smears from two minutes to nine days and estimating the number of polonium atoms present in each aggregate. This should give us a relative measure of size and we hope to find some method of determining the actual dimensions of the particles. This information, if it can also be obtained for other body tissues will be very helpful in calculations of maximum permitted dose of polonium.

The autoradiographic technique can also obviously be extended to the study of radium, neptunium, thorium and several fission products, all reported to contain "radiocolloids".

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*The amount absorbed from a 500 μc/kilogram dose given orally is roughly comparable to the 16 μc/kilogram intravenous dose.

In addition to the autoradiographic study of polonium aggregates and the aggregates of other metals, other methods such as dialysis, ultrafiltration, etc. will be used to supplement the results from autoradiography.

Problem Code: R.M.4 (Miscellaneous Project Metals)

Section Code: 3110

The Charcoal Adsorption Method for Measurement of Radon in the Breath:

Background: Since the refining of uranium requires processing of radium containing ores, and since a body content of radium in amounts as small as 0.9 μg has been reported to cause crippling bone changes in an exposed individual, it is of considerable importance to have available a method of measuring the accumulation of radium by plant employees.

The measurement of radium as contained in the body is most readily performed by collecting and analyzing the breath for radon, a disintegration product of radium. The procedure currently used is to collect the expired breath in a one liter bulb and to measure the radon by counting its alpha activity in a gas chamber.

This procedure is technically exacting on two scores. In the first place, care must be taken in collection of the sample so that it is a representative sample of expired air and so that it is free from contamination by ore dust.

The second difficulty is concerned with the measurement of the radon contained in the sample. Present practice is to set .1 μg of radium as the maximum permissible body content and one micro-microcurie of radon per liter of expired air as the equivalent maximum permissible breath level. The gas chamber alpha counters currently used to make these radon measurements in this laboratory are similar in design to those in general use. The background is about 0.3 count per minute. It is found that the net sample count obtained from one micro-microcurie of radon (after about two hours in the chamber) is 2.6 counts per minute. It is obvious that under these circumstances long counts are required to obtain a result free from large errors due to randomness alone.* This restriction applies particularly to measurements of fractional maximal permissible dose levels.

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*If the radon is measured in equilibrium with its short half-life alpha-particle emitting daughter products Radium A and Radium C', 1 micro-microcurie of radon is associated with 6.6 alpha disintegrations per minute. When measured in an alpha counter gas chamber, the maximum number of counts one would expect to obtain would be about 4.4 counts per minute. This comes about since Radium A and Radium C particles acquire a positive charge and are deposited on the surface of the metallic cathode within a time short in comparison with their half-lives. Thus, one might roughly expect to count all the disintegrations from radon gas but only one-half of the disintegrations

(continued on page 62)

As a consequence of these difficulties in the measurement, radium body content determinations have generally proved to be erratic and unreliable. It has been recognized for some time that radon could be concentrated from the air by two methods: (1) adsorption on activated charcoal, (2) condensation in a liquid air trap. Since concentration of radon from the breath seemed a reasonable approach to development of a better method for measurement of radium, we have, for the past two years, been exploring such a procedure. The charcoal adsorption method recommended itself over liquid air condensation as being adaptable to simpler field manipulations and transportation of the sample to a central analysis facilities, and it is this method that we have studied.

Our work during the calendar year 1947, may be briefly characterized as a study of the following variables as related to the efficiency of collection and release of radon; the amount of activated charcoal required, its grain size, appropriate containing vessels, velocity of gas flow, necessary temperature, and time for release. As a result of these studies, a method was worked out which would permit concentration of radon from about 30 liters of breath with the radon gas at tolerance level and passing through the charcoal at normal breathing rates. The subsequent release by heating and measurement of radon gave recoveries from 95 to 102 per cent for twenty runs.

Work done during the calendar year 1948: During the calendar year 1948, we have modified and further developed the method in order to make it applicable for measurements in the field. This work began with the modification of an industrially produced face mask to accept a charcoal cartridge of suitable design for our purpose. Further calibration studies were carried out with the new cartridge. Several workers exposed to radium were brought to Rochester and a series of measurements were carried out with results satisfactorily reproducible, for example, the results of a set of measurements made on one of these individuals was 13 ± 4 per cent tolerance. A field trip was made to a radium refinery where parallel air bulbs and charcoal cartridge samples were taken on six different individuals with different degrees of exposure in their employment record. The checks obtained in the measurement by the two methods were unsatisfactory due, we believe, both to the inherent inaccuracy in the air bulb sampling technique, and to contamination of the charcoal cartridges by standing. During the last few months of this year, we have been concerned with improving the charcoal cartridge method by devising and testing hermetically sealed storage containers and with the construction of a duplicate of our present alpha counting equipment.

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from Radium A and Radium C'. In practice the chamber voltage gradient and the pulse height discriminator may be set so as to reject pulses below a certain amplitude in order to discriminate against background counts originating from the chamber walls. An alpha chamber so adjusted is operated in this laboratory with a background of about 0.3 count per minute and a net count of about 2.6 counts per minute per micro-microcurie of radon in equilibrium with Radium A and Radium C'. Therefore, at 20 per cent of maximum permissible breath radon level and a one liter sample of expired air, this chamber would count about .5 count per minute net. To establish this result plus or minus a relative deviation of about 10 per cent requires that background and sample be counted for somewhat over six hours.

Proposals for future work: We are proposing to take two or three more field trips to test further the method from a point of view of reproducibility on the same individual and general agreement with results obtained with breath bulbs (using special precautions to get a representative sample of expired air). It is possible that for highest accuracy, we may have to construct a dry box in order to manipulate the charcoal in a radon-free atmosphere.

It is anticipated that this part of the program should be completed about July 1, 1949.

The next phase of the program should be periodic breath radon measurements on about twelve selected individuals. This might be carried over a period of a year with tests every two months. It may be that these tests should be supplemented where possible with gamma activity measurements on the individuals concerned. During this time, periodic standardization and blank runs will be scheduled. Depending on the progress made, it might appear advisable to ship some cartridges out to the health officer at a suitable plant with instructions as to the method of collecting charcoal samples and compare the results of such a test with samples taken on the same individuals by a crew sent out to the plant from our group.

Measurement of the Radium Content of the Bodies of Individuals Without Known Exposure:

Background: As a result of a study of injuries sustained by dial painters and other occupationally exposed individuals for whom the radium body content was measured, a maximum permissible level of 1×10^{-7} grams of radium per whole body has been commonly accepted. This level may include a certain margin of safety since in a group of patients studied by Drs. Aub, Martland, and Evans, the lowest body content retained in an individual showing definite bone changes was 9×10^{-7} grams of radium. On the other hand, in this group no individual having a body content of 5×10^{-7} grams of radium or less showed clinical symptoms.

To contrast with this picture of the radium body content of exposed workers, it is interesting to note the findings of A. Krebs who measured the radium body content of eighteen individuals who died with no known exposure during their lifetime. He found body contents ranging from less than 1×10^{-9} to 4×10^{-8} grams of radium, with an average of 1.4×10^{-8} grams.

If these findings have a general application, the margin between the level that may be regarded as "safe", for example, 1×10^{-7} grams and the radium load carried by some "unexposed" individuals, 4×10^{-8} grams, is uncomfortably narrow. Since the exposed group was tested only for gross symptoms, it might well be that at or below the "safe" level there would occur more insidious effects, such as a shortening of life span of genetic aberrations. For these reasons, it was decided to check Krebs' findings on such crematory material as we could secure from local hospitals.

Work done during the calendar year 1948: This problem was started at midyear and so far we have secured known aliquots of the body ashes of seven individuals. The material is in various stages of preparation for measurement. Two samples have been measured and checked. The results were respectively, 3.6×10^{-10} grams of radium per whole body. If the findings of subsequent analyses confirm this order of magnitude (about 1/40 of Krebs' average value

PROGRAM U.

URANIUM

The emphasis in establishing safety standards for uranium exposure is clearly shifting in the direction of radiation effects. Before undertaking a long-time inhalation exposure study designed to explore the importance of radiation in uranium exposures, a great deal of basic information must be obtained to enable us to set up the critical conditions of exposure. Several of the factors that determine the toxicity of insoluble uranium dusts have been currently under investigation.

Problem Code: U.1 (Physical and Chemical Properties)

Section Code: 3220

Uranyl-Citrate Complex:

Because of the importance of the uranyl-citrate complex in the development of "tolerance" to injected uranium, a series of chemical investigations on the nature of this complex was conducted.

Titration studies indicated that uranium combines with dicarboxylic acids to form stable ring structures containing five, six and seven members. In addition, alpha hydroxy acids apparently form ring structures with uranium through the ionization of the hydroxyl group. Thus, in uranyl-citrate a double ring structure is formed utilizing two carboxyl groups and the one hydroxyl group of the citric acid molecule. Evidence was also obtained which would indicate that the complex exists to a large extent as a dimer, particularly at elevated pH. Polarographic studies indicated that the dissociation of uranyl citrate to free uranyl ion was extremely low and confirmed the concept of dimerization.

The results of these chemical studies would indicate that uranyl-citrate may be assigned an important role in the detoxication of uranium.

Problem Code: U.3 (Toxic Limits)

Section Code: 3210

Investigations on the General Problem of the Effect of Particle Size of Dusts on their Toxicity:

The important hygiene problem of what is the relation of the size of an airborne dust to its toxic potentialities is one that has only recently become capable of precise experimental solution. This is chiefly because

only recently suitable sampling devices, notably the Cascade Impactor and oscillating thermal precipitator, have become available. These instruments permit non-selective sampling of dusts and fumes of wide particle size distribution range. Attack on the problem of the relation of particle size to toxicity was made through the use of uranium dusts. The dust was so selected that one, UO_2, represented a highly insoluble substance to which considerable exposure is expected among Atomic Energy Commission personnel; the second, UO_3 compound, although highly insoluble in water is relatively soluble in body fluids. These two compounds were selected because it appeared reasonable that entirely different effects might be obtained on the basis of their differences in solubility. Studies on these two substances are nearing completion, but to complete the investigation, tests of a still more soluble compound, UO_2F_2, is contemplated.

Two particle size ranges were tested, one below 1 micron and the other above this limit, because previous experimental work had shown that distinctly different effects were observed above and below this value. Special methods were required for the preparation of suspensions of both UO_2 and UO_3 dusts. The two size fractions showed a small amount of overlapping; 13-15 per cent of the particles above 1 μ were in the 2 μ fraction. Rabbits and rats were used in the test because past experience had shown the former to be a species highly sensitive to uranium poisoning. The rat was used because although more sensitive, this species had the advantage of a more uniform response to exposure. The use of both species thus provided a range of potential responses that considerably facilitated the solution of the experimental problem. In separate studies, the animals were exposed to particles of each dust dispersed in an exposure unit as an aqueous aerosol so attenuated that essentially dry dust resulted. Air samples in the chamber taken during exposure by the filter paper dust sampler for concentration and the Cascade Impactor for particle size showed variations in any day's run to be within the size limits given above. Exposures were made for 6 hours daily, 5 days a week for approximately one month during which time the animals' daily responses in weight, urinary protein and amino nitrogen and semi-weekly responses in blood NPN and urea nitrogen concentration as evidence of uranium injury were measured and compared with their pre-exposure control values as well as with similar values from suitable groups of control animals.

UO_2 Dust Particles: A series of four studies with UO_2 dust were performed (Table I - Page 67). The results of this series were most enlightening. Two facts of outstanding importance in the control of industrial dust were found: (1) the toxicity of an insoluble uranium dust is related to its particle size; particles below 1 μ are more toxic than are those above 1 μ, and (2) uranium injury is almost entirely a function of the mass of the inhaled particles below 1 μ in size and little, if at all, related to the total concentration of all sizes to which the animals were exposed.

The effects appear to be related to the greater amount of deposition in the lung. Table I shows that the deposition of uranium from the dust of 0.5 μ mass-median size was of the order of 10 times as great as that from dust of approximately 2 μ at the same exposure concentration (columns II and IV). On the other hand, tissue analyses shown in Table I do not indicate any correlation between particle size and the amount of uranium in the femur

Table I

Deposition of UO_2 in Tissues of Rabbits and Rats in Micrograms of Uranium per Gram of Wet Tissue

		I	II	III	IV
Mass-Median Particle Size, μ		0.5	0.5	1.0	2.0
Exposure Concentration (all sizes) mg/m³		22	80	80	80
Concentration of sizes below 1 μ (approx.) mg/m³		19	68	30	14
Exposure Hours		140	191	191	192
LUNG:	Rabbit	76	2,180	373	46
	Rat	305	1,233	510	157
KIDNEY:	Rabbit	1.0	1.2	2.6	1.1
	Rat	2.0	3.0	3.2	1.2
FEMUR:	Rabbit	1.8	2.2	4.8	2.7
	Rat	1.2	4.0	3.1	1.9

or kidney of either species. It is believed the greater toxicity with the smaller particle size is related to the higher reactivity of the smaller particle size.

UO_3 Dust Particles: Similar studies performed on hydrated UO_3 aerosol suspension, a compound differing markedly from UO_2 in its being soluble in body fluids, showed no such particle size effects. Results of studies with particles of mass-median size of 0.5 U and 1.8 U, which are still incomplete, indicate that not only did the smaller particles not show greater toxicity but they showed less than the larger particles. Among all criteria except weight loss, larger particle size produced greater mortality, higher elevation of blood NPN and urea nitrogen and urinary protein than did the smaller sizes. Whether this will be found due to increased retention of the larger particle sizes or to differences in particle surface remains to be shown.

Thus, has been brought to light a clear distinction between the toxic effects of particles soluble or insoluble in body fluids; particles below 1 μ augment toxicity only if the uranium dust is highly insoluble in body fluids.

Problem Code: U.4 (Fate)

Section Code: 3210

Retention and Distribution of Uranium Dusts in Animals:

Further insight into the problem of the effect of particle size and concentration on retention and distribution of uranium dusts was afforded in a special approach to the problem wherein an especially designed apparatus permitted intensive study of dust retention in single animals. The problem under investigation was to determine the amount of uranium dust retained for short periods (5 minutes) and to learn how the amount of inhaled particulate matter is modified by dusty atmospheres of known characteristics, such as concentration and particle size. Little information is available in this field owing to the difficulty of preparing, dispersing and measuring dusts of graded particle size.

Pulmonary retention was determined in rabbits by exposing them individually to UO_2 particles suspended in air at mass-median particle sizes of 1, 0.5, and 0.2 μ and at dust concentrations ranging from 10-250 mg/m^3. An apparatus, fitted with a system of electronic controls, has been constructed in which a rabbit breathes dusty air in a face-piece. The valves are operated in such a way that the exhaled air is drawn off through a filter paper sampler at a regulated rate to maintain the air pressure within the face-piece essentially equal to that of the atmosphere. A duplicate line continuously samples the same volume as the atmosphere is being inhaled, permitting calculation of the quantity of dust retained by the rabbit.

Retention: Five findings of particular applicability to the industrial dust problems of the Atomic Energy Commission installations were made:

(1) Uranium dioxide dust was retained by rabbits at a rate of 10 μg per minute per kg. body weight at a concentration of 100 mg/m³. This figure was of particular usefulness in calculating the amount of retained dust from an atmosphere of known concentration.

(2) The retention of uranium dioxide is directly proportional to the volume of air breathed by the animal and is independent of the rate of breathing.

(3) Retention varies as the 3/2 power of the concentration over the range from 10 to 250 mg/m³.

(4) There is no significant difference in the retention of uranium dioxide particles 1, 0.5, and 0.2 μ in diameter.

(5) Variations in respiratory volume may be equivalent to a five-fold variation in effective concentration and variation between individual animals may be equivalent to a two-fold variation in effective concentration.

Distribution: Pilot experiments made by injection of uranium dioxide indicate significant differences occur in the rate at which the three sizes are eliminated from the lung. Approximately 50 per cent of the larger sizes disappear from the lungs of rats in from 1 to 3 days following the administration of doses of UO_2 of from 20-30 mg/kg; on the other hand, 50 per cent of the dose of smaller sizes (0.2 to 0.3 μ) may remain in the lung after from 3 to 4 weeks. Evidence is accumulating that this early, rapid removal of uranium from the lung is a mechanical process by which particulate uranium moves from the lung to the gastro-intestinal tract. This hypothesis is being tested in rabbits exposed by inhalation and utilizing the retention apparatus above described as a means of exposure. Limited data from one series of 12 rabbits tends to confirm this hypothesis.

Problem Code: U.5 (Mechanism of Toxic Effect)

Section Code: 3220, 3260

Mechanism of Uranium Poisoning:

The mechanism of uranium poisoning has been studied in two complimentary programs -- one having to do with the deposition of uranium in bone and the other having to do with the metabolic effects of uranium.

Uranium in Bone: From the long-term point of view, the only site of significant storage of uranium is the skeletal system. Accordingly, a series of investigations was conducted to determine the mechanism of the deposition of uranium and the factors controlling this process.

The evidence would indicate that uranyl ion exchanges for calcium in the surfaces of the bone mineral substance. The resultant bone: uranium combination apparently involves a linkage between the two, adjacent phosphate groups and each uranyl ion. The dissociation of this complex is extremely low.

Animal studies indicated an unequal distribution throughout the skeleton, with highest concentrations of uranium appearing in those areas exhibiting the most active calcification. Acid and alkaline diets did not affect, and a rachitogenic (low phosphorus) diet increased slightly the rate of mobilization of uranium from the skeleton. The biological half-life of skeletal uranium in the young rat was estimated to be of the order of two months.

Although a great deal of information is already at hand, some further work is required to clarify the problem of the radiation hazard resulting from uranium deposited in bone. These studies should be aimed at the determination of the maximum accumulation of uranium in bone at various levels of daily exposure to uranium, preferably by inhalation.

Metabolic Effects of Uranium: Much of the work of the Physiology Section (3260) was devoted to elucidating the mechanism of action of uranium on living cells. The primary sites of action of uranium in animals are the cells of the proximal renal tubule. Because of serious technical difficulties, the types of information that may be obtained concerning the action of uranium on kidney cells are limited to renal function and to histopathological studies. The yeast cell, on the other hand, is an excellent biological system offering many technical advantages. It has therefore been used to investigate in detail the characteristic effects of uranium on living cells.

Uranium has been found to almost completely inhibit the utilization of hexoses such as glucose, fructose, and galactose by any route, glycolytic or respiratory. But uranium did not inhibit the utilization of stored glycogen, the synthesis of glycogen from alcohol, or the aerobic metabolism of alcohol and acetate. Uranium must be acting on metabolic steps which are unique to the hexoses and not concerned with glycogen, alcohol or acetate metabolism. Accordingly, only the early steps in metabolism of sugar can be involved.

By several techniques, it has been possible to show conclusively that uranium inhibits sugar metabolism by combingin with certain groups on the surface of the cell which are concerned with the initial steps in sugar metabolism. The dissociation constant of the uranium complex with cell surface groups hasbeen compared with the complex which uranium forms with a wide variety of substances. The constant for the uranium hexametaphosphate complex most closely approximates that for the yeast-uranium complex. The constants for polyphosphates, both organic and inorganic, are about 10 to 20 times as high. Those for protein carboxyl groups, dicarboxyllic acids and ester phosphates (including nucleic acids) are 100 to 6000 times as high. Polyphosphates, such as adenosine triphosphate are known to play an important role in metabolism. Recently it has also been shown that polymetaphosphates are also involved in an as yet unknown manner.

The cell surface groups complex not only with UO_2^{++}, but also with other bivalent cations, such as Mg^{++}, Ca^{++}, and Ba^{++}.

The total number of cell surface groups per yeast cell is about 5×10^{-7}. These groups form a reversible one-to-one complex with uranium with a dissociation constant of about 3 to 5×10^{-7}. The rate of metabolism is directly proportional to the number of freegroups. If half of the groups are inactivated, the inhibition is 75 per cent, etc.

Further information concerning the relationship of cell groups to metabolism is supplied by kinetic studies and by studies of temperature characteristics of metabolism in the presence and absence of uranium. These studies are as yet incomplete.

Although the mechanism of action of uranium has not asyet been completely and unequivocally defined, a hypothesis can be presented which seems best to fit the available facts. At the present time, it is not even possible to say that this is the only reasonable hypothesis. Basic to the present hypothesis is the assumption that the initial metabolic reactions between the yeast cell and external substrate (sugar) take place at the cell surface. These intital reactions are considered to function in the active transport of the substrate into the interior of the cell and are possibly analogous to the mechanisms of resorption of sugars from the gut and from the kidney tubule. The active transport mechanism is probably not part of the classic Embden-Meyerhof schemes. Uranium blocks the sugar transport mechanism by complexing with polyphosphate groups on the cell surface which are a necessary part of the enzyme systems involved. It may either prevent transphosphorylation or offer a steric hinderance. A similar general mechanism could explain the action of uranium on the proximal tubule.

In addition to the direct studies on uranium, a number of subsidiary studies have been made. The existence of other enzymes on the cell surface of yeast has been definitely demonstrated, namely, the phosphatases and sucrase. In the case of the phosphatases, a large variety of compounds can be split, including adenosine triphosphate, inorganic polyphosphates and various ester phosphates, such as sugar phosphates. These phosphatases can be competitively inhibited by low concentrations of molybdate or tungstate, although glucose metabolism is not thereby affected. The function of the surface phosphatases seems to be the splitting of phosphate compounds which cannot be metabolized directly, yielding organic residues which can be metabolized. For example, sugar phosphates cannot be metabolized directly, but after the phosphate group has been split off, the glucose residue can be utilized.

The presence of sucrase on the cell surface was demonstrated by using uranyl nitrate to inhibit hexose metabolism. Uranium-poisoned cells can split sucrose into glucose and fructose, and these products can be quantitatively recovered in the supernatant. If the sucrase were inside the cell, this would not be so.

In connection with studies of the effects of uranium on sugar metabolism, a program has been undertaken to utilize the technique of paper chromatography to separate and identify phosphorylated intermediates of metabolism. It is now possible to separate and visualize a number of compounds, such as phosphorylated sugars, glycerophosphate, pyrophosphate, adenosine triphosphate, adenylic acid, and orthophosphate. The technique is not as yet perfected to the state where tissue extracts can be completely analyzed.

Problem Code: U.6 (Methods of Detection of Poisoning, Prophylaxis, Treatment, and Protection)

Section Code: 3260

Sensitive Tests of Uranium Poisoning:

The ratio of amino-acid nitrogen to creatinine (AAN/C ratio) in the urine has been established as a simple, sensitive test for kidney dysfunction resulting from uranium poisoning. The test requires only a spot sample of urine. The minimal intravenous dose of uranium necessary to give a response is 0.02 mg/kg in rabbits. The time course of the response of the AAN/C ratio varies with the amount of uranium given and with the number of exposures. Generally there is a peak response in 2 to 4 days followed by a return to lower but still abnormal values. After a damaging dose of uranium the ratio does not return to normal even after several months.

PROGRAM Be.

BERYLLIUM

Beryllium poisoning in its two forms, specifically, acute pneumonitis and chronic granulomatosis, have in common a primary disability of the lung. Consequently, a large proportion of the efforts so far has been directed toward inhalation toxicity studies. Beryllium sulfate was first examined.

Problem Code: Be.1 (Physical and Chemical Properties)

Section Code: 3210

Beryllium Content of Coal:

Samples of soft coal from a nearby power plant were analyzed for beryllium content after air samples taken some distance from the project's scrubbed, effluent gas stack revealed no decrease in beryllium concentration in the air with increasing distances from the project building. Two samples of coal were analyzed, one on October 4, 1948, and the other on December 2, 1948. The coal was obtained from a Pennsylvania mine in the Westville No. 2 Freeport seam. Spectrographic analysis on the first sample showed 1.3 μg Be/g of coal. Analysis of the second sample showed:

Coal	4.3 μg Be/g
Ash	25 μg Be/g
Average Ash	12%

Therefore, for every gram of coal, approximately 1 μg of beryllium would appear to be liberated into the air from the stack. As 12 to 70 tons of coal are burned per day, depending upon weather conditions, this corresponds to the liberation of from 10 to 60 grams of beryllium per day. If these figures can be used as a guide for estimating the beryllium output of other soft coals, a figure of 25 per cent of the analyzed beryllium content of the coal apparently escapes in stack gases.

Problem Code: Be.1 (Physical and Chemical Properties)

Section Code: 3220

Physical Chemistry of Beryllium:

To obtain information on the chemical behavior of beryllium in the animal body, the solution chemistry of beryllium under physiological conditions has been studied.

It has been demonstrated that beryllium exists only to a negligible extent as a free ion at pH 7. Rather, the metal either precipitates as a hydroxide or forms soluble complexes with various anions. Of the many types of compounds studied, only complex hydroxy acids and polyphosphate compounds appear to be effective complexers. Thus far, only the reaction between beryllium and citrate (as an example of a complex hydroxy acid) has been studied. In the presence of an excess of citrate, beryllium is completely soluble and ultrafiltrable. When beryllium is present in excess, a stable colloid is formed (presumably a complex hydroxide) which, though apparently soluble, will not pass an ultrafilter. Polarographic studies have been conducted to determine the dissociation constant of the soluble beryllium citrate complex.

From the above, it is clear that the major constituents of body fluids have but little affinity for beryllium. We might predict, therefore, that the solubility of beryllium in tissue fluids would be very low and, following intravenous injection, it would be likely that colloidal hydroxide would be formed. Animal experiments with Be7 to date have confirmed these predictions.

Analytical Chemistry of Beryllium:

Although the spectrographic method for the determination of beryllium is adequate for many typesof experiment, the need for a precise analytical method is still urgent. A whole series of color reactions has been investigated in detail and, as a result, we have at hand six quantitative procedures for the estimation of beryllium in amounts from 0.2 to 30 μg with a precision varying from 3 to 15 per cent standard error. Each of these procedures has special advantages and disadvantages. They all have one shortcoming in common, i.e., they are all subject to interference by moderate quantities of most inorganic substances.

A large part of our effort, therefore, has been directed to the study of procedures for the isolation of beryllium free of interfering materials. Although some measure of success has been attained, to date, the methods are too time consuming and tedious to be applicable to routine use.

Problem Code: Be.1 (Physical and Chemical Properties

Section Code: 3320

Beryllium Oxide Crystals:

X-ray diffraction studies have been made of beryllium oxides as well as beryllium fumes produced under certain special conditions. Such studies indicate a difference in crystal patterns with beryllium oxides produced by calcining at different heats. It is shown that heat treatment increases the crystal size as well asreduces the impurity of the compound. Such results correlate well with results of electron microscopy and measurement of particle size and area by physical methods. A complete analysis may be found in a future Rochester Quarterly Technical Report.

Problem Code: Be.2 (Toxic Effects)

Section Code: 3250

Pathologic Anatomy of Beryllium Poisoning:

This work has been done in collaboration with the Industrial Hygiene Section and consisted of examination of animals sacrificed or dying after exposure of the animals to different beryllium compounds. The lesions observed have been limited largely to the lungs. Following inhalation of the soluble beryllium compounds, the pulmonary lesions of many species consist of an exudate made up of edema, phagocytes, lymphocytes and a few neutrophiles. Interstitial inflammation and fibrosis were observed. The extent and severity of the exudate varied with the dosage, period of inhalation and species. These pulmonary lesions in many respects resemble those occurring in the acute beryllium pneumonitis of humans. The insoluble beryllium compounds have produced no lesions of significance.

Hematology of Beryllium Poisoning:

When small amounts of beryllium sulphate are injected intravenously, the number of circulating platelets is increased and the coagulation and prothrombin times of the blood are decreased. Studies are being carried out on the effects of other metals on blood coagulation, and on the mechanism of the hypercoagulability of the blood.

Problem Code: Be.2 (Toxic Effects)

Section Code: 3260

Effect on Cellular Metabolism:

The effect of beryllium on isolated cells has been studied on a small scale. Beryllium in low concentrations has no effect on metabolism of yeast or on growth of E. coli. In relatively high concentrations it inhibits metabolism and growth only slightly. The only specific action of beryllium seems to be that on the cell-surface phosphatases of yeast, which are inhibited. Beryllium has no marked, reproducible effects on frog and turtle heart preparations. It does not seem to compete with Ca^{++} in this system.

None of the effects of beryllium on isolated cells and tissues seem to shed any light on the mechanism of action of this substance on the human lung.

The development of techniques for evaluating beryllium damage to the lungs, and for characterizing beryllium toxicity in physiological terms is of prime importance. With this objective in mind, a program of study was recently

initiated. The first efforts of this program have been directed toward developing techniques for continuous measurements of O_2 tensions both in expired air and in blood. The most promising method seems to be the electro-reduction of dissolved O_2 at a micro-platinum or other electrode under certain fixed conditions. Considerable progress has been made in adapting the method to the desired purposes, but a number of problems still must be solved before it can be used on a practical scale.

Problem Code: Be.3 (Toxic Limits)

Section Code: 3210

The Character of the Acute Response in Animals Inhaling $BeSO_4$:

Cases of acute and chronic toxicity, often ending fatally, have recently been ascribed to exposure to compounds involved in the production of beryllium. No unequivocal proof existed in the literature that beryllium is the etiologic agent, or that beryllium per se is toxic, for reports exist imputing the toxicity of beryllium compounds of the acidity of their anions. The situation obviously required immediate re-examination, and the truth of existing reports confirmed or denied.

Accordingly the acute toxicity from inhalation of beryllium sulfate mist in animals was determined in large-scale experiments wherein the toxic limits of exposure and the characterization of the acute response were established. Beryllium sulfate was chosen because it represented a typical soluble beryllium compound from which plant medical histories of injurious exposure were known; a mist was employed because of close simulation to the state in which the sulfate occurred in neutralizing and centrifuging operations in the beryllium plants. A series of 4 concentrations (100, 50, 10, and 1 mg $BeSO_4 \cdot 6H_2O$ per m^3 of air) were tested in daily six-hour exposures for periods of from 14 to 105 days. Particle size of the mist was from 0.5 to 1.3 μ.

Marked species variation in response was noted. At levels of 100 and 50 mg/m^3, 80-100 per cent mortality occurred during weeks 1-6 in the cat, dog, rat, goat, and monkey, 60 per cent (weeks 1-2) in the guinea pig, 10-50 per cent (weeks 2-5) in the rabbit, mouse and hamster; all swine and fowl survived. Overall mortality chiefly confined to the rat, at 10 mg/m^3 was 14 per cent; at 1 mg (0.04 mg Be/m^3) no mortality was observed in any species and weight losses were recovered at the end of 90 days exposure although certain evidences of poisoning were still present.

The monkey, dog, cat, and rabbit showed acute pulmonary inflammatory response, resembling that in man; the rat gave an atypical response. Effects that were severe and rapid in onset at the 100 and 50 mg levels were minimal and slower in developing at the 10 mg level changing to moderate with continued exposure and, at the 1 mg level, were nonexistent in certain species at the end of 105 days exposure. The rabbit showed two consistent pictures of progressive injury with increasing exposure. Highest levels produced severe

proteinuria and changes in blood protein values, not apparent at lower levels. At the lowest level, reduced blood oxygen tension, alteration in blood cell lipid ratios and possibly serum alkaline phosphatase give promise of suitability as diagnostic aids in early or mild forms of beryllium poisoning. A hypochromic macrocytic anemia appeared in the dogs. Beryllium concentrations in 20 tissues were determined and showed on the 10 mg level that the tissues containing significant deposition in the rat were in decreasing order: lung, pulmonary lymph nodes, liver, tooth, kidney, femur. Other tissues showed insignificant accumulation at the end of 95 days. Among species, rat lung contained the most, that of the guinea pig next, the dog least, (11, 6, 4 μg/g respectively).

From these studies, three facts of importance have been noted: (1) beryllium is unquestionably a toxic element. The response to dosage progressed in a manner typical of such agents with the production of the typical acute lesion. Moreover, a control study with $NaHSO_4$ that simulated conditions of acidity (pH 1.5), concentration (100 mg/m^3), particle size (1 μ), and duration of exposure (14 days) of beryllium sulfate failed to produce lesions in animals similar to those seen in beryllium exposures. (2) To date, although the acute phase is similar, the typical picture of chronic pulmonary granulomatosis seen in humans has not been reproduced in animals from inhalation of the soluble beryllium sulfate despite continued daily exposure for periods of 3.5 months. (3) Certain species, notably the guinea pig and goat, appeared to possess the capacity to recover from the acute phase of poisoning in the face of continued daily exposure to concentrations of the beryllium mist that initiated the response, a finding not reported to occur in man.

On the Correlation between Toxicity by Inhalation and the Physical Properties of Beryllium Oxide:

A suggestion from Mr. Eisenbud of the New York Operations Office that one special beryllium oxide distinguished from others by a lower temperature of calcining was responsible for the human cases, led to an intensive examination of physical, chemical and toxicological properties of a series of beryllium oxides.

Early in the program the need for the selection for experimental test of beryllium compounds implicated in producing toxic exposures in plant personnel came face to face with the perplexing situation that individuals exposed to beryllium oxide in one plant did not develop beryllium poisoning whereas at another plant the same compound appeared to be a distinct health hazard. Indeed, survey experimentation confirmed the innocuousness of the oxide in the first instance in animals. Accordingly, beryllium oxide was freed of implication in the toxic exposures and was disregarded as a compound with potential toxicity. Continued report of toxic exposures in the second plant that pointed unmistakably to the oxide could not be set aside and further investigation suggested that different methods of preparation may be at the basis of the differences in the responses at both plants. This hypothesis was tested in this study that involved the combined techniques of physical chemistry, electron microscopy, x-ray diffraction analysis, surface area, density and porosity measurements and the inhalation exposure of animals. As a result of

these studies, a plausible interpretation of the previously confusing situation is that the toxicity of inhaled BeO appears to depend upon the physical properties of the material. The processes currently employed in the production of BeO involve calcining a beryllium salt at high temperatures. Differences in the physical properties of the product are the result of differences in the nature of the salt used as a starting material and in the temperature and duration of calcining.

Dogs, guinea pigs, rabbits and rats have been exposed 6 hours daily for 12-15 days to an atmosphere containing approximately 85 mg/m³ of BeO aerosol. Even at this relatively high concentration, one grade of BeO appeared to be virtually non-toxic. This material was fired at 1350° C and was composed of unit particles having an arithmetic mean diameter of about 0.7 μ, with aggregates of low porosity (5.2 per cent). A second grade of BeO gave definite evidence of toxicity. This material was fired at 1100° C and comprised unit particles of about 0.5 μ diameter, with aggregates of markedly greater porosity (35 per cent. Two of 20 rats died during the exposure period, while 3 of 5 rats, held for observation following exposure, showed a marked increase in their leukocyte count due to an absolute increase of neutrophils and lymphocytes. One of 4 dogs showed a transient low blood exygen tension following exposure, and the lungs of this animal, which was sacrificed terminally, exhibited extensive lesions. Dogs and rats exposed to an aerosol of BeO produced by calcining $Be(NO_3)_2$ at 400° C exhibited weight loss during exposure, 1 of the 2 dogs showed a significant reduction in blood oxygen tension, and 8 of 40 rats died. This material was made up of unit particles of about 0.4 μ mean diameter and aggregates of very high porosity (134 per cent). The small sizes were confirmed by x-ray diffraction analysis, which also revealed trace quantities of a foreign substance identified as silica. On heating of the material at 1000° C, grain growth occurred followed by the disappearance of the silica lines.

Another possible complicating factor, that is not easily amenable to solution is the possibility of small amounts of highly toxic beryllium compounds (e.g., beryllium hydroxide or nitrate) occluded in the latter preparation which would contribute significantly to the observed toxicity.

Problem Code: Be.3 (Toxic Limits)

Section Code: 3230

Oral Toxicity of Beryllium:

The inhalation exposures always involve a certain amount of exposures by mouth. A limited oral toxicity study is useful in supplementing the inhalation program and providing data which are useful in interpreting the results of inhalation exposures.

Aims of the Program:

1. Data to be obtained from experiments in which laboratory animals were given beryllium orally could be expected to serve a two-fold purpose in the overall problem of beryllium toxicity.

(a) A picture of the fundamental histopathological changes brought about in the body by various beryllium compounds could be obtained. Feeding studies provide an opportunity for the uniform, controlled intake of a chemical substance by a normal route in the body, thus giving full opportunity for all tissues to be affected and for all animals in the experiment to be equally exposed. By comparison, administration of a chemical by the route of inhalation cannot be expected to give consistently uniform pathological results in all exposed animals, because it is often impossible to maintain either a non-fluctuating exposure concentration or a uniform concentration of the test material in all parts of the experimental exposure chamber.

(b) The establishment of toxic levels of various beryllium compounds when given orally would make possible the determination of the possible role of ingestion as a factor to be considered in the interpretation of data obtained from inhalation experiments.

2. Because the case histories of some women suffering from beryllium poisoning indicated pregnancy as a possible contributing factor, it seemed worthwhile to investigate the possible role of pregnancy in beryllium-poisoned laboratory animals.

Program:

1. Groups of albino rats (10 male and 10 female per group) have been fed various dietary levels of beryllium sulfate ($BeSO_4.6H_2O$), beryllium carbonate ($BeCO_3.2(OH)_2.3H_2O$), beryllium metal, and two grades of beryllium oxide (fluorescent and refractory of SP) for periods of about one month. Preliminary studies with dogs involved the simple experiment of feeding one level of a given beryllium compound to a single dog. Beryllium sulfate and two grades of beryllium oxide were so tested.

2. To test the possible effect of pregnancy on beryllium poisoning 4 groups of female weanling rats (25 per group) were treated as follows:

Group I -- Controls - no beryllium administered.

Group II -- Injected intraperitoneally with a single dose of 1 g beryllium oxide (fluorescent grade)/kg body weight.

Group III -- Same treatment as Group II.

Group IV -- Injected intraperitoneally with a single dose of 1 g beryllium oxide (refractory or SP grade)/kg body weight.

In groups I, II, and III, each female rat was then caged with a male for the duration of the experiment. The male was removed during each period of pregnancy and returned to the female after the birth of each litter, the young being discarded. The females in Group IV were not caged with males, thus preventing pregnancies in this group.

Results Obtained from Program:

Rats Fed Beryllium Sulfate Hexahydrate: No effect on growth was noted in rats fed this compound at a dietary level of 0.5 per cent. No rats died. Rats fed 2.0 per cent beryllium sulfate in the diet when compared with controls exhibited an average weight depression of 30 g for males and 10 g for females. Such weight depressions in male rats perhaps serve as a significant criterion of toxicity; the 10 g depression in females is of doubtful significance. Complete inhibition of growth resulted in rats fed 5.0 per cent beryllium sulfate in the diet and 2 rats died in the 30-day experimental period. When 10.0 per cent beryllium sulfate was given in the diets of male and female rats, rapid weight loss occurred and all of the animals were dead in 9 days.

No pathological changes of significance occurred in the tissues of rats fed 0.5 and 2.0 per cent beryllium sulfate in the diet. At the end of a 30-day period on a diet containing 5.0 per cent beryllium sulfate histopathological changes were detectable in liver, kidney, gonads, and bone. The bone changes were also clearly evident radiographically. When rats were placed on a stock diet containing no beryllium, after previously ingesting 5.0 per cent beryllium sulfate in the diet for 30 days, and sacrifices were made at 10, 20, and 60-day intervals, rapid and progressive repair of kidney, liver, and gonads was demonstrated. Complete recovery of these organs was evident at the end of 60 days after removal of beryllium sulfate from the diet. Repair of the bone injury after return of the animals to a stock diet was not clear cut. At the end of the 30-day period on a diet containing 5.0 per cent beryllium sulfate, a rachitic-like condition was present accompanied by a thickening of cartilage plates and a decrease in osteoblastic activity. At this time of marked inhibition of osteoblastic activity, there was observed near the head of each of the tibias a plate of fused irregular inactive bone. Upon removal of the beryllium sulfate from the diets of the rats this bony plate remained, although there was a return of normal osteoblastic activity and apparent recovery from the rachitic-like condition.

Rats Fed Beryllium Carbonate: At a dietary level of 0.5 per cent beryllium carbonate the rats grew equally as well as the controls. No animals died. At a dietary level of 2.5 per cent, there was a slight decrease in average body weight when compared with the controls (males 19 g, females 11 g). Such weight depressions are of questionable significance. There were no deaths. When 5.0 per cent beryllium carbonate was fed in the diet a very serious growth depression occurred, amounting to about 100 g in the males and about 50 g in the females, when compared with the controls. One male and 2 females died.

The only definite pathological changes attributed to the ingestion of beryllium carbonate were rachitic-like changes in the long bones. This rickets-like condition was confirmed by radiographs. Some liver damage was

present in approximately 1/3 of the animals terminally sacrificed; our pathologist declared this finding to be of questionable significance.

Rats Fed Beryllium Oxide (Refractory or SP Grade): At dietary levels of 0.5 and 2.0 per cent beryllium oxide (refractory grade) the rats grew as well as the controls. At a level of 2.0 per cent of the diet an average growth depression of about 35 g occurred in the males when compared with the controls; in the females the corresponding weight depression amounted to approximately 20 g. No deaths occurred. At present the pathological studies on these animals have not been completed.

Rats Fed Beryllium Oxide (Fluorescent Grade): At dietary levels of 0.5 and 2.0 per cent beryllium oxide (fluorescent grade) no significant depressions of body weight occurred in rats at the end of 30 days. There were no deaths. When 20 per cent of the fluorescent grade oxide was fed in the diet, male rats had an average weight depression of about 50 g when compared with the controls; in the females the corresponding weight depression amounted to about 20 g. Pathological studies on these animals have not been completed.

Rats Fed Beryllium Metal: At dietary levels of 0.5 and 2.0 per cent the rats grew as well as the controls. At a dietary level of 20 per cent an average growth depression of about 30 g occurred in males when compared with the controls; in the females no significant depression occurred. There were no deaths. Pathological studies on these rats have not been completed.

Dogs Fed Beryllium Sulfate Hexahydrate: When beryllium sulfate hexahydrate was given in the diet of a dog at a level of 1 g/kg/body weight per day for a period of about 2 months, there were no clinical effects apparent and histopathological studies disclosed no abnormal conditions in the tissues.

Dogs Fed Beryllium Oxides: When beryllium oxide (fluorescent grade) or beryllium oxide (refractory or SP grade), respectively, was fed to a single dog at a dosage level of 5 g/kg/body weight/day for a period of about 2 months, neither clinical nor histopathological effects were observed.

Female Rats Injected Intraperitoneally with Beryllium Oxides: At the end of approximately one year following the injection of a single intraperitoneal dose of beryllium oxide in weanling female rats an average weight loss of about 20 g was evident. Such a weight depression in mated females is of questionable significance. During this experimental period 1148 pups in 138 litters were born to the controls; 883 pups in 114 litters were born to the females injected with refractory grade oxide; 855 pups in 117 litters were born to the females injected with fluorescent grade oxide. These differences in total pups born during the experimental period represent decreases of 23 and 25 per cent, respectively, when compared with the controls. The statistical significance of these figures has not been determined. Nineteen of 50 females injected with fluorescent grade beryllium oxide died as compared with 3 of 25 injected with refractory grade beryllium oxide and 2 of 25 controls.

Problem Code: Be.4 (Fate)

Section Code: 3220, 3250

Distribution and Excretion of Beryllium Sulphate Using Be7 as a Tracer:

Beryllium sulphate was administered intravenously to rats and rabbits; the rate of blood clearance, the urinary and fecal excretion and the distribution to organs was determined by counting techniques. In some cases non-radioactive beryllium sulphate was added to the isotopic beryllium sulphate. More than 95 per cent of the beryllium was cleared from rabbits' blood during the first 5 hours after administration; the rate of clearance was more rapid when isotope alone was administered than when carrier was added. The urinary excretion of beryllium was greatest during the first 6 hours after administration; thereafter small amounts, less than 1 per cent, were excreted daily. In both rabbits and rats a higher per cent of the isotope was excreted when isotope alone was given than when carrier was added: 28 per cent and 11 per cent, and 35 per cent and 25 per cent, respectively. The total fecal excretion over a 7-day period was approximately 10 per cent of the dose in the rats and 2 per cent in the rabbits. When carrier was administered with the isotope approximately 35 per cent was found in the skeleton of the rat and 51 per cent in the skeleton of the rabbit; when isotope alone was administered the skeletal recovery was 48 per cent and 54 per cent. The amount of isotope recovered from the liver and spleen of the rat was insignificant when isotope alone was administered and amounted to 12 per cent and 2.5 per cent, respectively when carrier was added. Significant amounts of beryllium were present in the bone marrow when carrier was administered with the isotope. The kidneys contained less than 1 per cent of the dose.

Problem Code: Be.5 (Mechanism of Toxic Effect)

Section Code: 3210

The "X" Factor in Beryllium Poisoning:

Dr. Gardner had the hunch that part of the mysterious character of beryllium poisoning arose from the existence of a factor, "X", which, added to a beryllium exposure, was followed by beryllium poisoning but in the absence of which, beryllium exposure was not always in a causal relation to the well-known clinical beryllium diseases. The search for a possible factor "X" has so far been unsuccessful. The following tests have been made:

(1) Effect of HF.

(2) Effect of physiological stress (excercise)

(3) Effect of infection:

gram-negative rod
tuberculosis
hyaluronidase

(4) Pneumonitis from intratracheal oil administration

(5) Rutin

Potentiating Effect of Inhalation of HF Gas on Beryllium Sulfate

Poisoning in Rats: Industrial exposures to beryllium are commonly not homogeneous but consist of mixtures of other potentially toxic agents. In some processes in the manufacture of beryllium, hydrogen fluoride (HF) or other soluble fluorides have been shown not only to be present with the beryllium but actually to exist in amounts several-hundred-fold greater than that of beryllium. Toxic exposures have been shown to occur also among plant personnel at sites where these mixed exposures existed. The study described below was performed to determine whether HF had a potentiating effect on beryllium poisoning by inhalation. The results obtained in rats confirm this hypothesis.

Eighty rats were exposed in groups of 10 and 20 each to either beryllium sulfate or to HF separately, or in combination on alternate days or daily for one month, a period of sufficient duration to determine completely the effects of exposure. The concentration of beryllium sulfate hexahydrate selected approximated 10 mg/m^3. This concentration had previously been established as giving 50 per cent mortality in rats in a period of 3 weeks. Exposure concentrations of HF were to approximate 7 mg/m^3, a concentration that likewise had been previously shown to produce either inconsequential pulmonary changes in rats or no response at all.

The 20 rats that were submitted to both agents were exposed on alternate days for 6 hours, first to the beryllium aerosol and then to the fluoride gas for a total exposure period of 62 hours for each aerosol. This procedure was purposely adopted partly to avoid analytical difficulties that would otherwise occur in a mixed exposure of the two aerosols and partly because a definite interpretation of toxicologic results would be made more certain if exposure to each agent was less than that of the controls on the reasoning that if mortality occurred from reduced exposure (lowered C.T. values), the HF would have potentiated beryllium toxicity, HF being certainly not lethal at the C.T. values employed. Serving as controls for this study were 4 additional groups of rats; one group of 20 was exposed daily to HF at concentrations approximating 7 mg/m^3 (124 exposure hours), another group of 20 rats was exposed to beryllium sulfate hexahydrate mist at approximately 10 mg/m^3, a third and fourth group of 10 rats each were exposed on alternate days to the above concentrations of either HF or beryllium sulfate.

The toxicologic findings were clear-cut and unequivocal. Results of mortality, weight response and histologic examination all were consistent for any given group and were sharply different between the group exposed to the mixed agents as compared with the groups exposed to the agents separately for the same exposure time. As for mortality, precisely the same number of rats died that were exposed to both agents on alternate days as died from beryllium sulfate alone and that were exposed daily. Mortality began on the 13th and 10th days in each group, respectively, but both groups reached the identical rate of mortality on the 20th day (40 per cent), and no change

occurred in this variable thereafter. Thus, the same mortality was produced from one-half the total exposure to beryllium plus HF as was produced by double the amount of exposure to beryllium alone. No deaths occurred in rat groups exposed to HF alone daily, or to beryllium sulfate alone on alternate days. One rat of a group of 10 exposed to HF on alternate days, died, however, from some cause unrelated to the exposure. Substantiating this evidence of potentiating toxicity of HF for beryllium were the weight-response data. The only rat groups to show weight changes lower than the pre-exposure mean values were the same 2 groups showing the 40 per cent mortality. Plots of the weights of the other 3 groups never dipped below this pre-exposure mean and in 2 groups showed progressive increase in weight upon continued exposure. Similar evidence substantiating the main conclusion was obtained from histologic examination of the animals dying from exposure and those sacrificed at its termination. Rats exposed to beryllium sulfate showed minimal to moderate pulmonary disease, and those exposed to the combination of both beryllium and fluoride showed pulmonary changes similar in character and degree to those exposed daily to beryllium alone. Exposure to beryllium on alternate days resulted in definite pulmonary lesions in only a few instances, none in a few cases and equivocal effects in the remainder.

Thus, it may be definitely stated that since pulmonary injury and associated effects were as great in rats receiving one-half the exposure to beryllium sulfate when HF-exposure was superimposed (and under conditions that either agent alone produced no changes) as the effects produced by rats receiving twice the exposure of beryllium sulfate alone, that HF has a potentiating effect on beryllium action in the body.

It was not ascertained whether inhalation of HF gas induced additional intake of beryllium thus predisposing the animals to greater injury. It appears more probable that HF would reduce pulmonary exchange.

Effect of Physiologic Stress in Beryllium Poisoning:

Effect of Exercise: Case studies of beryllium industrial workers indicate that physical activity may increase the tendency to acute poisoning. Efforts have been made to produce a comparable effect in animals. The hamster was chosen as the experimental species because previous inhalation exposure studies at high concentrations of beryllium sulfate mist (100 and 50 mg/m^3) had indicated that hamsters were more resistant to this lung irritant than were other animal species suitable for such a study. If mortality could be induced in this species through its added stress of exercise, then it was felt that a striking case for such stress in beryllium exposure would be demonstrated.

Exercise was provided by placing the animals in a rotating cage of 21" diameter which revolved at the rate of 2.3 revolutions per minute or 138 revolutions per hour. In order to avoid over-exertion of the animals, the cage was rotated for 20 minutes each hour, followed by 40 minutes of rest during the 5 daily exposure hours. This procedure was followed during the conditioning period and during the dust exposure phases of the study. The animals were exposed to beryllium sulfate mist at a concentration of 10 mg/m^3 of air.

The mortality results of two studies of this type are given in Table I -- Page 86. In each instance an equal number of exercised and non-exercised animals were used, the latter group serving as beryllium exposed controls, e.g., placed in the chamber in a stationary exposure cage. It is seen that identical results were obtained in each experiment, namely, that all animals subjected to exercise succumbed, where only 1 in 6 died from exposure without such exercise. Deaths occurred in both exposed groups in from 9 to 12 days; exercise thus did not speed the time of death.

The results of this hamster experiment indicate that the combined effect of exercise and beryllium sulfate inhalation exposure are more toxic and produce more severe pulmonary lesions than exposure alone.

Effect of Infection: The relationship of a concurrent pulmonary infection has occasionally been proposed to explain the course of beryllium pneumonitis. Gardner, et al have reported the isolation of an acid-fast gram-negative rod from the lungs and blood stream of humans with beryllium disease. An attempt was made to survey briefly the bacterial nature of the lungs of animals given beryllium by intratracheal administration or through the inhalation route. Using sterile precautions, a loop streak was obtained from the lungs of recently sacrificed animals and cultures were made from blood agar, Douglas broth and an anaerobic meat culture.

More than 35 rats, rabbits, dogs, and guinea pigs were studied. The number of contaminated samples were no more than 2 or 3 and the contaminants were readily identified as staphylococci or streptococci. However, from the lungs of a rat receiving beryllium metal intratracheally several months previously and exposed to beryllium sulfate by inhalation and which was sacrificed when found to be dyspneic and moribund, a positive pure culture of a gram-negative rod was obtained. Specific bacteriologic characteristics of this organism have been given in detail in Rochester Report No. M-1974. At that time, it was tentatively believed that the organism was anaerogenic salmonella enteriditis. From the lungs of a rabbit exposed to beryllium sulfate by inhalation, there was obtained another motile gram-negative rod whose characteristics varied but were in many ways similar to the organism previously obtained. The cultures from the lungs of other sacrificed animals were sterile.

The organism, described as a salmonella and obtained from the beryllium-treated rat, was grown for 48 hours on blood agar plates and a suspension of the organism in saline was made and was administered through the intratracheal route to 300-gram male rats. Ten rats were given the organism alone (Group 1). Eight rats were given the same number of organisms plus 22 mg of beryllium metal (Group 2). Four were given the same number of organisms plus 5 ml of hyaluronidase (Group 3). Group 1: of the 10 rats, 5 died within 6 days after treatment. Group 2: 6 of the 8 in this group died within 4 days post-treatment and one succumbed three months later. Group 3: 3 of the 4 rats died within 5 days post-treatment.

All rats administered the suspension of the unknown organism showed a very marked leukocytosis and evidenced an obvious toxic course. At autopsy, the lungs were hemorrhagic, consolidated and yielded on culture a pure growth of the same organism instilled. When the surviving animals were sacrificed, 7 months later, frequent abscesses and congestion of the lungs were to be seen.

Table I.

Effect on Exercise on Mortality of
Beryllium-Sulfate-Exposed Hamsters

	EXPERIMENT 1			EXPERIMENT 2		
	No. Exposed	No. Dying	% Mortality	No. Exposed	No. Dying	% Mortality
Exercised	6	6	100	12	12	100
Non-exercised	6	1	17	12	2	17

From more than 35 sterile cultures taken from the lungs of beryllium-treated rats, rabbits, dogs, and guinea pigs, 2 positive cultures were obtained. One of these was from a rat and it was tentatively identified as an anaerogenic salmonella enteriditis. From the lungs of an exposed rabbit, another gram-negative rod which had several similar quantities was isolated. Intratracheal instillation of a culture of the organism obtained from the rat proved to be very toxic to other rats. The simultaneous administration of beryllium metal or hyaluronidase appeared to potentiate the fatal effects of this organism.

Effect of Superimposed Beryllium Exposure on Tuberculosis in Guinea Pigs: In 1935, Loomis and Bogen reported an enhancing effect of various soluble beryllium compounds on the extent of tuberculous infection in guinea pigs and further suggested the treatment of guinea pigs with beryllium to increase the sensitivity of this animal in testing tuberculous sputum. Because of this report and the present industrial interest in the toxicity of beryllium, it was thought advantageous to confirm this observation.

Equal numbers of male and female guinea pigs were selected and distributed among groups of 10 each. One group was infected with 0.005 mg of M. tuberculosis, strain H37 (ATCC 8236). These animals served as control-infected animals. Two other groups of guinea pigs received the tuberculous infection in doses of 0.5 and 1 mg subcutaneously and intra-abdominally, respectively, and in addition received weekly exposure to beryllium sulfate at a concentration of 50 mg/m³ for from 2 to 6 hours daily. A 4th group received no infection with tuberculosis but was exposed to beryllium sulfate only. A 5th group received neither infection nor exposure to beryllium sulfate and served as normal weight controls for the entire group. Exposure to beryllium was continued for 2 1/2 months. Weights were taken weekly and observations were continued for a period of 4 months from the start of the infection. At this time autopsies were performed on the animals and the results of previously obtained nodular size correlated with the histologic results from stained sections.

The microscopic examination of the tissues of the guinea pigs failed to show that weekly exposures to 50 mg/m³ of beryllium sulfate had any effect on the course of tuberculosis in the guinea pig. The lymph nodes draining the area of infection showed in all groups the same type of tissue reaction, namely, proliferative tubercles some of which were caseous. The liver and spleen of a few animals in each of the 3 infected groups contained a few tubercles of miliar size and of proliferative type but no significant differences between the groups, although the dosage of tubercle bacilli among the groups differed widely.

No pulmonary tuberculosis was found and the pulmonary lesions usually observed from the inhalation of beryllium sulfate were either minimal or absent; this finding has been previously observed in the guinea pig, which is a relatively resistant animal to inhalation to beryllium dust. Accordingly it may be concluded conversely that the tubercular infection did not predispose guinea pigs to beryllium poisoning.

The findings at gross autopsy and measurement of nodular size made throughout the study showed no differences attributable to the different treatments among the groups. Moreover, the weight response of all treated groups showed a parallel rise in growth following the third week of the study which was at all times somewhat less than that of the untreated controls. The beryllium-exposed animals showed the poorest growth response of all groups. In general, however, the weight response was consistent with the histologic findings.

These results with experimental animals agree with the general impression of industrial physicians that beryllium workers with tuberculosis are not more susceptible to beryllium poisoning.

Effect of Hyaluronidase: The invasiveness of certain types of micro-organisms is dependent upon a "spreading factor" known as hyaluronidase. Some consideration was given to the possibility of stimulating the pneumonitic penetration of otherwise benign micro-organisms by furnishing them with an artificial supply of hyaluronidase. It was anticipated that with the combination of such an effect, a more specific approach might be made to the effects upon beryllium disease in the lungs of experimental animals.

Pure cultures were grown of a variety of micro-organisms including several types of pneumococci and pertussis. Suspensions of these in saline solution, with or without the addition of 2.5 to 5.0 mg hyaluronidase, were given intratracheally to adult rats. The daily pattern of the total white leukocytic count and differential count was observed.

A summary of several of the tests is given below. It may be stated that pneumococci Type VI and VII were also tested but without demonstrating any toxicity to rats with or without hyaluronidase. (See Table II - Page 89).

The concurrent administration of hyaluronidase to a variety of types of pneumococci and H.pertussis gave somewhat variable results. In one or two trials, the pneumonitic virulence of these organisms appeared to be increased but the results appeared to be somewhat inconsistent.

Effect of Peanut Oil: The peculiarities of the susceptibility of beryllium disease in humans suggested that certain uncontrollable factors might be of some importance. One route of increased beryllium susceptibility can conceivably exist in individuals bearing a concurrent pulmonary infection. Another could possibly involve an increased beryllium penetration which would mobilize the second or third line of body defense.

The intrapulmonary administration of oils in general tends to produce a pneumonitis. In particular, however, peanut oil has been known to contain a special irritant and is capable of producing a marked pneumonitic involvement.

Table II

The Effect of Hyaluronidase on the Invasiveness of Micro-Organisms in the Lungs of Rats

Given	No. of Animals	Mortality	Died	EFFECT ON		
				WBC Count	Diff. Count	Pathology of Lungs*
H.pertussis plus hyaluronidase	4	1/4	1-17 days	2/4 elevation slight	No change	Slight consolidation
H. pertussis	3	1/3	1-14 days	1/3 slight elevation	No change	Slight consolidation
Pneumococcus Type II plus hyaluronidase	3	3/3	2-4th da. 1-7th da.	2/3 slight elevation	2/3 high polymorph.	Marked fibrinous pleurisy and pneumonia
Pneumococcus Type II	3	1/3	7th day	No elevation	No change	"
Pneumococcus Type XV	5	0/5	0	" "	" "	Slight consolidation
Pneumococcus Type XV plus hyaluronidase	5	0/5	0	Slight elevation 3/4	9-16% eos. in 3/5 animals	Slight consolidation
Pneumo Type II	5	1/5	1-7th da	No elevation	No change	Fibrinous pleurisy
Pneumo Type II plus hyaluronidase	5	4/5	1/1st da 1-2nd da. 1-4th da. 1-9th da.	Slight elevation 3	No change	Fibrinous pleurisy

* Observed on autopsy of rats which succumbed.

An extensive survey was made of the reaction of a large number of rats to quantities of 0.1 to 0.8 ml of castor oil, peanut oil, mineral oil and olive oil for control purposes. Daily leukocyte counts and mortality were used as criteria. The only specific indicators of any toxicity were observed in rats receiving the larger quantities of peanut oil. The affected animals manifested a leukocytosis persisting for several weeks. (See Table III - Page 91).

To 8 rats (Group 1) were given 100 mg of beryllium metal intratracheally 2 to 3 months previously. 0.5 ml of peanut oil was given through the same route. To 5 rats (Group 2) beryllium metal and 0.2 ml of olive oil were given. To 5 rats (Group 3) beryllium metal and 0.3 cc olive oil and 0.5 cc peanut oil were given. To 5 rats (Group 4) beryllium metal and 0.3 cc of olive oil plus 0.2 cc peanut oil were given. To 4 rats (Group 5) a 0.3 ml of olive oil plus 0.5 ml peanut oil were given. To 5 rats (Group 6) a 0.3 ml of olive oil plus 0.2 ml of peanut oil were given. To 6 rats (Group 7) 0.1, 0.3, and 0.5 ml of peanut oil were given.

No distinct changes in weight were noted on the above animals.

The simultaneous intratracheal administration of beryllium metal plus olive oil and peanut oil had no significant effect on the mortality from beryllium disease in those animals over a period of 9 months of observation. However, a more marked leukocytosis was observed in most animals from the addition of the oil to the beryllium metal. Peculiar to those animals which had received beryllium previous to the administration of peanut oil, a significant eosinophilia developed.

Rutin as a Prophylactic and Therapeutic Agent in Beryllium Poisoning in Dogs: One of the greatest needs in the current beryllium exposure problem is that of an effective prophylactic or therapeutic agent. Attempts to fulfill this need were focused on rutin, a plant flavanol, because of its particular chemical configuration and physiologic properties. One of these latter properties is its ascribed capillary antifragility action. This action should prove beneficial in beryllium poisoning poisoning which is characterized in part by hemorrhage and edema of the lungs. A second property of potential beneficial value in beryllium poisoning is its phenolic structure permitting complex formation with beryllium. Indeed an especially sensitive microcolorimetric method employing rutin as the analytical reagent has been developed in this laboratory. Further evidence on the potential effectiveness of rutin in beryllium disease was furnished by an experiment in which beryllium combined with serum was shown to be capable of removal by dialysis in the presence of rutin; removal of beryllium from the serum proteins did not occur in the absence of rutin. There was thus a number of indications that rutin might prove effective as a therapeutic or prophylactic agent in the treatment of beryllium poisoning.

Accordingly, an inhalation exposure experiment was performed employing 10 dogs, 5 of which received daily by capsule 100 mg of rutin in 2 daily divided doses for 2 weeks prior to the beginning of exposure to beryllium sulfate mist at a concentration of 25 mg/m^3. A closely similar group of dogs, some of which were litter mates of those in the untreated group, received the regular diet

Table III

Effect of Potentiating Agents on Beryllium in Lungs of Rats

Group	Substances Administered	No. of Animals	No. Died	Survived	EFFECT ON		
					WBC Count	Diff. Count	Pathology
1	Be, 2 mos. previous	8	1-12 days	2/8*	Elevated (2/2) 5-14 days, occasional elevation for 7-9 mos.	Eosinophils rose to av. of 15± % at 20 days at intervals throughout	3 plus consolidation. Frequent abscesses
2	Be plus 0.2 ml olive oil	5	1-21 days 1-50 days 1-6 mos.	3/5	Elevated (4/5) on 3rd day, occasional elevation for 7-9 mos.	Occasional blast cell seen	"
3	Be plus 0.3 ml olive oil plus 0.5 ml peanut oil	5	1-15 days 1-8 mos.	2/5	Slight irregular elevation (3/5) between 17-25 da.	Frequent early forms, blast cells erythroblasts	"
4	Be plus 0.3 ml olive oil plus 0.2 ml peanut oil	5	1-13 days 1-3 mos.	2/5	Slight irregular elevation (3/5) between 14-20 da.	Frequent early forms, blast cells erythroblasts	"
5	Control 0.3 ml olive oil plus 0.5 ml peanut oil	4	2-4 mos.	2/4	Slight irregular elevation (1/4) on 16th day	Occasional toxic form, blast and erythroblast	Slight congestion
6	Control 0.3 ml olive oil plus 0.2 ml peanut oil	5	2-4 mos.	2/5	Slight elevation (2/5) between 11-17 days	Rare blast cell	"
7	Control 0.1, 0.3, 0.5 ml peanut oil	6	None (duration 5 mos.)	0	Slight irregular elevation (2/5) between 1-20 da.	Rare blast cell	"

* Remainder died of causes unrelated to the experiment.

without rutin but received the exposure to the beryllium sulfate simultaneously with the treated dogs. Exposure to beryllium sulfate mist was continued daily for 6 hours for a period of 35 days. During this time, rutin was administered daily, at first in 100 mg amounts, later during the last 2 weeks of the experiment in 300 mg daily dose.

There was no significant difference in the weight response of the animals of the 2 groups at the termination of the beryllium sulfate exposure. During the course of exposure, however, animals receiving no rutin showed a decided loss in weight from the first to the third week but after this period the weight of the 2 groups was essentially indistinguishable. Differences in mortality in the control and treated groups were not remarkable. Two dogs of the beryllium-exposed group died, one on the 17th, and other on the 35th day of exposure; but one of the 5 rutin-treated dogs died on the 22nd day of exposure. This difference, though indicating a possible favorable response to rutin treatment, was not considered significant.

Inasmuch as rutin has been claimed to maintain the integrity of the capillary, a special attempt was made to determine differences in the amount of edema or hemorrhage in the lungs of the dogs receiving rutin compared with those of the controls. No such effect was observed; rather the lungs of the animals receiving rutin showed somewhat more edema than the controls. Furthermore, the character of the inflammatory response was similar in both groups and no difference in the degree of extent of the pulmonary lesions could be demonstrated. Rating the degree of injury from 1 to 4, indicating progressive change from mild to extensive pulmonary involvement, of 5 beryllium-exposed but not rutin-treated animals, 2 each gave a 1- and 2-plus reaction and 1 showed a 4-plus reaction; of the 4 animals examined in the rutin-treated group, no animal showed a 1-plus response, one each showed a 2-plus and a 4-plus response and 2 showed a 3-plus response. Thus, rutin instead of improving the condition of the pulmonary lesion from beryllium did appear to have exacerbated the condition. Further evidence bearing out this observation was furnished by spectrographic analysis for beryllium content of certain tissues of the exposed and treated dogs. Results of these analyses appeared to show that rutin rather than ridding the tissues of beryllium tended to prevent elimination of this element. Amounts of beryllium in the lung, bone, liver, and kidney were for the beryllium-exposed animals, respectively, 4.9, 0.56, 0.24, and 0.08 $\mu g/g$. For the same tissues of the rutin-treated dogs, values of beryllium were correspondingly 5.6, 0.72, 1.2, and 0.09. Thus, in each tissue, somewhat higher average values for beryllium were found in the rutin-treated dogs.

It is therefore, concluded that from the basis of histologic evaluation and results of spectrographic analysis of the tissues for beryllium that rutin is ineffective either as a prophylactic or therapeutic agent in the treatment of beryllium injury in dogs.

Problem Code: Be.6 (Methods of Detection of Poisoning, Prophylaxis, Treatment, and Protection)

Section Code: 3210

An Investigation of Clinical Criteria in Beryllium Poisoning:

At present no reliable biochemical methods exist for the diagnosis of early or mild forms of beryllium poisoning. Such methods present special difficulties in discovery and development because beryllium is a pulmotoxic agent and few function tests exist for measurement of lung dysfunction, and none that are of service for detecting early beryllium poisoning. Accordingly, a broad survey of clinical criteria was undertaken in animals undergoing exposure. This included determination of changes in urinary protein and amylase, blood non-protein nitrogen, urea nitrogen, serum protein, serum albumin-globulin ratio, fibrogen, serum calcium and phosphorus, acid and serum alkaline

PROGRAM Th.

THORIUM

Problem Code: Th.1 (Physical and Chemical Properties)

Section Code: 3210

Rapid Colorimetric Estimation of Thorium in Microquantities:

A method has been developed for the rapid colorimetric determination of thorium in microquantities, based on the formation of a stable colored complex with excess carminic acid in acid solution. The spectral absorption of the complex at 560-590 mμ and pH 4.0-4.2 is directly proportional to the concentration of thorium over the range 2.5-25.0 μg/ml. The method has the advantages that the colored complex between thorium and carminic acid forms almost instantaneously in solutions at room temperature, eliminating the necessity of heating to accelerate color development, and the color is stable indefinitely at ordinary temperatures.

The reagent is a complex polyhydroxyanthraquinone derivative which acts as an acid-base indicator in aqueous solutions, undergoing a color change from orange to violet in the range of pH 3.45 to 7.45. A similar color change occurs in the presence of thorium. The optimum pH for determination of thorium is probably at or below 3.45, but satisfactory results have been obtained with 0.5 M sodium acetate-acetic acid buffer at pH 4.0-4.2, the choice of pH being governed by the advisability of working in the range of maximal buffer capacity of the solutions.

Ferrous and ferric iron interfere seriously in the determination of thorium, as does aluminum, also. The sensitivity for ferrous iron is about the same as for thorium, 1:400,000, while that for ferric iron and aluminum is about one part in 750,000. K, Na, Ca, Mg and the common anions with which thorium forms soluble compounds do not interfere in the method, which cannot be used, however, in the presence of anions which precipitate thorium as water-insoluble compounds, or in the presence of certain organic substances, e.g., citrate and tartrate, which form complexes with thorium in which the strength of the coordinate bond is greater than with carminic acid.

Problem Code: Th.3 (Toxic Limits)

Section Code: 3210

Inhalation Toxicity of Thorium:

Interest in the toxicology of thorium stems from the possibility that the element may have wide use as an alternative source of fissionable material, and

from the fact that Atomic Energy Commission personnel are exposed to a possible hazard in the separation and purification of thorium metal. The literature contains conflicting accounts regarding toxic effects following the use of thorotrast in roentgenography as well as following exposure to thorium dust in mining and refining operations. It seemed desirable, therefore, to undertake a series of experiments with the object of characterizing the toxic response to inhaled thorium dusts and defining the acceptable limits of exposure. Aerosols of thorium nitrate and thorium dioxide have been used initially as representative soluble and insoluble thorium compounds encountered in the production of pure thorium metal. The toxicological studies were delayed because of the necessity for developing a satisfactory quantitative method for the detection of thorium in microgram quantities.

Acute Toxicity of Inhaled Thorium Nitrate Dust: A series of three pilot experiments has been completed in which 85 laboratory animals, comprising 6 species, were exposed to an atmosphere containing 75 mg $Th(NO_3)_4/m^3$. The purpose of these experiments was to determine whether acute toxic effects are produced by inhalation of thorium nitrate dust in relatively high concentration. In the first experiment, 25 female mice, 20 rats, equally divided with regard to sex, 10 male guinea pigs and 3 rabbits were exposed 6 hours daily for a total of 68 hours during 11 1/3 calendar days. Three rabbits and 20 male hamsters were exposed 6 hours daily for a total of 60 hours during 10 calendar days in the second experiment, and in the third and final experiment, 4 female dogs were given the same exposure.

The thorium nitrate was dispersed as a dry dust prepared by micropulverization of material purchased from the Maywood Chemical Company. Spectrographic analysis of a sample of the Maywood product by the National Bureau of Standards indicated the thorium content of the material to be 41.55 per cent by weight. The overall mean concentration of dust as thorium in these experiments, therefore, was 35.5 mg/m^3. Since the thorium nitrate was processed more than two years ago, it contained appreciable amounts of mesothorium.

In each experiment, the period of exposure was preceded by a 2-week conditioning period during which the animals were placed for 6 hours daily in a chamber which was held at the same temperature and relative humidity as the exposure chamber, but which contained no thorium nitrate dust. The weight response of the animals, urinary protein, blood NPN, and urea nitrogen, and the hematological picture were followed as toxicological indices during both the conditioning period and the period of exposure.

There were no deaths among any of the animals during the period of exposure and all were sacrificed terminally with the exception of 2 dogs which are being held for further observation. The dogs were the only species in which toxic signs were observed during exposure. Retching, gagging and occasional vomiting were observed periodically beginning on the third day of exposure. There were no significant changes in weight among any of the 6 species of animals and anorexia was not observed even in the dogs. The clinical chemical determinations revealed no significant changes in blood NPN and urea nitrogen or in urinary protein content at any time during the conditioning and exposure periods or during the period of observation following exposure in the case of the 2 dogs, which were held for this purpose.

Negative hematologic findings were reported except for the dogs. There were no significant changes in the red blood cell counts of any species. All of the dogs exhibited leukocytosis at some time during the exposure period or immediately thereafter. The average increase in the white blood cell count of all 4 animals was about 50 per cent, the peak of the response occurring at different times in different individuals. In the 2 dogs which were sacrificed, the highest leukocytic count occurred during the second week of exposure, whereas in the 2 animals that were held for further observation the peak of the response was observed during the first week following exposure. In 3 of the animals a subsequent decrease in the white cell count was observed after the peak had been attained. The appearance of atypical granulocytes in the blood films of the dogs has been interpreted as evidence of possible damage to the hemopoietic tissue. These cells are characterized by the basophilic staining and vacuolization of their cytoplasm, and by the presence of atypical eosinophilic granules and globules, of varying size and staining property. The atypical granulocytes first appeared in the blood films on the seventh day after the beginning of exposure at which time they constituted 6 to 10 per cent of the total granulocytes. In the 2 dogs which were kept for observation, the proportion of abnormal granulocytes reached a maximum of 9 to 12 per cent, 5 1/2 weeks after exposure, at which time 4 to 5 per cent of metamyelocytes with bizarre nuceli were observed. The atypical granulocytes were still present to the extent of about 5 per cent in blood films from these dogs 4 1/2 months after exposure.

The only significant pathological changes in the tissues of any of the animals occurred in the lungs of the 2 dogs which were sacrificed terminally. In one of these, a giant cell reaction was seen about the small bronchi, the giant cells containing a crystalline material and resembling the giant multinucleated cells which have been observed previously in animals following intraperitoneal and intratracheal administration of thorium nitrate. The lungs of the other dog showed predominantly a mononuclear type of reaction with only a few scattered giant cells containing crystals.

Acute Toxicity of Inhaled Thorium Dioxide Dust: Two pilot experiments have been completed in which 81 laboratory animals, comprising 5 species, were exposed 6 hours daily for a total of 60 hours during 2 calendar weeks to an atmosphere containing 49.4 mg ThO_2/m^3. The mean atmospheric dust concentration as thorium was 43.4 mg/m^3. The purpose of the experiments was the same as in the pilot tests with $Th(NO_3)_4$, and an attempt was made to adjust the dust concentration so that the exposures in the 2 series of experiments would be as nearly as possible identical with respect to thorium concentration. The distribution of animal species in the 2 completed tests was as follows: (1) 10 male guinea pigs, 20 hamsters, 25 female mice, and 20 rats, equally divided with respect to sex; (2) 6 rabbits. Preceding exposure, the animals were conditioned 6 hours daily for 10 days during a 2-week period in a chamber held at the same temperature and relative humidity as the exposure chamber, but in an atmosphere free from ThO_2 dust. The thorium dioxide was dispersed in the exposure chamber as a dry dust prepared by micro-pulverizing material obtained from the U. S. Atomic Energy Commission, Iowa area, and containing 83.4 per cent of thorium, with a loss on ignition of 2.5 per cent.

Toxicological indices followed during both the conditioning and exposure periods were the weight response of the animals, urinary protein excretion, blood NPN and urea nitrogen, and complete hematologic studies on blood films from 3 of the rabbits. There were no deaths among any of the 5 species, and

animals were sacrificed terminally except the 3 rabbits on which hematologic studies are being followed. The gross pathological findings were negative at autopsy. In general, moreover, the clinical chemical determinations yielded negative results. In the case of the rats, a distinct depression of growth was observed in both males and females following the initiation of the dust exposures. The weight changes in the other species were not significant.

Problem Code: Th.4 (Fate)

Section Code: 3220, 3250

Distribution and Excretion of Thorium:

These studies were performed using the UX_1 as a radioactive tracer. The isotope plus thorium sulphate were administered by various routes. All studies indicated that thorium was largely insoluble in body fluids; when given orally it is excreted in the feces, when given intramuscularly most of the thorium is recovered from area of injection, when given intratracheally it remains in the lungs. Following intravenous administration approximately 95 per cent of the dose is recovered from the liver, spleen, and bone marrow, suggesting that the reticulo-endothelial system is the site of deposition.

PROGRAM F.

FLUORIDE

Problem Code: F.4 (Fate)

Section Code: 3210, 3220

To supply evidence useful in the litigation arising from an alleged loss of a fruit crop several years ago, a number of problems have been opened. Since excessive blood fluoride levels were reported in human residents of the same area, our principal effort has been devoted to describing the relationship of blood fluorides to toxic effects.

The principal defect in undertaking this study originally was the lack of a method for determining the fluoride content of blood.

Determination of Fluoride in Blood:

A reproducible, accurate method suitable for routine work was developed which depended upon a preliminary separation of the fluoride from the organic components of the blood by distillation from concentrated sulfuric acid at 134-137° C, evaporation and ashing of the distillate in platinum in the presence of lime, and a final redistillation of the fluoride from concentrated perchloric acid. The fluoride content of a suitable aliquot of the distillate is done by a modification of the thorium nitrate -- alizarin red titration.

It is necessary in the absence of information to determine first the content of fluoride in normal human and animal blood to serve as a base line for the toxicity studies.

Fluoride Content of Normal Human Blood: Application of this method to the determination of the fluoride content of 20 specimens of nonfasting blood of donors whose water supply contained 0.06 ppm fluoride showed a range of from 0-9 micrograms of fluoride per 100 ml of blood; 45 per cent of these bloods contained no fluoride. In contrast to these observations, the analysis of 12 bloods from donors whose community water supply contained 1.36 ppm fluoride showed only 8 per cent of the samples to contain no fluoride; 42 per cent of the samples contained 3-6 micrograms of fluoride per 100 ml. However, the range of values found for the group was no different from the range of 0-9 µg F/100 ml found for the previous group of samples. These data may indicate that the mean blood fluoride level is related to the fluoride content of the community water supply; the data are being extended to include samples from localities whose drinking water contains still greater amounts of fluoride.

Fluoride Content of Normal Dog and Rabbit Blood: In the course of experimental work, 75 analyses were obtained on 30 normal dogs and 20 analyses were obtained for 13 normal rabbits. Food analyses showed the diet of the dogs to contain 13.1 ppm of fluoride; that of the rabbits contained 3.4 ppm. Drinking

water contained 0.06 ppm of fluoride. Approximately 80 per cent of the bloods from both species were found to contain less than 10 μg F/ml. An additional 13 per cent of the dog bloods and 20 per cent of the rabbit bloods contained 10-20 μg F/100 ml. A small number of dog samples contained relatively large quantities of fluoride, ranging up to 70 μg/100 ml.

Routes of Fluoride Administration: Two routes of administration of fluorides have been used: (1) Rats have been given large doses of sodium fluoride intraperitoneally; (2) Rabbits and dogs have been placed in atmosphere of hydrogen fluoride so that inhalation exposures were made.

Blood Fluoride Levels in Rats Following the Intraperitoneal Injection of NaF: Groups of 20 female rats weighing approximately 134 g were injected intraperitoneally with 37.8 mg NaF/kg, the approximate LD50 for rats of this sex and weight. At regular time intervals following the injection, the survivors in different groups were sacrificed and the fluoride content of the pooled blood sample determined. It was found that the fluoride content reached a peak of approximately 900 μg F/100 ml within 30 minutes following the injection; the level then decreased again. Twenty-four hours after the injection, the blood content was again at a normal level of 0-3 μg F/100 ml. In spite of the fact that the maximum blood level is reached within a half hour after injection, significant numbers of fatalities did not begin to occur until 4-5 hours after the injection, by which time the blood fluoride level was reduced to approximately one-tenth of its maximum value.

Blood Fluoride Content Following Exposure to Hydrogen Fluoride: The complete absence of reliable data on blood fluoride levels in previously reported studies of toxic effects of hydrogen fluoride leaves an undesirable gap in the information available regarding the metabolism of fluorides. In order to obtain data of this nature, two 5-day exposures of animals to hydrogen fluoride were completed with blood fluoride analyses being made at regular intervals throughout the exposure period.

The exposure of rabbits to approximately 29 mg HF/m^3 for intervals of 1 to 5 days resulted in a five-fold increase in the blood fluoride level. The blood level reached a plateau after 1 day of exposure and did not increase with continued exposure. The fluoride content decreased immediately following termination of the exposure, but was still significantly above the normal for at least 3 days after the animals were removed from the hydrogen fluoride atmosphere.

When dogs were exposed for intervals of 1 to 5 days to approximately 20 mg HF/m^3, however, the blood fluoride content showed a progressive increase through the 4th day; to a maximal value of 291 μg F/100 ml at the conclusion of the 5th day of exposure, the blood level showed a precipitous drop to 103 μg F/100 ml. The plateau effect so striking in the rabbits was not noted with the dogs; moreover, the maximal level occurring in the dog was approximately 2.5 times as great as was the highest level seen in the rabbit. Five days after termination of the exposure the blood levels were still 3-5 times greater than the pre-exposure levels.

Urinary Excretion of Fluoride:

Work by various investigators has shown that under usual conditions of exposure to fluoride, as in cryolite factories, magnesium foundries or in communities whose water supplies contain appreciable quantities of fluoride, the greater portion of the ingested or inhaled fluoride is excreted by the kidneys. However, these investigations have ignored the relation, if any, between the functional status of the kidney and the corresponding urinary fluoride excretion.

The Effect of Renal Dysfunction on the Urinary Excretion of Fluoride in the Rabbit: The effect of uranium-produced nephritis on the urinary excretion of fluoride has been studied in rabbits receiving: (1) a subcutaneous injection of 0.3 mg U/kg as uranyl nitrate; (2) the same dosage of uranium in addition to 15 ppm fluoride in the drinking water; and (3) 15 ppm fluoride in the water supply. Control urinary fluoride excretion was also determined.

Within 3 to 4 days after injection of the nitrate, the uranium excretion of fluoride in those rabbits receiving only the injected nitrate dropped from a pre-injection level of 0.35 mg F/total daily urine sample to a level of 0.05 mg U/total daily urine sample (a sever-fold decrease). In those rabbits receiving both uranium and fluoride the urinary excretion dropped from a pre-injection level of 1.9 mg F/total daily sample to a level of 0.25 mg F/total daily sample, which again is approximately a seven-fold decrease. The urinary excretion returned to the pre-injection level in both groups approximately 12 days after the injection.

Fluoride content of the tooth and bone indicated that the presence of uranium in the kidney may inhibit the deposition of ingested fluoride in the bone and thus offer a degree of protection against fluorosis. The fluoride contents of the tooth root, femoral epiphysis and jaw alveolar bone for rabbits receiving only added fluoride were singificantly higher than in comparable tissues from rabbits treated with both uranium and fluoride, despite the greater quantity of fluoride consumed by these latter animals. Other supporting evidence for the protective effect of added fluoride on uranium poisoning was: (1) a two and one-half-fold lower blood urea nitrogen; and (2) lowered mortality in those rabbits receiving both agents, as compared to the results obtained in rabbits receiving only the uranium injection.

The Determination of Urinary Fluoride Excretion as a Possible Test for Renal Dysfunction: The determination of the urinary excretion of small doses of ingested fluoride has been measured in two individuals with normal renal function, and in two patients with known abnormal kidney function. One patient with chronic glomerulonephritis was only able to excrete 20 per cent of the ingested fluoride; the second patient with chronic pyelonephritis with hypertension could excrete none of the fluoride. The two control individuals excreted 45 and 51 per cent of the dose of ingested fluroide.

These preliminary data indicate that the measurement of the urinary excretion of a test dose of fluoride may prove of value in detecting certain types of renal disorders.

Fluoride in Bone:

The problem of the mechanism of deposition of fluoride in bone has never been settled. This problem is doubly important: It is basic to an understanding of fluoride toxicology and also to the use of fluoride in the prevention of dental caries. A program of investigation was designed to test whether fluoride ion underwent ionic exchange with some grouping in the bone mineral substance. It was found that, for the most part, fluoride exchanges for hydroxyl ion in the apatite lattice of bone. This process is analogous to that observed for uranium.

PROGRAM I.S.

ISOTOPES

Problem Code: I.S.1 (Tracer Chemistry)

Section Code: 3120

Studies of Protein Metabolism in the Normal Dog Using C^{14}-Labeled Lysine:

Background: Studies of protein nutrition and protein metabolism are of fundamental importance in seeking an understanding of a variety of phenomena of basic concern to modern medicine. Blood protein production and utilization, wound healing, convalescence, the resistance of kidneys and liver to specific toxic agents, and the physiological burden of pregnancy are among the outstanding fields of interest in which it is already recognized that adequate protein nutrition and an understanding of protein metabolism are critically involved.

Because under normal conditions the concentrations of the various blood protein components are maintained within narrow limits by physiologic mechanisms, it was impossible by classical methods to measure either the rate of production, or the rate of disappearance of a given protein component.

The element carbon is an integral chemical component of the amino acids from which the proteins are fabricated by the body. If one could label or tag carbon atoms and thereby label amino acids, the incorporation in a protein of such a labeled amino acid would become apparent to an investigator equipped with the necessary tools for detecting and measuring the presence of such a label.

The nuclear reaction pile has made available C^{14}, an isotope of carbon, which is chemically indistinguishable from ordinary carbon, C^{12}, and which is labeled by virtue of the fact that each C^{14} atom has a known probability of emitting a beta particle detectable by suitable measuring instruments. The organic chemist has synthesized amino acids (such as the lysine used in our studies) with the C^{14} atoms in a known position in the amino acid molecule. Such amino acids can be fed to animals and are absorbed and metabolized just like the chemically identical amino acids of the food proteins.

1. The Distribution of C^{14} Activity 24 Hours After Ingestion of -C^{14}-Labeled DL-lysine: C^{14} labeled DL-lysine has been fed to normal dogs and the rate of appearance of the labeled amino acid in the various blood protein fractions becomes a measure of the apparent rate of production of the various blood and plasma protein fractions. By the same token, after the single dose of labeled amino acid has been built up into proteins, the relative rates of disappearance of the activity from the red cells and plasma protein fractions becomes a measure of the disappearance of the fractions themselves. Such conclusions are valid insofar as it is impossible for single amino acids to

"slip in and out of" the giant protein molecules without complete destruction of the protein molecule. In other words the loss of a radioactive amino acid label is an indication and a measure of the loss of a whole protein molecule and its replacement by an identical but non-radioactive molecule. These considerations are applicable to the blood proteins, but in the case of the tissue proteins evidence exists for the replacement of single amino acids in tissue protein molecules without complete or very profound degradation of the parent molecule.

Out studies in the normal dog have demonstrated rapid and extensive incorporation in blood and tissue proteins. In a typical experiment about 3 per cent of the fed dose of radioactive DL-lysine was present in the circulating plasma proteins at the end of 24 hours; at this time the serum albumin activity per gram was about twice that of the globulin fraction. However, determinations on specimens removed at regular intervals during the 24-hour period suggested that the globulin fraction was actually being formed and, therefore, turned over at a greater rate than albumin. This conclusion was confirmed by other studies mentioned below.

The labeled blood plasma of such an animal was removed with heparin as an anticoagulant and used to trace the fate of plasma proteins when injected into a recipient animal (See I, 2).

The distribution of the isotopically labeled amino acids fed to normal animals yields a body of data essential for evaluation and comparison of results to be obtained from animals in various abnormal or pathological states.

2. The Fate of C^{14}-Labeled Plasma Proteins When Injected Intravenously Into a Normal Dog:

Background: A number of investigators have used isotopically labeled plasma proteins to trace their removal or disappearance from the circulation of normal animals into which they have been injected. As a rule, these studies made no attempt to distinguish between the various plasma protein fractions and in some instances, the proteins were treated chemically in a manner which might seriously alter their native state. In general it was found that one-half of the injected proteins had been removed from the circulation (and replaced by non-isotopic proteins) in 24 to 48 hours.

Work done during the calendar year 1948: In two experiments normal dogs were injected intravenously with plasma obtained from a normal dog which had previously been fed C^{14}-labeled lysine. Analysis of samples of blood plasma removed at intervals reveals that one-half of the injected albumin had been removed from the circulating blood in 55 to 58 hours, and one-half of the globulin fraction had been removed in 38 to 44 hours. Thus, even in the normal dog the plasma globulin fraction is turned over more rapidly than the albumin. This conclusion has been verified by a study of the albumin and globulin activities of the dog in which a single feeding of labeled lysine was made as in I, 1 of this report, and samples of the plasma taken at intervals over a period of five weeks.

Studies of the type just described are of significance in any attempt to understand the pathological physiology of certain disease states (e.g. chronic nephritis, hepatic cirrhosis, etc.) in which it has been impossible to ascertain whether the abnormally low plasma albumin is the result of abnormal loss, or a result of failure to produce albumin at a normal rate.

Proposal for future work: By injecting plasma proteins of sufficiently high specific activity, it is hoped to follow the utilization of the plasma protein by the various tissues. This will subject to experimental scrutiny the hypothesis that the circulating plasma proteins are normally in a dynamic equilibrium with the tissue proteins which equilibrium may be disturbed in disease.

3. The Fate of C^{14}-Labeled Plasma Proteins When Injected into the Peritoneal Cavity of the Experimentally Ascitic Dog:

Background: Certain disease states such as cirrhosis of the liver and carcinomatosis are associated with abnormal accumulations of fluid in the abdominal cavity known as ascites. The ascitic fluid is very rich in proteins; in fact an animal or human may have an ascitic accumulation containing several times as much albumin as is present in the entire circulating blood.

The availability of C^{14}-labeled plasma proteins makes it possible to study the rate of exchange of the protein between the abdominal cavity and the circulating plasma.

Work done during the calendar year 1948: In a typical experiment, C^{14}-labeled plasma protein was obtained from a normal donor dog previously fed C^{14}-labeled lysine. The plasma was injected into the abdominal (peritoneal) cavity and the rate of appearance of the labeled plasma in the circulating blood was followed at intervals. The data show more rapid movement of the (smaller) albumin molecules into the circulation; furthermore, the exchange between the abdominal cavity and the blood occurs so rapidly that within 25-30 hours a steady state is approached in which the blood level of labeled albumin becomes constant.

Proposal for future work: Techniques are known for perfusing surviving livers with blood under known oxygen tensions. Thus, it is possible to maintain the liver under conditions closely approximating the in vivo state. It is proposed to use surviving livers to study directly their contribution towards the production of specific plasma protein fractions. Conversely, by perfusing a normal surviving liver with blood containing labeled plasma proteins, the role of the liver in metabolism of the plasma proteins can be studied.

4. Studies of the Protein Stability and Life Span of the Dog Erythrocyte:

Background: Until the advent of isotopic tracer methods, the average life span of the red cell was ill determined. Values ranging from about 15 days to 150 days or more were quoted. The most reliable figure, based on quantitative studies of blood hemin formation and bile pigment excretion, was 125 to 130 days.

Work done during the calendar year 1948: C^{14}-labeled lysine was fed in a single dose to a normal dog previously rendered anemic by repeated bleeding. Under the stimulus of the anemia, the dog rapidly made new red cells incorporating the fed isotopic amino acid. The animal was then bled only small amounts for C^{14} assay at intervals over a period of 150 days. The results, based on the decrease of the C^{14} content of the circulating blood cells, indicate an average life of 115 days for the erythrocyte protein as an entity not exchanging with extracellular components. This figure corresponds so well with the best life span figures, that it indicates these experiments have independently measured the life span of the red cell. In addition it strengthens the opinion that red cell protein is not actively altered during the life of the circulating normal dog red cell. This makes the red cell unique among all the cells of the organism.

Furthermore, on the breakdown of the red cells at the termination of their "life span" the hemoglobin protein is not preferentially re-utilized for the formation of new red cells.

Proposal for future work: Cogent circumstantial evidence exists that the protein component of hemoglobin, viz., globin, can be utilized to meet the protein needs of the organism. By giving labeled hemoglobin (laked red cells) parenterally, it will be possible to study the sites of hemoglobin breakdown and utilization.

5. The Intermediary Metabolism of Lysine:

Background: It is known that many amino acids undergo metabolic conversion to chemically related but different metabolites before their incorporation into proteins. Hence, without isolation, adequate chemical characterization and identification, and isotope assay of the individual amino acids, one cannot be certain of the exact chemical binding of the C^{14} administered as C^{14}-labeled lysine.

Previous attempts by other workers seeking to detect the conversion of lysine to other amino acids were unsuccessful, chiefly because of the methods employed.

Work done during the calendar year 1948: Accepted methods for protein hydrolysis and amino acid isolation have been adapted and improved for the small scale isolation of lysine, arginine, histidine, glutamic, and aspartic acids. It has already been found that lysine may be converted into at least three other amino acids. Hence, the total C^{14} activity of a protein from an animal fed C^{14}-labeled lysine can be resolved into components attributable to lysine, glutamic acid, aspartic acid, and arginine, as well as at least one more unknown component, probably the amino acid proline.

The observation that lysine may be converted to arginine by the animal accounts for one possible source of the latter when the diet contains little or no arginine.

Proposal for future work: It is expected that the duration of the problems on protein metabolism will be indefinite (minimum of three years).

II. The Preparation of Antisera to Normal Tissue and Tumor Components:

1. The Use of Radioactive Tracers in Following This Reaction Between Tissue and Tumor Components and Specific Antisera:

Background: Attempts to treat neoplastic disease (cancer) with antisera were first made over fifty years ago. More recently antisera for normal tissues have been prepared and within the last year the preparation and immunochemical assay of antisera for normal adult tissues, embryonic tissue and tumor tissues have been described. In one report the preparation of anti-rat kidney serum labeled with radioactive iodine was described and presumptive evidence for its preferential localization in the rat kidney after injection was presented. There is considerable room for a careful quantitative study of the reaction between isotopic-labeled antisera and normal tissue and tumor components, first to demonstrate the specificity of the reaction in vitro and then to study the reaction in vivo. If a highly specific fixation of radioactively labeled antiserum can be affected, it will open up a possibly effective mode of therapy for cancer.

Work done during the calendar year 1948: Extracts of normal rat liver and kidney tissue have been prepared and methods of producing antisera in quantity have been worked out using a precipitin reaction for controlling titres of antibody.

The work on the radioactive iodination is now in progress.

Future proposal of work: It is estimated that the duration of this problem will be about two years.

Problem Code: I.S.2 (Autoradiography)

Section Code: 3171

Autoradiographic Techniques:

Background: Autoradiography is a new field and offers a powerful tool to the pathologist, physiologist, biologist, and biochemist.

1. Stripping Film Technique: Stripping film is a film specially prepared so that the photographic emulsion is on a ten micra thick cellulose ester base which is, in turn, temporarily bonded to a thick supporting film to facilitate handling. On the occasion of its use, the emulsion and thin base may be stripped from the supporting film. This provides one with an emulsion coated on a thin water impervious base. This arrangement has been of great use in the development of autoradiographic techniques where it is desirable to mount the tissue and photographic emulsion in a permanent relation. A number of specific techniques have been developed depending on this device.

2. Calibration of Film: With the increasing use of autoradiographic techniques, it becomes important to determine the most satisfactory emulsion or emulsions for this purpose. Comparative studies of several films and plates have been made to determine the fastest emulsion with the lowest background and highest resolution for cytological, histological, and gross autoradiographs. The I^{131} beta spectrum was used; Eastman NTB emulsion was found best for cytological and histological work. Data is not yet finished to permit a choice for the best gross autoradiographic emulsion.

3. Autochemography: We have shown that several tissues, when fresh, fog a photographic plate giving a characteristic pattern. The various tissues fog at a different rate. Bone marrow, kidney, liver, etc., fog at various rates even in the deep freeze unit while blood does not fog. This we assume, is associated with one or several reducing agents present in varying quantities or having varying reducing potentials. This has been termed autochemography. It is obviously a matter of importance to gain information on this phenomenon since chemical emulsion fogging might otherwise be wrongly attributed to the activity of the specimen.

It is also a matter of interest in its own right. Experiments in progress have suggested that autochemography may have a place as a histochemical technique. The photographic plate is more sensitive than most chemical methods in that one has a chemical level of about 10^6 through the catalytic process of development.

A particular application which has been of interest to this laboratory section is the possible use of this tool in the diagnosis of cancer. A preliminary experiment has shown that a section of gut demonstrating cancerous tissue produced fogging. Methylene blue studies have shown that the blood of 80-95 per cent of proved cancer contains a reducing substance in the blood. Since plate fogging is believed to be due to reducing substances, it may be that a photographic plate method would have advantages over the methylene blue procedure.

4. Freezing Microtome Techniques: With short half-life materials it is desirable to reduce so far as possible, the time required for the histological preparation of the tissue, as well as to avoid losing the radioactive material in the fixing solutions normally used. This laboratory has, therefore, developed a technique for cutting a frozen section of seven micra thickness and transferring while thawed to a photographic plate in the darkroom. Another technique has been developed for cutting a ten micra section and, while keeping it in a frozen condition, transfer to a photographic plate in the darkroom. It is planned to carry the technique one step further, i.e., separate the tissue into individual cells moved laterally from one another.

Problem Code: I.S.2 (Autoradiography)

Section Code: 3171

Autographs of Blood Cells Containing C^{14}-Labeled Glycine:

Background: Since Altman, et al demonstrated that the alpha carbon of glycine labeled with C^{14} is incorporated into the hemin and globin moieties of hemoglobin, it was believed that the incorporated C^{14} in an individual blood cell could be demonstrated by an autoradiograph.

Work done during calendar year 1948: In collaboration with the Radiation Chemistry and Radiation Physiology Sections of this Division, an experiment was performed in which a rat was given 3 μc of glycine containing C^{14} in the alpha carbon atom. Blood was taken from the tail vein 24 hours after administration of the dose, diluted with serum made from dog blood, and smeared directly on an Eastman NTB emulsion. The smears were dried in air and fixed in methyl alcohol. After 67 days exposure, the plates were developed and cleared, and the cells were stained with Wright's stain.

Excellent autoradiographs were obtained. In general, it may be said that the percentage of cells of each type associated with definite autoradiographs declines in the order: lymphocytes, polymorphonuclear, leucocytes, erythrocytes. This difference may be associated with the fact that the rate of turnover of the different cell types decreases in the above order.

A second finding was that the concentration of silver grains appears to be higher in the case of the lymphocytes, which may be correlated with the higher concentration of nucleoproteins in the lymphocyte. This follows from the assumption that a large part of the C^{14} activity may be associated with the nucleoproteins built up in part from glycine.

This work has been published in Science, 108, 529 (1948).

Proposals for future work: The radiation Chemistry Section of this Division plans to continue its study of blood formation and this section proposes to cooperate in respects where autoradiographs may make valuable contributions.

Problem Code: I.S.3 (Therapy)

Section Code: 3110

Preparation and Measurement of Sr^{90} Films in Eyecups:

Background: This problem was undertaken in collaboration with Dr. A. H. Dowdy of the University of California Medical School and is concerned with a new device for treatment by radiation of surface conditions of the cornea

of the eye. It is desirable that the radiation used for this purpose produces its greatest effect within the first 1 or 2 mm of depth in order that the lens of the eye receive no injurious effects. It is furthermore expedient to administer the treatment in a period of time not longer than ten minutes. It seemed possible that a beta emitter, such as Sr^{90}* might be used in this connection.

Work done during the calendar year 1948: The first part of this problem involves the construction and measurement of a suitable applicator. This phase of the work has been tenatively completed and further modifications depend upon the clinical findings and tests which are in progress.

The details of the preparation of the applicators can be briefly summarized. To carrier-containing Sr^{90} solution, gelatin was added. Films of gelatin of uniform thickness were prepared and these films were cemented to the inner surface of the eyecups, each film covering approximately one 90° quadrant. An equal area of the same shape cut from the same film preparation was cemented onto a flat brass plate. Both films were covered with a layer of butyl methacrylate. Dosage measurements were made on the flat film with a specially constructed extrapolation chamber calibrated by means of x-irradiation. The dose delivered by the finished preparation was measured to be 12 r per minute average to the first millimeter of tissue.

Proposal for future work: If the clinical tests turn out satisfactorily, beta applicators of varied shapes, designed for a number of uses, may be made.

Therapeutic Use of Radio-iodine, I^{131}:

Background: This work is a continuation of a collaborative program involving the Department of Radiology of Strong Memorial Hospital. The program is concerned with the use of the eight-day half-life isotope of iodine partially or wholly to destroy thyroid tissue both in normal situ and metastatic. The division of labor is such that the Radiology Department critically selects suitable cases for treatment from the group of patients referred by hospital staff members. An agreement is arrived at in regard to the suitable dose. The personnel of this Section, having measured the activity of isotope shipments from Oak Ridge, make up the dose for the particular patient and analyze urine and biopsy samples, if any, for beta activity. Frequently gamma counts are made with a lead columnated G.M. tube at points of interest around the surface of the patient's body subsequent to administration of a tracer or therapeutic dose. Autoradiographs are made in all cases where samples of biopsy thyroid tumor are obtained.

Work done during calendar year 1948: In general we have used iodine, I^{131}, to treat three kinds of cases: Graves's disease, thyroid tumors, and a third

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*Sr^{90} emits beta particles of E_{max} = .65 MEV with a daughter product of Yttrium with a beta particle of E_{max} = 2.2 MEV.

group where destruction of thyroid tissue is indicated in treatment of some condition not involving thyroid disorders per se. The following summary indicates the scope of the treatment program for 1948:

	Graves's Disease Patients	Suspected or Verified Tumor Patients	Other
No. of total doses (Including tracer studies)	13	13	3
No. of therapeutic treatments	12	3	3
Amount in millicuries I 131 dispensed	89	104	25

In general, it may be noted that the treatment of the Graves's disease patients has been uniformly marked with success and enjoys the enthusiastic cooperation of the hospital staff. The tumor cases that we have treated have shown improvement. The question as to whether cures will be produced can only be answered after longer treatment and observation. The cases in which the thyroid was destroyed in order to treat heart disease have given satisfactory results.

Proposal for future work: It is proposed to continue this collaboration program with an increased emphasis upon measurements of a research nature in order to improve the clinical treatment procedures.

Effect of X-irradiation on the Ability of Rat Thyroids to Take up Iodine:

Background: Since this Section of the local Project in collaboration with the Radiology Department of Strong Memorial Hospital has undertaken a program employing radio-iodine as a therapeutic tool, the question of the radiation resistance of the thyroid gland is important for our work as well as the work of many other therapeutic users of I^{131}. As a first objective, we proposed to answer experimentally the question "What is the maximum dose of x-irradiation which has no effect on the rat thyroid as measured by its ability to take up iodine and to bind iodine in organic form?".

Work done during the calendar year 1948: Our method of investigation has involved the local x-irradiation (250 k.v. filtered so that the half value layer was equivalent to 2.15 mm copper) of the thyroids of rats placed on an iodine-low diet, and the test of thyroid function by administering I^{131} in a small single dose at varying intervals of time after irradiation. The animals were sacrificed four hours after administering the iodine dose, and the excised thyroid glands were analyzed for total I^{131} content and separately for dialyzable and non-dialyzable fractions.

The present status of the work is that tests have been completed for 1000 r dose of x-irradiation and are underway at the 3000 r level. The findings are that thyroid function is not impaired at the 1000 r dose level. On the basis of the incomplete data for 3000 r, it appears that there is likewise no effect at this dose.

Proposal for future work: It is proposed that the studies at the 3000 r level be completed. It may be difficult to extend the x-irradiation to higher doses due to systemic effects found in spite of careful columnation of the x-ray beam.

As a corollary to this dose study, it is proposed that autoradiographs be made of rat thyroids and human biopsy thyroid specimens to study the space distribution of iodine, I^{131}, in the gland. Emulsion grain counts per unit area would be made on an area of the autoradiograph sufficiently large to be representative of the tissue. This should obviously be done as a function of time after the administration of the dose and perhaps as a function of the dose.

PROGRAM O.S.

OUTSIDE SERVICE

Problem Code: None

Section Code: 3310

The nature of the work carried out under Code 3310 (Outside Service) during 1948, has been the supplying and processing of film badges for radiation monitoring, and liason work with the New York Operations Office involving the analysis and reporting of various samples collected in the plants under their direction. During the year the majority of the film monitoring was taken over directly by the New York Office so that by the latter half of the year we were processing only 1/8 of the amount previously received. These few films were from places like Brookhaven, Columbia, California and the University of Rochester Physics Department where the monitoring involved radiations additional to beta and gamma.

The balance of the work performed by this Section was concerned with the receiving of samples approtioned to other Sections, chiefly, 3110, 3150, and 3210 for analysis and final reporting of results. Included also was the preparation of certain sampling containers and shipment of such sampling equipment to various sites. The number of different types of samples handled by this Section for the calendar year 1948, are as follows:

Smears:	20
Urines:	
Uranium	718
Fluoride	338
Beryllium	53
Beryllium dust	2197
Uranium dust	168
Radon air	354
Radon breath	9
Fingerprint	916
Soil:	
Uranium	104
Beryllium	4
Radium	5
Liquid:	
Uranium	54
Fluoride	31
Beryllium	3
SO_4	17

Miscellaneous: (Tissues for analysis - done by Dr. Steadman)	31
Films	14,031

PROGRAM P.H.

PROJECT HEALTH

Problem Code: None

Section Code: 3330, 3351

Activities in the Project Health group have consisted in establishing a medical code for personnel of this Project, for pre-employment and termination examination of individuals employed here, and the necessary first aid for individuals employed here, and the necessary first aid for individuals injured on the job.

The second activity in cooperation with the Hematology group has been concerned with the careful evaluation of possible exposures to beryllium and its compounds to Project personnel working on the inhalation chambers. On such areas, an occasional small concentration of beryllium in the atmosphere has been observed. Careful examinations, x-rays, hematology, and analysis of beryllium concentration in 24-hour urines have been carried out. To date no apparent abnormalities have been noted although this project is continuing.

PROGRAM H.P.

HEALTH PHYSICS

Problem Code: H.P.1 (Research and Development)

Section Code: 3320

Investigation of Film Behavior:

The major interest in the area of developmental health physics has involved the thorough investigation of the use of photographic film of varying types in the accurate measurement of ionizing radiations of wavelengths ranging from 1 angstrom to 0.001 angstrom. Investigations were directed first to the x-ray measurements, inasmuch as generators were available.

The plan of attack has consisted in the main of the measurement of absorption curves from the generators. Such measurements ultimately reveal the half-value layers for specific pre-filtration. With such values known, all data could then be referred to the respective half-value levels for the specific wavelength ranges. Using the available .14 MEV. and a 1.0 MEV generators, films were exposed in algebraic progression uniformly developed together with sensitometric control strips and measurement of each exposure correlated by simultaneous measurement of ionization by a Victoreen "r" meter calibrated against a standard chamber (see later section).

Data obtained from this method were correlated with measurements made in a parallel experiment with personnel of the Department of Radiology in the medical School. The film packets studied were equipped with two copper filters 0.12 and 0.24 mm in thickness, thus making areas of three different densities available for interpretations. Other measurements were made simultaneously with pencil chambers and fixed area monitors. Such data were analyzed and a technique developed for reducing measured densities in film to absolute exposure values for the different energies received by the film. Details and tabulations concerning such measurements are given in the Quarterly Technical Report for July-September, 1948 (Rochester Report UR-45).

With the success of the present method plans are in progress to carry this investigation, not only to higher energies, but also to those complex energies found in the analysis of film following exposure to beta irradiation. To speed the carrying out of such measurements a photographic sensitometer has been designed and constructed. This apparatus consists of an aluminum cassett containing the photographic emulsion which is brought into a beam of the desired radiation by means of a stepping control. This latter control can regulate exposure either by algebraic or geometric progression. At the same time, simultaneous recording of the total radiation dose is achieved by the use of an integrating recording ionizing chamber. Plans call for the investigation not only of varying types of radiation but varying grades of sensitive compounds of emulsions to each specific type.

With the aid of the Bureau of Standards a standard type of free air ionizing chamber has been constructed and is being used in the previously discussed calibration scheme. Plans for the extension of the ranges of this instrument beyond that of the present 250 k.v. radiation are well under way, and it is hoped that ranged beyond 1 MEV. can be attained, thus allowing for measurement of gamma radiation from standard radium sources and the like.

Problem Code: H.P.2 (Service)

Section Code: 3320

Monitoring:

A separate activity of this Section has been concerned primarily with routine monitoring of the facility including contaminated areas, exhaust stacks and fixed ionizing generators. Surveys have indicated that no major radiation hazard exists.

Chemical analysis for possible chemical hazards has likewise been carried out on a routine basis for control of exposures, particularly beryllium compounds.

A small "hot" laboratory has been constructed and is supervised by this group.

PROGRAM C.S.

SPECIAL CLINICAL SERVICE

Problem Code: None

Section Code: 3312

During the year seven patients with chronic beryllium poisoning were studied, the major efforts being directed into two distinct areas: (1) respiratory physiology, and (2) metabolic study. In addition, for comparative purposes, one patient with chronic liver disease and one patient with pulmonary fibrosis with severe anoxia were studied. Results of all of these experiments have been reported in detail in the Quarterly Reports covering this period (Rochester Reports UR-21, UR-38, and UR-45). Significant findings on the respiratory studies of these various patients can be summarized as follows: The main deviation from the normal is hyperventilation, particularly during work; this compensating for the produced hypoxemia. The observed diminished arterial saturation is a function of abnormally increased alveolar-arterial gradient for oxygen and a contracted lung volume, the complemental air being particularly involved. Polycythemia may also be seen as a compensatory mechanism but is not observed in the majority of cases. Advanced cases of varying stages of cor pulmonale and occasionally congestive heart failure occur as a result of the increased pulmonary resistance. Of definite interest is the daily variation in the intensity of the dyspnea which can be only related to the psychological results of an occupational disease and the anxiety so produced. It is possible that definite alterations in the water and electrolyte exchange may produce increased awareness of dyspnea from a subjective viewpoint. Results of respiratory studies are reported in extensive detail in Rochester Report UR-43.

Results of the complete metabolic study on five patients and partial study on two additional patients yield the following information: Patients with chronic beryllium poisoning definitely present a picture of a generalized disease associated with excessive weight loss, hypoalbuminemia, evidence of liver disease as well as renal disease and laboratory evidence of disturbance in nitrogen metabolism. From respiratory studies, it is quite obvious that anoxia presents a definite complicating picture.

Careful study of the nitrogen metabolism of four patients, only three of whom exhibited marked respiratory embarrassment, all exhibited large weight losses. Nitrogen balance studies on very high nitrogen intake showed marked difficulty in establishing good evidence of nitrogen storage. Fecal nitrogens were in excess of the normal amount, suggesting difficulty in absorption; evidence of liver disease would suggest the possibility of difficulty in utilization, and anoxia of the bowel might likewise be contributory to faulty absorption and utilization. However, stool analysis for fat on two occasions was not observed to be high as would be expected if faulty absorption was a major factor. The possibility of increased excretion of nitrogenous substances into the gut could be raised, although this possibility has not yet been investigated. In view of the presence of liver involvement and possible faulty

metabolism based on such pathology, one patient with liver disease was investigated in a similar fashion and showed a marked ability to store nitrogen, particularly under the influence of testosterone. Adequate trial of testosterone did not cause appreciable storage in patients with chronic beryllium poisoning. At the present time the effect of anoxia on protein metabolism is being studied in individuals whose anoxia is not attributable to chronic beryllium poisoning. Results are not yet ready for analysis. Complete summaries of accumulated data on the metabolic experiments may be found in the Quarterly Reports covering this period, to which reference can be made for more detail.

The daily urinary 17 keto-steroid excretion was studied in three men and three women. In contrast to the normal value obtained in this laboratory, all showed marked decrease in keto-steroid excretion. In the women, values of 5.1, 2.8, and 4.7 (normal 6-20 mg/day) were noted. In the men, values of 3.0, 3.6-6.0, and 7.0-8.7 mg/day (normal 13-25 mg/day) were found. Urinary gonadotrophins in two men were determined, and subnormal values were also obtained. The significance of these findings and their relation to nitrogen metabolism is as yet unknown but is being further investigated. Complete details will be given in a future report.

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: 3110

Instrumentation Research and Development:

Background: This problem category includes machine shop and electronics shop work in the design and construction of new equipment to be used by the various Sections in carrying out their research programs.

Work done during the calendar year 1948:

1. C^{14} Program:

   (a) Built vane ionization chamber and electrical components according to Dr. W. F. Bale's design.

   (b) In connection with C^{14} counting method built special counter tubes, lead tube shield, quenching circuits, and other electrical components as well as glassware for tissue digestion and CO_2 handling.

   (c) Rebuilt proportional counter for tests investigating suitability of this method for C^{14} counting.

2. Radon Measurement Program (R.M.4):

   (a) Built an ignition furnace for heating charcoal cartridges.

   (b) Made mask parts for use in collection of breath radon.

   (c) Made hermetically sealed containers for charcoal cartridges.

3. Radon Measurement Program (O.S.):

   (a) Redesigned and rebuilt vane ionization chamber for air bulb measurements.

4. Sr^{90} Film Measurements (I.S.3):

   (a) Built small extrapolation chamber probe and probe chamber.

   (b) Built associated electrical circuits.

   (c) Built metal wells for preparation of films.

5. Miscellaneous Items of General Use:

   (a) Counter probe and electrical circuits for survey of laboratory bench-top contamination.

   (b) Hand counter tube and shield for checking hand contamination.

6. In Connection with Teaching Program:

   (a) Special apparatus to carry out measurement of range of α particles.

   (b) Cloud chamber to observe and photograph tracks made by alpha and beta particles.

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

Section Code: 3150

Development and Improvement of Spectrographic Methods of Analysis:

Background: Spectrographic methods have proved a very useful tool in the quantitative assay of such metals as uranium, beryllium, and thorium.

The uranium content of tissues is rarely large, even in tissues containing the greatest amount of uranium after long-time exposures. Amounts of uranium less than 1/10 of a μg frequently are encountered. Obviously the former uranium method with its sensitivity of about 0.2 μg could not be employed.

One of the most mystifying aspects of beryllium poisoning is the extraordinarily minute traces of beryllium present in the tissues, for example, in the lung of patients dying from chronic granulomatosis or from acute pneumonitis. No sensitive chemical method is available. In the experimental animals, likewise, only the minutest traces of beryllium are found in many of the tissues following exposure to beryllium dust-laden air. Providentially, the spectrographic method for beryllium is one of, if not the most, sensitive analytical methods known.

The probelm of thorium distribution and excretion is complicated by the extraordinarily small quantities of thorium which ordinarily are found in the tissues. There is no chemical method applicable.

Work done during the calendar year 1948: The sensitivity of the methods developed in this laboratory for the determination of uranium was increased by about a factor of ten. The present sensitivity is about 0.02 micrograms uranium. The method is described in the Rochester Report UR-41.

The sensitivity of the method developed in this laboratory for the determination of beryllium, in urine in particular, was increased by about a factor of five. The smallest amount measured is 0.002 micrograms. Sensitivity for urine measurement is about 0.01 micrograms per liter using a 500 ml sample. A comprehensive abstract of the method was written up as a Rochester Report UR-18, and made available to industrial analytical laboratories. Standardization measurements and consultations were carried out in collaboration with several spectrochemical laboratories outside the Atomic Energy Commission for the purpose of unifying the methods of analyses to be used in the beryllium industries.

The sensitivity of the method developed in this laboratory for the determination of thorium was increased by about a factor of ten. The present sensitivity is about 0.01 micrograms.

Proposal for future work: It is proposed to continue this research in order to improve the spectrographic methods of analyses of materials of interest to the Project.

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

Section Code: 3161

Research and Development of Electron Microscope Techniques:

Background: Requests are made to this Section for electron micrographs of a great variety of materials. In order to get satisfactory results, it is necessary to maintain an active program on research and development of new handling techniques.

Work done during the calendar year 1948: Below are listed the improvements in technique which have been realized during the past year:

1. A special method was developed for electron micrographs of glass beads. These beads were used in calibration of equipment for measurement of the surface area of particles.

2. A photo-electric device was developed which measures the intensity of illumination of a fluorescent screen in the path of the electron beam and provides a means of predetermining the correct exposure of plates in the electron microscope.

3. A standard microtome was altered to make possible the cutting of histological sections of the order of 0.1 to 0.2 micro in thickness.

4. A magnetic beam splitting device was constructed which is attached externally to the electron microscope. The magnetic

field from a pair of coils separates the electron beam into two components which, if the instrument is not correctly focused, results in two images of the specimen. When exact focus is achieved, the two images move into coincidence. The effect is similar to that of a range finder of a camera.

5. A technique for the easy production of silica films for use as a specimen support was developed.

6. An instrument to mark the electron microscope specimen was devised which makes possible the localization of the particular area for examination in the optical and electron microscopes. It also simplifies the finding of the same area in the electron microscope whenever desired.

7. A preliminary investigation of a new photographic emulsion in collaboration with Eastman Kodak promises an improved plate for electron microscopists. Thus far, it is indicated that the plate will have greater sensitivity to the electron beam, greater contrast, and a several-fold gain in resolution over the best plates used in the past.

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

Section Code: 3320

Neutron Detector:

By arrangement with the University this Section has been developing a program on health safety for the new 130-inch cyclotron. Early evaluation of the proposed program indicated a necessity for a suitable neutron indicator capable of measuring high intensity radiation.

An instrument has been designed in which it is hoped that adequate compensation for gamma radiation can be gained similar to that of other instruments developed elsewhere. This instrument consists of two ionizing chambers of different sensitivity in which the central collectors are connected and the outer electrodes have opposing high potentials. If an imbalance of the central electrodes is produced by a neutron beam, the current so produced is amplified by a vacuum tube electrometer and direct coupled amplifier, and the output fed either into a milliammeter or a continuous paper recorder. This instrument is now under design and appears to react well to the energy beam produced by the cyclotron. Calibration for high energy neutrons is to be attempted by means of the C^{12} (n,2n) C^{11} reaction occurring at high energies. More complete results of these studies will be reported elsewhere.

Problem Code: I.N.2 (Service)

Section Code: 3110

Instrumentation Service:

Background: This problem category includes building and repair of standard electronic and mechanical equipment where no new design or research is indicated. It also includes routine measurements performed for other Sections of the Radiology and Biophysics Division.

Work done during the calendar year 1948:

1. Measurement of Polonium Foil Samples:

   (a) 1412 foil samples were analyzed for alpha activity.

2. Service and Repair of Counters:

   (a) Six counters in use by this Division have been maintained in operating condition.

3. For Radon Measurement (R.M.4):

   (a) A duplicate alpha gas chamber and associated electrical circuits were built.

4. Construction of Miscellaneous Items:

   (a) Eight G.M. tubes were built, filled, and are being tested.

   (b) A scaling circuit was constructed for use in testing new G.M. tubes.

   (c) An alpha plate counter electronic circuit was built.

   (d) A standard ionization chamber power supply was constructed.

   (e) A small portable ionization chamber was constructed.

   (f) Twelve screw thread remote filling devices for pipettes were constructed.

5. Glass Bench:

   (a) Glass bench supplies were purchased and glass bench facilities maintained as a Division service.

Problem Code: I.N.2 (Service)

Section Code: 3150

Spectrographic Service Analyses:

Background: The results of analyses made for personnel of the Rochester Project have been incorporated in the research programs of several Sections. Analyses have been done mainly for beryllium.

Work done during the calendar year 1948: These analyses are divided into three principal types:

1. Analyses of human urines from patients studied by the Division of Medical Services. Number of Analyses - 102.

2. Analyses of animal tissues for distribution studies by the Division of Pharmacology and Toxicology. Number of Analyses - 401.

3. Air dust analyses for control of beryllium dust chamber concentrations for the Pharmacology Division. Number of analyses - 1505.

Work planned for the future: It is anticipated that this service will continue for an indefinite period.

Problem Code: I.N.2 (Service)

Section Code: 3161

Electron Microscope as Used for the Examination of Dusts:

Background: The electron microscope was installed for the purpose of studying the morphology of the numerous particulate materials (dusts) used in the various animal exposures.

As an example of the usefulness of this approach, the measurements on beryllium dust may be cited. Some time ago, Mr. Eisenbud of the New York Operations Office came to us with the suggestion that one of the grades of beryllium oxide might be responsible for much of the poisoning found in beryllium workers. It immediately became important to see whether any differences, physical, chemical or toxicological, could be discovered between the various grades of beryllium oxide. A dramatic demonstration of the difference in particle size was immediately discovered by the use of the electron microscope. The refractory grade has large, massive, chunky particles; the fluorescent grade has open, basket-like, mosaic-surfaced particles; the special material heated only to 400° contained innumerable, extremely minute flakes. From other sources, evidence is accumulated that the increases in toxicity are

paralleled by increases in particle area to volume ration, that is, by decreases in particle size.

Work done during the calendar year 1948: During the past year, a total of about 950 photographic plates were taken in connection with the measurement of particle size. Studies were made on the following dusts:

1. Beryllium oxide
2. Beryllium hydroxide
3. Uranium oxides
4. Beryllium fluoride
5. Silica dusts
6. Rutin crystals

Proposal for future work: It is proposed to continue the use of the electron microscope for the examination of small particles as a service to local and outside groups.