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Health and Biology - General

THE UNIVERSITY OF ROCHESTER
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QUARTERLY TECHNICAL REPORT

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Submitted by: Andrew H. Dowdy, M.D.,
Director

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INTRODUCTION

This is the first regular Quarterly Technical Report to be issued by The University of Rochester Atomic Energy Project. The scientific work presented herein has been coded at the program and problem levels according to the scheme given on Pages 6 and 7. 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 Page 10 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. Since only progress in specific scientific problems is being reported herein, the cumulative work of special service units is not given separately. Their contributions appear or are implied in the reports on problems in which they participated.

EXPLANATION OF PROGRAM AND PROBLEM CODES

The scientific work of the University of Rochester Atomic Energy Project has been coded at the program and problem levels. The programs, in general, indicate broad fields of investigative or service activities while the problems indicate divisions of these fields. Although no consistent method of division into problems was possible, an attempt was made to achieve a natural division in the sense that each problem would encompass a subject normally written up and generally considered as a unit. The program 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 quarterly report of our research and service activities, all material being assembled according to the program and problem codes. The contribution of each section to a Quarterly Technical Report will be prefixed by the section number, however, to permit reference to the administrative organization if necessary.

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

PROGRAM AND PROBLEM CODES

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

- X.R.1 Tolerance Studies (Dose levels, 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 & ULTRAVIOLET)

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 Materials

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 Effect
- 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 Effect

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 Effect

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

Atomic Energy Project
THE UNIVERSITY OF ROCHESTER

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

Section Code	Section	Section Head
3110	Instrumentation	John B. Hursh
3120	Tracer Chemistry	John B. Hursh
3130	Radiation Physiology	Thomas R. Noonan
3140	Radiation Chemistry	Kurt Salomon
3150	Spectroscopy	Luville T. Steadman
3160	Radiation Mechanics	Francis W. Bishop
3180	Metabolism	Samuel H. Bassett

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

Section Code	Section	Section 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, M.D.

Section Code	Section	Section Head
3310	Industrial Service	J. Russell Hayes
3311	Industrial Survey	J. Russell Hayes
3312	Clinical Problems	Joe W. Howland

3320	Health Physics	J. Russell Hayes
3330	Project Medical Service	Joe W. Howland

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

Section Code	Section	Section Head
3402	Embryology	James Wilson
3410	Mouse Genetics	Donald R. Charles
3430	Surgery	Paul E. Rekers

V. DIVISION OF ELECTIVE RESEARCH (3500): Henry A. Blair

PROGRAM X.R.

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

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

Section Code: 3130

The Susceptibility to Acute Irradiation as Influenced by Previous Chronic Irradiation:

Background: In the monthly report for January 1947 (M-1945) the Radiation Physiology Section reported experiments designed to investigate the effect of previous chronic x-irradiation on the tolerance of rats for acute x-irradiation. The experiments reported below differ from the earlier work in that longer chronic exposures are produced before testing with the acute exposure.

Method: Reference is made to the monthly report for January 1947 (M-1945) for details concerning the experimental method. Essentially the design of both experiments is the same. Groups of albino rats, containing equal numbers of each sex, were irradiated with 250 kilovolt roentgen rays daily except Sundays for a given number of treatments. The daily dose was either 10 r or 20 r. After a specified number of treatments, the irradiated rats, together with a suitable control group, were exposed to 600 r. The half-value layer of the irradiation was the same for both chronic and acute exposures. Total and differential leukocyte counts were done on eight animals randomly selected from each group during the week preceding acute irradiation.

Results: The group designations and chronic treatment schedule are shown in Table I.

TABLE I

Group	Original Number of Animals	Treatment
III NCA a	16	10 r/day for 75 exposures
III NCA b	16	10 r/day for 150 exposures
IV NCA a	16	20 r/day for 75 exposures
IV NCA b	16 (a)	20 r/day for 150 exposures
V NCA a	16	0 r/day for 75 exposures
V NCA b	15 (b)	0 r/day for 150 exposures

(a) Two animals died prior to acute irradiation

(b) One animal died prior to acute irradiation

In Table II are shown the hematological data for each group, just prior to the acute irradiation.

TABLE II

Group	Treatment	Total Leukocytes (per cu mm) Mean Value	Absolute Neutrophils (per cu mm) Mean Value	Absolute Lymphocytes (per cu mm) Mean Value
III NCA a & b	10 r x 25	12,959	4312	8,428
IV NCA a & b	20 r x 25	8,672	3583	5,000
V NCA a & b	Control	19,897	5436	14,285
III NCA a & b	10 r x 75*	13,603	5063	7,751
IV NCA a & b	20 r x 75*	10,938	4553	5,885
V NCA a & b	Control	23,388	6184	16,690
III NCA b	10 r x 150	22,438	9021	11,491
IV NCA b	20 r x 150	12,144	4401	7,471
V NCA b	Control	25,757	9083	15,259

*Counts done on last five days of chronic exposure.

In Table II mean values which differ significantly from their respective controls are underlined as follows:

Significant ($.05 > P > .01$) _____.

Highly significant ($P \leq .01$) _____.

TABLE III

Group	Exposure	Mean Leukocyte Count (per cu mm)	Cumulative Group Mortality (in per cent) 15 days	30 days	60 days
III NCA a	75 x 10 r	13,603	18.75	31.25	31.25
IV NCA a	75 x 20 r	10,938	50.00	62.50	62.50
V NCA a	75 x 0 r	23,388	6.25	6.25	6.25
III NCA b	150 x 10 r	22,438	6.25	25.00	37.50
IV NCA b	150 x 20 r	12,144	35.71	57.10	71.40
V NCA b	150 x 0 r	25,757	7.14	7.14	14.29

The mortality data from this experiment and that previously reported have been combined in Table IV.

TABLE IV

Treatment	30 Day Mortality (percent)	Ratio	Significance of Ratio
50 x 20 r	10	0.50	Not significant
50 x 10 r	15	0.75	Not significant
100 x 10 r	45	1.50	Not significant
150 x 10 r	25	3.46	Doubtfully significant
75 x 10 r	31.25	5.00	Doubtfully significant
150 x 20 r	57.10	7.90	Significant
75 x 20 r	62.50	10.00	Highly significant

$$\frac{\text{Percent mortality, experimental group.}}{\text{Percent mortality, appropriate control group.}}$$

Discussion: Based upon the data presented in Table IV, it may be concluded that chronic irradiation at levels of 20 roentgens per day for 75 or more treatments significantly increases the susceptibility of the rat to acute irradiation. Irradiation at 10 roentgens per day may increase susceptibility to acute irradiation after 75 treatments. Since susceptibility to acute irradiation was tested at only one dose level, there is still the possibility that a shorter series of treatments and/or a lower daily dose of chronic irradiation may increase susceptibility to a different dose of acute irradiation. On the basis of these limited data (including the hematological finding in Table II), it would appear that chronic irradiation at levels of 10 and 20 roentgens per day will produce definite changes in the blood picture before a significant change in the susceptibility to acute irradiation occurs.

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

Section Code: 3140

Ossification of Irradiated Birds:

Background: It has been known for some time that the sexual cycle of the pigeon is closely related to ossification in the long bones. At a certain stage in the ovarian cycle, ossification of the long bones is augmented to such an extent that the marrow cavity may be almost obliterated. At a later stage of the sexual cycle, this bone is progressively resorbed until the normal condition is attained.

In earlier experiments pigeons which had been irradiated with x-radiation at various dose levels were autopsied. The observation was made that, despite extensive atrophy of the ovaries, the long bones were at the stage of maximum ossification. Since the effect of irradiation appeared to be that of injuring the ovary without affecting the long bone process which has been believed to be dependent on the normal function of the ovary, it was felt worthwhile to conduct further experiments. The experiment reported below is the first in a series for which the time of irradiation will be controlled with respect to the phase of the ovarian cycle.

Method: The ovarian region of two female pigeons was irradiated with 5000 units of x-radiation three hours after the first egg was laid. At this stage of the ovarian cycle, the complementary long bone picture characteristically shows a predominance of osteoclasts as compared with osteoblasts.

This stage seemed to be a suitable one for irradiation since the long bone ossification process was near a minimum. Both pigeons were sacrificed 30 hours after the first egg was laid (27 hours post-irradiation), and the extent of the correlation between injury to the ovaries and failure of the normal growth of the ossification process was studied histologically.

Results:

a. Chemical Findings: Chemical analysis of the blood for calcium content revealed that radiation had not affected the calcium level which was 13.2 mg. per cent and 12.9 mg. per cent in the two pigeons respectively. These values agree with those reported in the literature for the stage investigated, and again substantiate our previous observations that irradiation of the order of magnitude employed does not affect blood calcium levels in pigeons.

b. Histological Findings: In comparing ovarian follicles of the irradiated pigeons (S-1 and S-2) with ovarian follicles from a non-irradiated female pigeon not in the egg-production period (S-01), no significant differences were present. Small numbers of degenerate epithelial cells were scattered throughout the mucosa of the oviduct in S-1 and S-2. ... The shell glands of these pigeons appeared normal.

An examination of the femoral bone sections revealed a stage of rather marked destruction of the medullary bone which had at one time formed trabecular network throughout the femoral marrow. In the bone marrow the osseous trabeculae were present throughout the cavity, but in many cases were thin, ragged, washed-out, and disconnected, broken up into small pieces, having lost the formation of an intercommunicating network. The trabeculae, or pieces and bars of medullary bone, were generally rather strongly basophilic. The medullary bone had practically lost all connection with the bone of the shaft.

The destruction of medullary bone reflected the rather marked osteoclastic activity. There were relatively large numbers of osteoclasts as compared with osteoblasts, and very little, if any, new bone was being formed. Much of the medullary bone was covered with osteoclasts, some long and stretched out to one cell thickness and others compact, more round, and collected, with varying numbers of nuclei. Considerable numbers of osteocytes were in various stages of liberation from their lacunae by the dissolution of the osseous matrix, and some were in the act of fusing with osteoclasts. The marrow spaces unoccupied by bone contained hemopoietic tissue.

Discussion: The preliminary experiment reported above obviously needs to be supplemented by examination of a non-irradiated female pigeon sacrificed 30 hours after the first egg is laid. Such an experiment will be performed as soon as the pigeons have finished their moulting period. Pending this crucial comparison, no definite conclusions can be drawn. Further experiments are planned at different radiation dose levels and with varying intervals before sacrifice.

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

Section Code: 3150

Absorption Spectra of the Blood of Irradiated Animals:

Background: This work was done as part of a general program designed to throw light on the mechanism of injury from x-radiation. The specific object of the pilot experiments reported below was to find out whether there were any changes in the blood plasma detectable by absorption spectrum measurements when animals were given relatively large doses of irradiation.

Method: Three adult rabbits were irradiated with single doses of 250, 500, and 1000 r units of x-radiation respectively. The animals were sitting in wooden boxes and received total body radiation. The target to skin distance was 40 cm., the filtration was 0.5 mm. Cu and 1.0 mm. Al, and the tube was operated by 140 k.v.

Initial blood samples of about 20 cc. were taken from each of the three animals. Six days were then allowed before irradiation in order that cells and plasma proteins might be regenerated. A second set of blood samples was taken a few hours after irradiation and a third set was taken a few days later as shown in the accompanying tables. These samples were all by heart puncture. The rabbits after irradiation received no care or treatment differing from that of storage animals.

Each blood sample was divided into two parts. One part was allowed to clot so that serum was obtained, the other was subjected to a plasma protein separation procedure, outlined previously in Report No. M-1973, whereby the more or less conventional fractions designated as albumin, pseudoglobulin, euglobulin, and filtrate were prepared and kept in solution. Appropriate amounts of these samples, were examined for their absorption spectra in the ultraviolet, visible, and infra-red regions of the optical spectrum.

For the visible and ultraviolet a Bausch and Lomb medium quartz spectrograph with sector photometer was used. The absorption cell length was 5 cm. In general, serum samples, about 3 cc., were diluted with water to 0.1 and to 0.01 their original concentrations. At the higher concentration some samples showed oxyhaemoglobin due to hemolysis but this was not related to the irradiation.

With the lower concentrations the characteristic protein absorption curve which has a broad maximum at 2750 A was obtained. The plasma fractions in 5 cc. solution were generally diluted to 0.2 or 0.1 the concentration. Similar protein curves were obtained for each including some protein absorption in the filtrate. The optical densities for the maxima of the absorption peaks are listed in the tables. The optical density is related to the mass of the protein present and for a given fraction is proportional

to the amount, but different proteins cannot be compared without knowing the specific absorption and this we have not worked out as yet for the conditions of the experimental procedure.

For the infra-red measurements a Beckman IR-2 spectrometer was used. About 5 cu. mm. of the samples were slowly dried on thin sheets of mica producing a deposit about 3 mm. in diameter. The absorption spectrum from 1 to 9 microns was obtained on the mica alone and then on the mica plus sample. Because of absorption in the mica a small correction is necessary. The protein fractions are characterized by curves having bands at 3.3 and 7.2 microns whereas the serum has bands at 3.0 and 6.2 microns, and is different in appearance. Again the densities of the peaks are recorded in the tables for the various samples and, although the quantity of sample is not measured, the change in density for a given fraction measures either the relative amounts of protein present or shows that the composition has changed because the optical spectra are associated not primarily with the proteins as such but with the amino acid components.

Results: The results are tabulated in Table I and Table II.

Discussion: It is apparent from the data that fairly large changes occur in the densities of the absorption bands both in the ultraviolet and in the infra-red and that these changes are more pronounced in the protein fractions that in the serum which is a mixture. It will be noted that the maximum densities for the same fraction of the initial samples show some variation from animal to animal. The variations to be expected in the same animal without irradiation will have to be established by careful control experiments. However, discounting for the lack of complete controls, the data are striking and the magnitude of some of the density changes are strong evidence that the alterations are significant and due to the effect of x-irradiation.

However, these preliminary experiments do not appear to establish any significant relationships between the changes measured and the time after irradiation or the size of dose. Furthermore, there does not appear to be any obvious correlation between the ultraviolet and the infra-red data.

TABLE I

Optical Density at 2750 A and Per Cent Changes

Rabbit No.	X-Ray Dose	Sampling Time (Hours)	Serum		Albumin		Pseudoglobulin		Eoglobulin		Filtrate	
			Density	Per Cent	Density	Per Cent	Density	Per Cent	Density	Per Cent	Density	Per Cent
558	250r	-144	2.8		2.0		1.2		1.2		1.6	
		+16	1.7	-39	2.0	0	1.8	+50	1.8	+ 50	1.3	-18
		+96	2.6	-7	3.8	+90	2.2	+83	3.0	+150	1.4	-12
559	500r	-144	2.0		2.4		2.2		2.6		1.5	
		+6	2.6	+30	2.0	-20	2.4	+9	1.5	-35	1.2	-20
		+96	2.6	+30	2.6	+8	2.4	+9	2.1	-19	1.2	-20
553	1000r	-144	2.4		2.2		2.4		3.0		1.1	
		+5	2.0	-20	4.4	+100	2.4	+0	2.2	-27	0.5	-35
		+96	2.2	-9	2.8	+27	2.2	-8	3.4	+13	1.1	+0

NOTE: Zero time = Time of irradiation.

TABLE II

Optical Density at 6.0 microns and 7.2 microns, and Per Cent Changes

Rabbit No.	X-Ray Dose	Sampling Time (Hours)	Serum		Albumin		Pseudoglobulin		Eoglobulin		Filtrate	
			Density	Per Cent	Density	Per Cent	Density	Per Cent	Density	Per Cent	Density	Per Cent
558	250r	-144	1.6		0.31		0.82		0.09		1.5	
		+16	1.2	-25	0.38	+23	0.24	-70	0.62	+600	1.4	-7
		+96	1.9	+19	1.19	+284	0.44	-46	--	--	1.9	+3
559	500r	-144	1.4		0.18		0.29		0.16		1.9	
		+ 6	1.3	-7	0.47	+160	0.62	+110	0.34	+113	1.9	+0
		+96	1.4	+0	0.45	+150	0.53	+83	0.31	+95	---	--
553	1000r	-144	1.3		0.36		0.26		0.11		0.7	
		+5	1.2	-8	1.10	+206	0.84	+223	0.22	+100	1.1	+57
		+96	1.0	-23	0.87	+140	0.53	+104	0.72	+550	1.9	+170

Problem Code: X.R.3 (Therapy)

Section Code: 3430

Marrow Transplantation:

A first draft of a final report on transplantation of marrow has been submitted. This report has been revised and is now ready for retyping and subsequent distribution. A summary of the report follows:

1. The subject of transplantation of mammalian tissue to mammalian hosts has been critically reviewed and the various theories propounded.

2. Single dose, total body radiation of 550 r delivered to dogs may be regarded as a dose lethal to 50 per cent of the animals within 8 to 23 days following radiation. Animals surviving to the 25th day after radiation in all probability will recover.

3. Hematological changes of most of the peripheral blood elements and histopathological changes of the bone marrow can be detected readily within 2 days or less and reach a maximum depression within 7 to 14 days of radiation. Depression of the lymphocytes appears early and is marked while depression of the erythrocytes is late and mild.

4. Recovery from radiation may be detected from examination of the peripheral blood by the 21st day or slightly earlier and from examination of the bone marrow by the 28th day or possibly sooner.

5. Clinical signs of radiation intoxication and recovery therefrom parallel the hematological and pathological findings.

6. Histological examination of the bone marrow shows early degeneration of the more primitive cells of the marrow parenchyma with subsequent disappearance of all types of parenchymatous elements. So-called "reticulum proliferation" has been poorly demonstrated in this study.

[redacted]

10. Normal marrow transplantation to intramedullary marrow sites is without appreciable benefit to the radiated host. Such procedures induce intramedullary bleeding with resultant organization and fibrosis, rather than stimulation of bone marrow growth.

11. Bone and marrow transplantation results in death and replacement of the graft. The newly formed bone marrow probably arises from persisting portions of the original marrow, rather than the transplanted marrow.

12. Normal marrow transplantation to normal or radiated spleen is without appreciable benefit. Residual deposits of the marrow fat only can be demonstrated.

13. Stimulated bone marrow transplanted intravenously results in multiple heterotopic bone spicules within the pulmonary veins of the lungs. The evidence suggests that concomitant with this type of marrow transplanting the mortality is 43 per cent whereas the mortality of the control group is 67 per cent. Likewise, the total white blood cell count, the absolute lymphocytes, and the reticulocyte values of the peripheral blood are increased slightly in the transplanted group of animals.

14. Surgical procedures induce an additional load upon the radiated host. However, these two procedures do not appear to inhibit the formation of callus and subsequent bone growth.

15. An explanation for the inability to transplant bone marrow successfully was studied further. Such failure could not be adequately explained on the basis of lack of demand on the part of the recipient for bone marrow, homogenicity, or selection of transplant site. The transplantation of bone marrow to such regions as the anterior chamber of the eye, the subcutaneous region of the ear, intra-medular regions, intraperitoneal sites, and within the medulla of long bones was uniformly unsuccessful.

16. The belief that tissue sensitivity can be reduced and rendered more acceptable for transplantation by passing the tissue through tissue culture media or subjecting the donor and/or host to radiation is not supported by these studies.

17. Autologous or homologous marrow transplanted to extra-medullary sites rapidly results in heterotopic bone formation.

18. Shielding experiments suggest a sparing action on the part of the protected marrow for the host and cytological studies lend further evidence in support of the belief that the effect of radiation on hematopoietic tissues is a direct one. Furthermore, such experiments indicate that total destruction of the bone marrow parenchyma requires more than a single dosage of 2000 r.

19. Transplantation of selected autologous, homologous and heterologous hematopoietic embryonal tissues to radiation hosts showed no evidence of alteration of the radiation intoxication in these hosts nor conclusive evidence of successful transplantation of these tissues in the short period of time studied.

20. Transplantation of selected autologous, homologous and heterologous tissues to non-radiated hosts and examined at least 60 days after transplantation frequently showed proliferating cartilage, some osteogenesis and occasionally a suggestion of early marrow formation.

Marrow Culture:

A first draft of a final report on cultivation of marrow by tissue culture technique has been submitted. This report has been revised and is now ready for retyping and subsequent distribution. A summary of this report follows:

1. Cultivation of normal adult bone marrow resulted in an immediate and abundant migration or proliferation of cells from the explant. For the most part these cells were of the myeloid series and in the latter stages of maturation. This migration persisted up to about 4 days after explantation when fibroblast growth began and overran the culture within 10 to 14 days.

2. The cultivation of bone marrow by roller tube and flask techniques shows no significant differences in the rate, the magnitude, or duration of growth. Slide cultures invariably showed less evidence of growth but were more suitable for detailed cytological examination.

3. A study of standard media such as chicken plasma, heterologous adult and/or embryonic sera, embryonic juices and salt solutions indicates that long-term cultivation of hematopoietic tissues cannot be sustained by the media and proportions so tested.

4. The absence or variations in concentration of dextrose has no appreciable inhibiting or stimulating effect on marrow cells.

5. High concentrations of O_2 and high concentrations of CO_2 initially promote growth, but the end result is about the same.

6. The freezing of media does not appear to effect the growth of cells in tissue culture.

7. Bone marrow, explanted from animals whose marrow had been stimulated by acute bleeding or subcutaneous injection of turpentine, shows a marked increase in cellularity.

8. The cultivation of marrow from radiated animals indicates that the production of marrow parenchymatous elements decreases with time, after radiation up to and including the 21st day of radiation. Beginning with the 28th day after radiation a production of parenchymatous cells is again noted.

9. Cultivation of the spleen results in an initial outpouring of round cells, probably lymphocytes. As these cells disintegrate, small granular cells with processes are seen. In all probability these latter cells are early fibroblasts.

10. The cultivation of embryonic spleen and bone marrow did not differ conspicuously from the cultivation of adult spleen and bone marrow.

Radiation Intoxication:

In dogs given 350 r of single dose total body x-irradiation, 64 per cent (16 of 25) of the untreated animals died with a wide-spread hemorrhagic diathesis. When the flavonol glycoside, rutin, was administered continuously pre- and post-radiation, 10 per cent (3 of 25) of the animals died. A reduced incidence of hemorrhagic signs was observed in these animals.

Problem Code: X.R.3 (Therapy)

Section Code: 3561 (Elective Research)

Folic Acid and Radiation Leukopenia

Reports have been completed on the clinical effects of folic acid on radiation leukopenia in human patients receiving radiation therapy and on the effects of folic acid given both prophylactically and therapeutically to cats receiving 200 r whole body radiation. A final report entitled, "Folic Acid Therapy. Results of a Clinical Study" has been submitted as a final report (Report No. M-19), declassified, and will be published shortly in the medical literature. It has been pointed out in this report that no discernible beneficial effect on radiation leukopenia in human patients resulted from folic acid therapy.

The animal experimentation showed no beneficial effect of folic acid given in large doses either orally or subcutaneously to cats receiving 200 r whole body radiation. This was true whether folic acid was given prophylactically prior to radiation or therapeutically following it. The data are not fully analyzed. A final report on this phase of the work will be made within a few months.

Problem Code: X.R.4 (Hematology)

Section Code: 3561 (Elective Research)

Adrenal Cortical Hormone and Thoracic Duct Lymphocytes:

A. During the period July 1 to September 30, 1947, the program dealing with the effect of adrenal cortical hormone on the numbers and rate of flow of thoracic duct lymphocytes was completed insofar as the experimental

work was concerned. Successful thoracic duct cannulations and measurement of lymph volume and numbers of lymphocytes over a period of several hours have been completed on the following groups of experimental animals (cats):

1. A control group of ten animals receiving no adrenal cortical hormone.

2. Five normal animals receiving adrenal cortical hormone after two-hour baseline determinations were obtained.

3. Five adrenalectomized animals receiving adrenal cortical hormone after two-hour baseline determinations were obtained.

4. Five normal and one adrenalectomized animal receiving adrenal cortical hormone after baseline determinations of more than two hours were obtained.

The data are not yet analyzed and no conclusions can be drawn as of September 30, 1947. It is contemplated that a final report on this phase of the study will be ready before or shortly after January 1, 1948.

B. A study on the effects of 200 r whole body radiation on the peripheral blood and bone marrow of the sternum, ribs, and femur has been completed. The data are being analyzed and a final report on this study should be available shortly after January 1, 1948.

Problem Code: X.R.5 (Genetics)

Section Code: 3411 (Discontinued)

The Effect of X-rays on the Mutation Rate in Drosophila melanogaster:

The finding of Caspari and Stern (Rochester Report No. M-1966) that the sex-linked mutation rate in the sperm of Drosophila melanogaster was not significantly altered by continuous x-irradiation of 2.5 r per day for 21 days initiated further work to attempt to discover why this result was at variance with the well-established rule for acute doses; namely, that mutation rate is linear with x-ray dosage. Uphoff and Stern (Rochester Report No. M-2000, not yet issued) irradiated females with 50 r from radium on the 21st day following insemination. A total of 46,232 offspring from the experimental flies and 44,601 offspring from the control flies were tested for lethal mutations. The experimental and control mutation rates were 0.2834 and 0.1682 per cent, respectively. This difference is statistically significant. It agrees with the linear rule and, therefore, disagrees with Caspari and Stern's results using chronic exposures. That the mutation rates for experimental animals, 0.2834 and 0.2848 per cent,

were very similar in the two experiments while the control rates were quite different, 0.1682 and 0.2489 per cent, suggests that some unknown factor invalidated the control rate with chronic exposure. This explanation is not satisfactory, however, because the low control rate of the acute experiment is not as typical as the higher chronic rate.

That the results can be explained by repair during chronic exposure is not supported by new work of Novitski (Rochester Report No. M-2001, not yet issued) in which the mutation rate was measured for sperm in spermatheeae of females irradiated with a single dose of 1000 r. No difference was observed between those cases in which fertilization occurred at once or was delayed three weeks. Consequently, no repair could be hypothecated.

At present it does not appear possible to conclude whether Caspari and Stern's chronic experiments are atypical or represent a real difference in response when the time of application of a given dosage of radiation is very prolonged.

This work has been discontinued in Rochester because Dr. Curt Stern has moved to the University of California, Berkeley, California.

Problem Code: A.R.6 (Embryology)

Section Code: 3402

Effects of Irradiation on Embryonic Development

A total of 36 rat embryos have been exposed to x-irradiation on the 10th day of gestation. These embryos were from nine different mothers in which the time of ovulation was determined to the hour, plus or minus one hour. (Recent studies of reproductive physiology in the rat have made possible such precise timing of ovulation.)

Special precautions were taken to eliminate possible variations in the quantity of irradiation reaching individual embryos. The abdominal wall of the mother was opened under anesthesia and the embryos to be irradiated were brought to the surface of the incision, oriented in a standard manner with respect to the path of the rays and all exposed at the same time. In this way there was no shielding by varying thicknesses of maternal tissues and intestinal contents.

At least half of the embryos of each litter was protected from irradiation by lead shields, thus providing unexposed controls in each litter containing exposed animals. The controls were given a sham "exposure" by carrying them through the same manipulations as were necessary with the experimentals, but no current was used in the machine. The mothers were entirely shielded from irradiation, except for a short segment

of uterus containing the embryos that were irradiated.

Three levels of dosage were used -- 50, 100, and 200 roentgen units -- in different litters; but at each level the intensity of irradiation was the same and only the time of exposure was varied.

Irradiated embryos and a suitable number of controls were taken from each litter at 48, 72, and 98 hours post-irradiation and weighed, measured, and fixed. They were then dehydrated in the usual way, serially sectioned and stained for histologic study.

Observations: To date 18 of the irradiated embryos have been sectioned and 10 of these have been studied. This number is not sufficient to permit any complete analysis of the effects of irradiation on the 10th day of gestation but some interesting observations were made. The embryos thus far studied include only those receiving doses of 50 and 100 r. The dose of 50 r was ineffective in producing morphological alterations that were visible on the second or third post-irradiation day. Before judging this dose completely ineffective, however, more animals must be exposed and some of them allowed to live beyond the third post-irradiation day.

Doses of 100 r caused alterations in the development of the eye that were recognized as early as the second post-irradiation day and which by the third day resulted in pronounced microphthalmia and often such bizarre malformations as displacement of the lens outside the optic cup. Three days after exposure this dose caused recognizable but less conspicuous abnormalities in other organs, such as the aortic arch arteries, the lung buds, and the urogenital system. Some of these latter effects may be attributable to retardation of developmental processes, particularly growth. These embryos as a whole were shorter of crown-rump length and weighed less than their litter-mate controls, a fact suggesting an overall retardation of development by x-irradiation.

No conspicuous changes either in blood-forming organs or in the cell content of circulating blood has been observed in the irradiated embryos. To definitely establish this point, however, a more detailed study than has yet been possible must be undertaken.

The major accomplishment thus far is that of having established a procedure that fulfills the needs of this type of investigation and which, at the same time, is sufficiently simple that it may be repeated indefinitely with little likelihood of variations. Embryos receiving the same dosage and removed the same number of hours after irradiation have shown surprisingly little variation, whether from the same or different litters.

Future experiments will involve the use of higher doses and further extension of the interval between exposure and the termination of pregnancy. When the effects of 10th-day irradiation are more fully understood, exposure on other days of pregnancy will be tried.

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

Section Code: 3561

Effect of X-radiation on Immune Mechanism in Rabbits.

Work was continued to determine the effects of roentgen irradiation on the immune mechanism of rabbits.

A. Data collected in regard to exposure of two groups of animals to 250 r whole body irradiation delivered eight hours before immunization with typhoid vaccine and sheep red cells were statistically analyzed. Each group consisted of nine animals, with five experimental and four control rabbits in the first group and four experimental and five control in the second group. The following points were established:

1. This amount of x-ray given eight hours prior to immunization definitely depressed the ability of the animal to form serum antibody. (The blood counts were followed and a significant depression of the peripheral blood lymphocyte level was shown which lasted three to four weeks.)

2. This depressant effect of x-ray on antibody production was transient in nature. One month after exposure the irradiated animals regained their ability to respond to immunization in the normal manner.

3. An anamnestic response of the antibody titre could not be elicited by either 20 r or 250 r whole body irradiation.

B. The effect of exposure to x-ray of animals in whom antibody production had already commenced is being studied in an experiment still in progress.

PROGRAM R.M.

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

Problem Code: R.M.1 (Polonium)

Section Code: 3130

The Development of Radioautographic Technique as a Tool for the Study of the Cellular Distribution of Polonium:

Background: The technique for production of radioautographs from tissue sections containing alpha-emitters such as polonium has been recently improved by mounting the tissue section directly on the photographic emulsion. This method has the disadvantage that subsequent staining of the tissue colors the emulsion and reduces the contrast for the alpha particle tracks. A new method has been developed to overcome this difficulty.

Method: The process consists of mounting a section of polonium containing tissue directly upon a photographic emulsion and exposing for a suitable length of time at about 10°C.

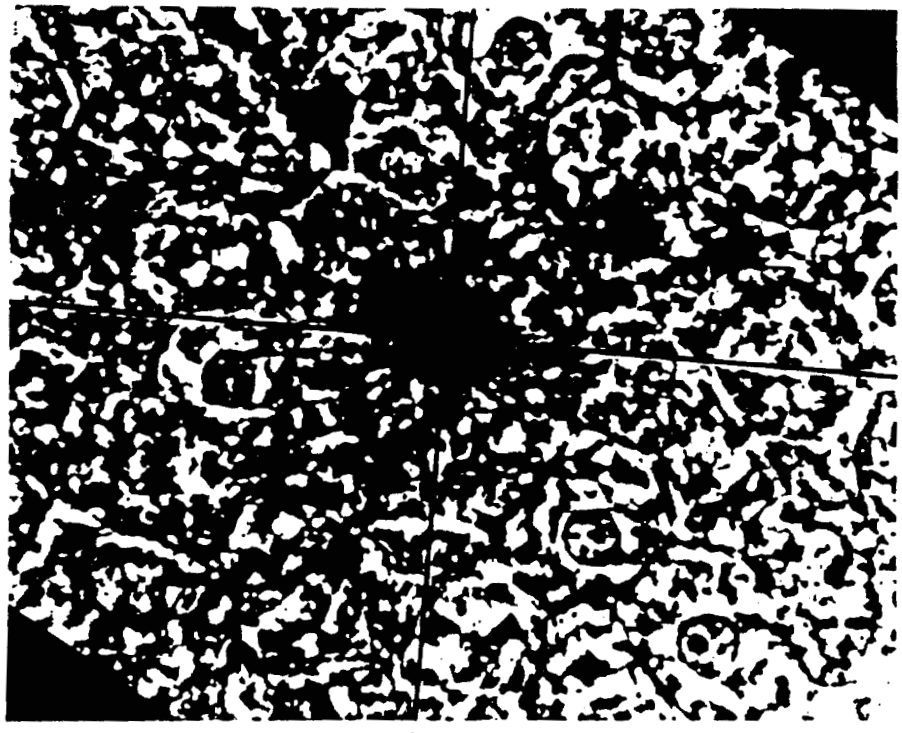
After exposure the plate with the tissue in place on the emulsion is run through xylene to remove the paraffin followed by an alcohol-water series to remove the xylene and alcohol. It is then developed for two minutes in D-19, passed through a stop bath and fixed. After fixing and washing, it is run back through the alcohol-water series to xylene instead of drying in air. Air drying can be used, but the former method is faster and the tissue can then be covered in balsam immediately.

When the slip is secure, the slide is ready for examination with the phase microscope. One can focus just above and just below the tissue emulsion interface and thus locate the alpha tracks relative to the cells of the tissue. The phase microscope enables one to see the tissue without staining, which eliminates the objection of staining the gelatin of the emulsion and thus obscuring the tracks. While it is possible to use phase illumination to see the tracks just below the tissue, they can be seen even better with bright field illumination. Thus, for a rapid survey at between 500 X and 1000 X magnification, one can use phase for both the tissue and tracks. For a more careful examination, it is advisable to use phase for the tissue and bright field illumination for the tracks.

For photomicrographic work, it is advisable to observe the tissue with phase and to use a dark field condenser for the tracks. The photograph on Page 30 is an example of this method. The phase photomicrograph was taken, printed, and toned in sepia. After this photomicrograph was taken, the field was not changed but only the focal plane of the objective lowered by 5 to 7 micra so as to bring into focus the end of the tracks

adjacent to the cells. A paraboloid dark field condenser was switched into place and the alpha track picture was taken. This was reprcduced as a reversible print and then superimposed on the sepia toned tissue photomicrograph with the cross hairs being brought into register. Both prints were made on a lantern slide, and the photograph was printed from the lantern slide. Thus, the sepia appears a light grey. The contrast between the black tracks and the sepia toned tissue is much more impressive when viewed in color.

Results: See photograph below.



Problem Code: R.M.1 (Polonium)

Section Code: 3120

The Effect of BAL and NDR 399 on Excretion of Polonium:

Background: Previous experiments (Rochester Report No. M-1935) have demonstrated that BAL and related compounds, when administered as an intramuscular injection to rats, cause a significantly increased excretion of polonium. These experiments elicited the curious finding that depending on whether the polonium was administered as an intravenous or subcutaneous injection, the compound of choice was BAL (NDR 133) or the urea derivative of BAL (NDR 399). This raises the practical question: "From the available data on rats, which of the two NDR compounds should be administered in treating a polonium accident case where the mode of introduction is unlikely to be exclusively intravenous or subcutaneous?" Since it is difficult to simulate the conditions of all the possible accidental ingestions of polonium in rat experiments, the question was approached from another angle. Experiments were set up in which polonium was administered to two rats as an intravenous and a subcutaneous injection respectively. Each rat received alternate doses of BAL and NDR 399. It was thought that if the presence of the other NDR compound did not impair the action of the compound of choice, such an alternate plan of medication might recommend itself for treatment of accident cases. Two such experiments were performed.

Method: Four male rats were used in these experiments. Two were injected subcutaneously and two intravenously at a polonium dose level of 15 uc/kg. Dummay polonium injections were delivered into bottles containing 100 cc. of 0.5 N HCL acid from which aliquots were taken for plating in order to determine the "theoretical" dose.

For the purposes of treatment with NDR compounds, 10 per cent BAL in peanut oil and 2.5 per cent NDR 399 in saline solution were injected intramuscularly as alternate doses into each of the four animals. A single dose of BAL was .16 ml./kg. and a single dose of NDR 399 was .64 ml./kg. The schedule called for a total of three doses per day and treatment was begun directly after the polonium injection and continued up to the date of sacrifice. The rats were kept in individual glass metabolism cages constructed so as to permit separate collection of feces and urine. Each collection period was 24 hours except for Sundays and holidays in which cases 48-hour samples were collected.

At the end of 20 (or 21) days the rats were sacrificed and selected tissues, including the injection site, were dissected out for analysis. The remainder of the rat carcass was digested and an analysis made.

The analyses were performed by plating out polonium on a silver foil and measuring the activity in an ionization chamber.[2]

Results: Tables I and II (Pages 34 and 35) present the ten-day excretion data for the four rats studied, and, in addition, data from previous experiments[2] for the sake of comparison.

The results are expressed in terms of per cent of dose. The term per cent of dose is a convenient means of characterizing the activity of an experimental sample. The activity of the sample is measured in terms of the activity on a standard polonium foil. Since the activity of the standard foil, the activity of the sample, and the activity of the dose, all decay with the same half life, it is apparent that the per cent of dose figure is independent of the time at which the measurement is made. It is further true that the sum of the activity measurements for the excreta and the tissues made at sacrifice, if expressed in terms of per cent dose, should equal 100 per cent dose.

It is laboratory practice[3] to arrive at the dose by carrying out such a summation and the "dummy injection" data is used for check purposes only.

The data for the animals injected subcutaneously is expressed in per cent absorbed dose which is arrived at by subtracting from the total dose the net activity found at the injection site at the time of sacrifice.

The rats were sacrificed at the end of 20 days and analyses were made of polonium content of blood cells, blood plasma, liver, kidney, spleen, bone, bone marrow, lung, muscle, skin, testis, intestines, and residue carcass. The results from these analyses show, in general, distributions similar to those detailed in the progress report of December 1946 (Rochester Report No. M-1935).

It is interesting to note that the large storage of polonium in the liver after intravenous polonium and NDR 399 treatment, has no counterpart in the present experiments in which both BAL and NDR 399 were administered.

Discussion: The results show that:

1. In all four experiments the administration of alternate doses of BAL and NDR 399 results in a significantly greater ten-day excretion of polonium than is the case for the untreated animals.

2. In regard to intravenously administered polonium, the treatment with BAL and NDR 399 gives results on the average slightly less satisfactory than results when BAL alone is employed.

3. In the case of subcutaneously administered polonium, one experiment using BAL + NDR 399 gives excretion results comparable to use of NDR 399 alone; the other experiment gives results comparable to results obtained in other experiments using BAL alone.

It is obvious that further experiments would have to be performed before it can be said that use of BAL and NDR 399 in alternate doses is,

or is not, as effective as the use of the compound of choice alone. At best the present experiments might be interpreted as showing that the alternate treatment gives results not too inferior to use of the compound of choice and that in some cases of mixed routes of ingress of polonium into the body, this alternate treatment might present advantages.

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[*] For details of this method see Rochester Report No. UR-1659.

[**] It will be noted that in some of the experiments cited for comparison, the NDR treatment lasted 3, and in some cases only 2 days. On the basis of experiments to date, it may be said that continuation of the treatment beyond 2 days, results in little, if any, improved excretion.

TABLE I

Effect of NDR Compounds on Excretion of Polonium Administration as an Intravenous Injection

Rat No.	339		342		Average 330, 332, 333		Average 326, 327		Average 7, 19, 22	
Polonium Dose	15 µc/kg		15 µc/kg		22, 20, 9 µc/kg		12, 13 µc/kg		12, 15, 30 µc/kg	
NDR Treatment 1 dose BAL = .04 cc 10% BAL in peanut oil 1 dose NDR 399 = .16 cc 2.5% NDR 399 in saline	3 doses per day Alternating BAL and NDR 399 Treatment during entire period		3 doses per day Alternating BAL and NDR 399 Treatment during entire period		3 doses per day BAL only Treatment for 3 days for No. 330, 332; entire period 333		3 doses per day NDR 399 only Treatment for entire period		No Treatment	
% dose excreted per 24 hours.	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
Day No. 1	.07	.4	2.1*	.07	.63*	.89	.15	.4	.12	3.8
2	.08	3.7	.06	6.7	.18	4.9	.17	2.1	.05	3.7
3	.03	2.5	.13	5.8	.16	8.8	.03	3.5	.05	4.5
4	.15	9.9	.13	5.8	.23	8.9	.04	3.2	.06	3.4
5	.04	6.9	.17	6.0	.22	8.1	.05	3.3	.07	3.0
6	.05	8.8	.12	3.2	.18	5.8	.05	3.3	.07	1.8
7	.05	8.8	.15	5.0	.18	5.0	.15	3.1	.10	1.8
8	.09	4.0	.10	2.2	.12	3.5	.11	3.6	.11	1.7
9	.11	2.7	.60	3.8	.10	2.0	.07	4.0	.10	1.8
10	.10	3.1	.10	1.9	.09	1.6	.06	2.9	.05	1.6
TOTAL EXCRETION	.82	50.8	3.66	40.5	2.09	49.5	.88	29.3	.78	27.0

*Sample probably contaminated by feces.

TABLE II

Effect of NDR Compounds on Excretion of Polonium Administered as a Subcutaneous Injection

Rat No.	338		343		67		Average 61, 65, 317		Average 62, 64	
Polonium Dose	15 μc/kg		15 μc/kg		10 μc/kg		17, 11, 9 μc/kg		11, 12 μc/kg	
NDR Treatment 1 dose BAL = .04 cc 10% BAL in peanut oil 1 dose NDR 399 = .16 cc 2.5% NDR 399 in saline	3 doses per day Alternating BAL and NDR 399 Treatment during entire period		3 doses per day Alternating BAL and NDR 399 Treatment during entire period		3 doses per day NDR 399 only Treatment for 3 days		3 doses per day BAL only Treatment No. 61, 65 for 2 days; No. 317 for 3 days		No Treatment	
% absorbed dose excreted per 24 hours	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
Day No. 1	.12	5.1	.79	.51	.18	1.0	.16	.5	.44	1.0
2	.12	4.6	.10	3.7	.42	5.5	.27	2.4	.09	3.5
3	.11	4.8	.19	2.4	.71	5.5	.10	1.8	.07	2.5
4	.11	3.5	.19	2.4	.38	5.2	.06	2.1	.09	2.2
5	.09	2.2	.07	2.3	.43	3.5	.10	3.9	.08	1.5
6	.04	4.1	.13	1.6	.43	3.5	.10	3.9	.07	1.1
7	.04	4.1	.06	3.7	.24	2.9	.06	3.0	.08	.6
8	.09	.8	.07	2.1	.38	2.7	.08	2.3	.07	1.0
9	.05	1.8	.07	3.6	.19	1.4	.09	1.7	.11	1.5
10	.13	1.6	.07	2.2	.27	1.5	.10	1.6	.07	1.4
TOTAL	.90	32.6	1.74	24.5	3.63	32.7	1.12	25.2	1.17	16.3

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

Section Code: 3110

Measurement of Radon in Breath Samples:

Background: The method in present use for the determination of radium in the body of living human subjects is by measurement of the radon concentration in the expired breath. The problem as presented to this section was to devise a reliable method for measurement of concentration of radon in the breath at levels of one-tenth to one-third the accepted tolerance level of 1 uucurie of radon per liter of air.

Method: Figure 1 (Page 38) shows the apparatus that is used to adsorb the radon on the charcoal cartridge. An operator breathing into the glass bottle causes regular inflation and deflation of a balloon. The bellows-like action of the balloon draws the spirometer mixture of expired air and radon through the charcoal cartridge at a rate equivalent to the normal breathing rate. It differs from the real case of a subject breathing into the charcoal cartridge only in that the gas is passed through the charcoal on the inspiration rather than the expiration of the operator. Since the breathing pressure cycle in man is more or less sinusoidal in character, this difference is felt to be unimportant.

The charcoal used in the experiments to be reported is twenty mesh activated charcoal contained in a disc with copper retaining screens at the circular entrance and exit ports, and a side wall of brass. The diameter equals $3\frac{1}{8}$ inches and the thickness equals 5/16 inches.

In preparation for each experiment the operator fills the spirometer by expiration into it until approximately 35 liters of expired air have been collected. He then adds a measured quantity of radon (ranging around 35 uuc) to the spirometer. The gas mixture is stirred up with a small fan sealed into the spirometer bell. The radon-expired air mixture is then passed through the charcoal in the manner described above.

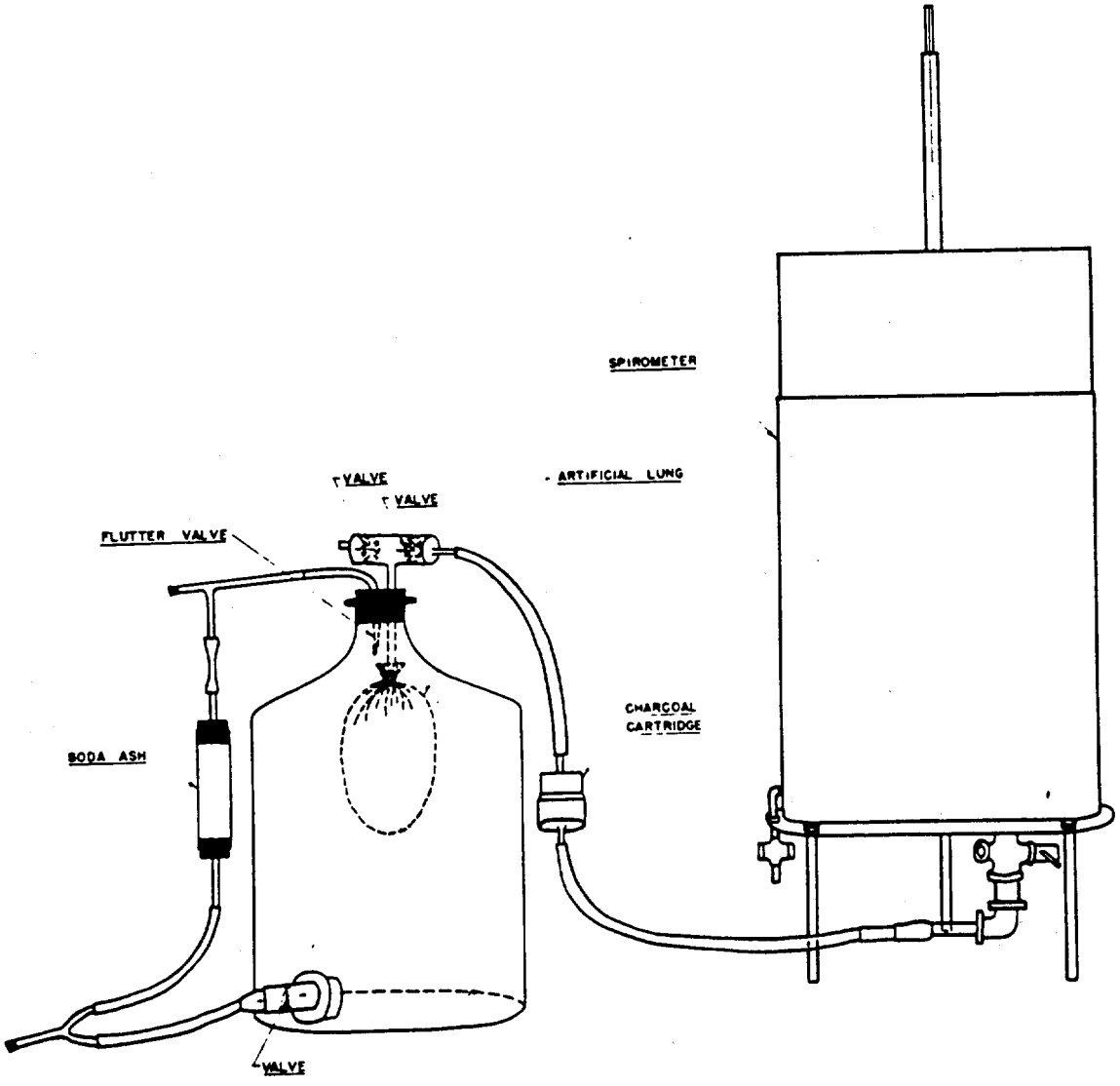
In order to recover the radon from the charcoal, the charcoal is transferred to a pyrex tube and heated in an ignition furnace at 350° Centigrade for thirty minutes. During the heating process nitrogen is slowly streamed through the charcoal and collected in a 1 liter sampling bulb. At the conclusion of the collection period, the sampling bulb is analyzed for radon in an ionization chamber or an α counter. In the results reported here, the routine ionization chamber technique was used exclusively since the total activity was sufficiently high and since this procedure is more rapid. A further series of experiments are underway using lower radon concentrations which necessitate measurement by the alpha counter techniques.

Results: Table I (Page 37) shows the recoveries measured in 19 runs.

TABLE I

Run No.	Average Rate of Breathing	Concentration of Radon	Recovery (per cent)
1	211 cc/sec.	1.14 uuc/l	128.3
2	248	1.16	108.2
3	192	1.24	111.9
5	191	1.14	96.3
6	219	1.14	94.7
7	200	1.04	80.4
8	234	1.01	102.4
9	238	0.97	111.5
10	240	0.99	103.1
11	258	0.95	110.5
12	256	0.96	93.2
13	243	0.96	107.9
14	258	0.96	105.5
15	237	1.02	93.8
16	260	1.03	95.0
17	238	1.03	98.3
18	234	1.04	92.3
19	225	1.05	98.7
20	221	1.00	105.0
21	217	1.01	78.9
22	204	1.01	106.4
23	237	1.04	112.6
Average of 22 runs	239	1.04 uuc/l	101.6

Figure 1



RADON SORPTION APPARATUS

PROGRAM U.

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

Section Code: 3210, 3220

Micro Uranium Method:

The study of the reaction of uranium with various proteins in solution has been continued. Two proteins have been investigated -- crystalline egg albumen and bovine serum albumin. The most recent work has involved rechecking selected data as to protein concentration, pH, and buffer concentrations, and extending the previous observations to more concentrated solutions of uranium.

Uranium Complex:

Polarographic studies of the uranium complex with citrate were completed. Citrate forms a soluble, complex ion, combining with UO_2 in a mole ratio of one. The complex probably exists in solution as a negatively charged dimer which dissociates only very slightly. The importance of the uranium citrate complex in the development of tolerance has already been established.

Titration experiments with ortho and pyrophosphate and other complexing groups have been carried out.

Measurement of Aerosols:

A model experimental dust chamber has been designed for testing instruments used in the determination of dust concentrations and particle sizes. This chamber of about 1 m^3 volume is cylindrical with a tapering inlet and outlet to give (a) a minimum of turbulence, (b) uniform dust distribution, and (c) uniform particle-size distribution. Construction is underway.

The electron microscope is in constant use. A projection box in the form of a 30-inch desk has been built, on which electron micrographs are projected with a ten-fold magnification. The optical system consists of a Colde-blower projector with a 4-inch lens and a plain surface mirror.

For electron micrographs, bulk dust samples are dispersed on a clean, dry slide (modified Green dispersion method). A thin film of collodion is formed over the specimen imbedding all particles. The film is lifted from the glass and transferred to the electron microscope grid.

Zinc, magnesium, brass, and beryllium fumes have been studied. The characteristic zinc oxide crystals, each bearing 2 or 3 spines, have been identified. The cubic crystals of magnesium oxide clump almost corner to corner giving a checkerboard appearance. Brass fume showed a mixture of material in which zinc particles could be identified. Beryllium fume produced by the arc of a beryllium rod against carbon showed a characteristic hexagonal-shaped crystal. Crystal clumping tended to occur at the ends of the crystals.

At present electron microscopic studies also include electron diffraction photographs.

In preparation for optical microscopy, stibnite, Sb_2S_3, with refractive index of 4.0 has been used to coat dust samples. It forms a stable, hard, optically clear imbedding medium. Smaller particles may be measured with such a medium; this effect has been described previously using selenium (refractive index 2.8).

The thermal precipitator has been redesigned to include an oscillating sampling plate along the lines suggested by E. Green, Porton Laboratories, England.

In cooperation with Dr. Joe W. Howland, of the Division of Medical Services, a series of industrial dust samples have been collected and analyzed from the Harshaw Chemical Company. Some high uranium values were found. Mr. R. N. Turner of this company and Mr. C. Berghaut of the New York Operations Office have each spent three days here studying the methods of dust collection and for the estimation of particle size.

A survey at the Lorain plant of the Brush Beryllium Corporation was completed and presented at the Sixth Saranac Symposium, September 29th to October 2, 1947. Plant processes were briefly described, analysis of the medical history and a summary of the types and characteristics of acute poisoning in beryllium workers were added to the physical and chemical data, such as atmospheric beryllium and fluoride concentrations and particle-size measurements.

Specific surface determinations have been made on various uranium dusts. UO_2, UO_3, and UF_4 have relatively low values (10ths of a m^2/g). Porosity factors were also low -- 1 to 2. UO_4 had a higher specific surface -- 5.9 m^2/g. A sample of powdered UO_2 was separated by a settling method into a coarse and a fine fraction. The coarse fraction had a specific surface of 0.47 m^2/g; the fine fraction showed a 10-fold greater specific surface, viz., 4.2 m^2/g. The porosity factors, namely, 6.8 for the coarse fraction, 14 for the fine fraction, show only a two-fold difference, indicating that the increased surface area is primarily attributable to reduced particle size.

Two toxicity studies have been made on the coarse and fine fractions just described. In one study the particles had a mass median size of 1.5 μ, range 0.9 to 3.0. In the other study the particle size had a mass median size of 0.45 μ.

During this quarter 672 uranium analyses have been done; 390 particle size samples; 131 urine and filter paper samples from the Harshaw Chemical Company; 6 urine samples sent by Dr. Wolf from the Middlesex Warehouse; 58 were occasional check analyses and 187 were standards.

Problem Code: U.2 (Toxic Effects)

Section Code: 3230, 3250

Uranium: Effect on Reproduction:

A group of experimental rats, caged in pairs, male and female, were given for one day only a diet containing 2 per cent uranium nitrate. Thereafter, they were fed the usual stock ration. A comparable control group has been observed simultaneously. Surprisingly enough, the experimental group has produced fewer litters and fewer pups per litter in the seven months following the initial administration.

Histological Studies:

A characteristic difference has been found in the degree of injury in the tissues (especially the kidneys) of several species of animals exposed to UO_2, depending on the particle size: large particles are much less toxic than small particles.

Problem Code: U.3 (Toxic Limits)

Section Code: 3210, 3220, 3230, 3250

Toxicity versus Particle Size:

Two studies designed to show the importance of particle size and surface area in the toxicity of uranium dusts were conducted at an atmospheric concentration of 80 mg/m^3 and average particle sizes of 0.45μ and greater than 1μ, respectively. Definitive results were obtained from exposures of animals at these narrowly separated particle-size ranges. Uranium injury was observed in rats exposed to dust particles less than 1μ in diameter but were not seen in rats exposed to particles greater than 1μ. Toxicity differences were not so clear-cut in the rabbits, but greater injury was found in those exposed to the smaller particles. Since the surface areas, 0.47 and 14.1 mg^2/g, respectively, show a large difference, perhaps surface area is more decisive than particle size as a factor in toxic response.

Two-Year Uranium Dust Study:

The two-year chronic exposure to uranium dust has been completed. Dogs and rats previously treated daily for one year to various soluble and insoluble dusts were subsequently exposed to an atmosphere containing 2 mg. of uranium/m^3 as uranium nitrate for an additional year. Preliminary data indicate no grossly observable injury during the second year of exposure. There were no deaths in either species that could be attributed to uranium exposure; the body weight, biochemical and hematological examinations were within the limits of normal value.

Skin Studies:

Uranium dioxide powder was introduced into incisions in the skin of rabbits and the wound healing observed. There was no interference with the healing, and, within experimental limits, all of the uranium placed within the skin was recovered from that site weeks later.

Anatomical Diagnosis:

During the quarter a number of reports have been prepared, in which the histological observations of various chronic uranium studies have been presented. These include examination of tissues of rats fed UO_2F_2 for two years; UO_2 and uranium nitrate for the same period. In addition, reports were sent out covering the appearance of the tissues in three of the year-long uranium dust studies.

Problem Code: U.3 (Toxic Limits)

Section Code: 3537 (Elective Research)

Relationship of Age to the Toxicity of Uranium Nitrate:

Previous work has indicated that 21-day-old rats are more susceptible than 28-day-old rats to uranium poisoning following ingestion or intraperitoneal injection of suitable doses. This resistance is promptly lost; rats 2 to 6 months old are much more susceptible than the 28-day-old rats. Rats 25 to 26 days old are nearly as resistant as the 28-day-old rats.

Problem Code: U.4 (Fate)

Section Code: 3201, 3220, 3210

Mechanism of Bone Deposition:

Using various chemical techniques, the nature of the mechanism by which uranium is bound to the surface of bone mineral has been clarified: Calcium and uranium compete for suitable combining sites (adjacent phosphate groups) on the bone crystal surface.

Problem Code: U.4 (Fate)

Section Code: 3530 (Elective Research)

Mechanism of Bone Deposition:

Radiocalcium adsorption on bone has been studied and it has been established that about 1/5 of the calcium in powdered bone is capable of entering into a fairly rapid exchange reaction with calcium in solution.

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

Section Code: 3210, 3260

Mechanism of Action of Uranium on Isolated Cells (Yeast):

Additional evidence for the action of uranium on the cell surface is available from studies of the osmotic behavior of uranium. As the uranium concentration is increased, the uranium uptake by the cells follows a series of asymptotic curves representing the dissociation constants of various complexes of uranium with groups on the cell surface. The inhibition of glucose metabolism is associated with the first asymptote, which represents the lowest dissociation constant of the cell - uranium complexes (about 10^{-8}). This reaction follows the form $U+C \rightleftharpoons UC$, where C is the complexing group. If the uptake of uranium by the cells were primarily a diffusion into the cells, then the relationship between uranium concentration and uranium uptake should be a straight line rather than a curve, or series of curves.

At high uranium concentration, although most of the uranium is complexed on the surface, some does penetrate into the cells, but this is apparently completely complexed by the adequate supplies of bicarbonate and

phosphates in the cell for it seems to exert no toxic effect. The inhibition of uranium can be completely reversed by washing the surface of the cell with competing complexers, even though the uranium inside the cells remains there.

Kinetic studies indicate that uranium inhibits the very first step in glucose metabolism, the reaction:

$$\text{glucose} + \text{ATP}^{*} \underset{\text{hexokinase}}{\rightleftharpoons} \text{glucose 1PO}_4 + \text{ADP}^{**}$$

*adenosine triphosphate **adenosine diphosphate

A competition actually exists between uranium and glucose for the ATP-hexokinase system. The uranium apparently ties up the phosphate groups of ATP, because the dissociation constant of the uranium complex associated with inhibition of glucose metabolism is about 10^{-8}, which is much lower than that for uranium-protein complexes. Preliminary data indicate that the ATP-U complex will have a dissociation constant of this order of magnitude.

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

Section Code: 3531 (Elective Research)

Relation of Cell Surface to Metabolism:

Yeast in the presence of glucose and tracer quantities of P_{32} (high in count, very low in concentration) takes up considerable quantities of P_{32}. If left for 24 hours, this P_{32} is distributed in a fairly uniform manner among the phosphate fractions of the cell. The cells can then be washed in unlabelled phosphate and the surface P_{32} is exchanged off. The major part of the P_{32} within the cell, however, is non-exchangeable (at least in a period of several hours). The cells are then suspended in glucose plus unlabelled phosphate. Small amounts of organic phosphate appear in the medium, but despite the high specific activity of the cell phosphates, these organic phosphates in the medium show practically no radioactivity. Thus, the organic phosphates did not leak out of the cells nor were they formed from cell phosphates. They must arise from the phosphate of the medium and must be formed at the cell surface. Present phosphate methods are inadequate to the problem of isolating the organic phosphate fractions. We are attempting to make use of the paper chromatographic technique to separate the phosphorylated intermediates of carbohydrate metabolism and have succeeded in modifying the standard phosphate methods so that colors can be developed on filter paper with only 0.1μg of P. If this

technique works, it will be used to identify the compounds formed by the yeast at the cell surface.

A second approach to this problem involves the study of phosphatases in yeast. By use of ATP with P_{32} incorporated in the molecule, it can be shown that an enzyme is present which splits the ATP to AA* and inorganic phosphate. The recovery of stoichiometric amounts of products in the supernatant, with no appearance of P_{32} in the cell, indicates that this enzyme is on the cell surface. Some of the characteristics of the enzyme are as follows:

a. Its pH optimum is 3.4 - 4.0 with no activity at 7.0.

b. It will hydrolyze ATP, ADP, and inorganic triphosphate, but not inorganic pyrophosphate.

c. It is inhibited by high concentrations of inorganic phosphate and by much lower concentrations of inorganic in the presence of AA*. AA alone does not inhibit.

d. The activity is directly proportional to enzyme concentration (first order) reaction, but the time course of the reaction involves a gradual slowing of the rate, which is associated with inhibition by the products of the reaction. The enzyme itself is not destroyed by the reaction.

e. Other enzymes exist on the surface of the cell which will hydrolyze alpha glycerophosphate and inorganic pyrophosphate, but not AA.

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*AA = ademylic acid

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

Section Code: 3260

Amino Acid Excretion in Uranium Poisoning:

The minimal dose of uranium to evoke a response in amino acid to creatinine ratio has been determined. The response to repeated doses of uranium has been evaluated and the mechanism of the response has been clarified.

SECRET

PROGRAM Be.

BERYLLIUM

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

Section Code: 3210, 3220

Analytical Methods:

Five analytical methods for beryllium have been investigated in some detail: three colorimetric, one fluorescent, and a spectrographic method:

Agent or Method	Limit of Sensitivity	Standard Error
	µg	%
Colorimetric (1,4 dihydroxy anthraquinone)	0.5	12
Colorimetric (aurin tricarboxyllic acid)	1.0	10
Colorimetric (alkanet)	0.2	5
Fluorescent (1 amino 4 hydroxy anthraquinone)	0.5	12
Spectroscopic	0.005	25
Radioactivity	0.001	5

The evaluation given in the table indicates approximately the present limits of usefulness of these methods. Many substances interfere with the chemical reactions and work is underway to provide a suitable procedure for the isolation of beryllium from biological material prior to its analysis.

A total of 503 beryllium analyses have been performed: 214 were cascade impactor samples; 213 were exposure chamber samples; and 76 were miscellaneous check and standard analyses.

Sampling and Measurement of Aerosols:

Because extreme difficulties were experienced in dispersing dry beryllium sulfate dust, the Laskin atomizing feed has been used to disperse the sulfate as a mist. This procedure duplicated conditions in the industrial

sulfating process and permitted better control of atmospheric concentrations than had been obtained with the dry dust. Strikingly, samples of the sulfate mist showed tiny crystals surrounded by a layer of moisture. In two pilot exposure studies concentrations of the order of 80 mg/m^3 have been obtained with uniform particle sizes, approximately 1 u in diameter, range 0.98 to 1.3 u.

Complex Formation:

Titration experiments have been carried out with various organic acids and beryllium. Only alpha hydroxy, dicarboxyllic acids (citric, maleic, tartaric) proved to be effective complexers of beryllium. Among those tried with negative results were: acetate, glycine, cysteine, protein, lactate, malate, succinate, etc.

Problem Code: Be.2 (Toxic Effects)

Section Code: 3250

Anatomical Diagnosis:

When beryllium sulfate is given intravenously, mid-zonal necrosis of the liver cells, necrosis of cells of the distal 1/3 of the proximal convoluted tubules of the kidney and degenerative changes in the cells of the hemapoietic system are produced. Following a single intravenous administration of beryllium sulphate, rather sharp changes occur in the elements of the peripheral blood. These consist of a secondary anemia (probably resulting from intravascular lysis of red cells), a leukocytosis, and an increase in the number of circulating platelets.

Following exposure of animals to beryllium sulphate dust (100 mg/m^3, 8 hours daily for 11 days), inflammatory pulmonary lesions are produced, which vary in intensity with different species. Pulmonary edema, a terminal bronchitis, and focal atelectasis are the most commonly observed lesions. The eyes of some species exposed to this dust develop conjunctivitis, keratitis, and corneal ulcers.

Problem Code: Be.2 (Toxic Effects)

Section Code: 3536 (Elective Research)

Production of Generalized Osteosclerosis and Osteogenic Sarcomas:

Repeated intravenous injections of beryllium sulfate have provoked in

rats a development of cancellous bone to the extent of nearly complete replacement of the marrow cavity.

Rabbits have been given various doses of beryllium compounds and are at present under observation awaiting the development of osteogenic sarcomas as reported by Gardner.

Problem Code: Be.3 (Toxic Limits)

Section Code: 3210, 3220, 3230, 3250

Preliminary Beryllium Sulphate Inhalation Studies:

A two-week pilot study was performed in 71 animals exposed 6 hours per day over a two week period to a misty atmosphere of $BeSO_4.6H_2O$ following a conditioning period of the same length of time. The concentration to which the animal group, consisting of 3 rabbits, 10 rats, 38 mice, 110 hamsters, and guinea pigs, was exposed was 99.8 mg/m^3 of the compound as determined gravimetrically. Deaths among the treated animals were distributed as follows: 10 of 10 rats, 3 of 10 guinea pigs, and 2 of 10 hamsters. There were no fatalities in the mouse or rabbit groups. The weight response data showed that all of the animals were adversely affected with the mouse showing the greatest change with a weight loss of 13% following exposure. The guinea pig and rabbit showed the least change with 2 and 3% loss, respectively. The biochemical and hematologic values from the rabbits and rats were unusually normal and consistent throughout the experiment, showing no indication of damage. Histologic results are not available, but gross examination of the tissues at the time of autopsy revealed widespread pulmonary edema in the rats and some alveolar congestion in the guinea pigs. The mice, both those studied serially and at the termination of the experiment showed little indication of damage.

In order to ascertain if differences existed between the susceptibility, albino and colored guinea pigs (10 of each, approximating a mean of 400 g) were exposed to a misty atmosphere of $BeSO_4.6H_2O$ at a concentration of 100 mg/m^3. Eleven old and heavier animals averaging 850 g were also placed in the chamber together with 12 rats. It was noted that all of the young guinea pigs died while but two fatalities were recorded in the mature group. There were no discernible differences noted in the effects of exposure upon either the albino or colored young guinea pigs, as deaths occurred with about equal frequency in both groups. All but one of the 12 rats died during the course of the experiment, with the deaths occurring in about the same chronological order as in the initial mist-exposure study, e.g., the first one being recorded on the 8th calendar day.

Skin Reactions:

As far as has been ascertained, beryllium does not provoke a

sensitization response. There is no absorption or practically none through the intact skin; however, once through the skin, soluble beryllium compounds produce a typical ulcer.

Feeding Studies:

Beryllium rickets has been produced in rats fed either beryllium sulfate or beryllium carbonate at a level of 5% of the diet; 2.5% beryllium carbonate did not produce rickets. When replaced on the stock ration, these animals apparently recovered and the rickets healed; however, in the following weeks an odd, hitherto-undescribed, radiolucent area appeared in the metaphyseal area and seemed to increase in saggital dimension with time. The nature of this lesion is unknown.

When large amounts (10%) of beryllium oxide or powdered beryllium metal were mixed in the diet of rats, no depression in growth was produced.

Acute Toxicity: Intraperitoneal Injection:

Aqueous solutions of a number of salts have been administered to albino rats intraperitoneally. The LD50's are approximately as indicated: BeF_2 - 12 to 15, $BeOF_2$ - 13, $Be(NO_3)_2$ - 500 to 600, $BeCl_2$ - 100 to 200 mg/kg.

Acute Toxicity: Intravenous Injection:

In order to attempt to reproduce previous work which showed that beryllium sulphate when injected intraperitoneally in an aqueous solution was more toxic than in a saline solution, 20 rats were given intravenously 7.2 mg/kg of beryllium sulphate in aqueous and 20 in saline. Ten rats of each group died and 10 survived.

Since guinea pigs seemed to be unusually resistant to beryllium sulphate when inhaled as a dust, it was thought worthwhile to determine roughly the intravenous toxicity in this species. Groups of 2 animals were given increasing doses of beryllium sulphate intravenously. These results indicated that the guinea pig was more susceptible than the rat, but less than the rabbit.

Problem Code: Be.4 (Fate)

Section Code: 3201, 3210, 3220

Intratracheal Studies:

During the past three months, certain findings have made advisable a revision of the original program of intratracheal administration of

beryllium compounds. A number of experiments which had appeared to be negative at first are now, after 6 to 8 months, producing a high mortality rate in rats. The immediate inference is that the detailed studies of the reaction of the body to beryllium should be planned to cover at least this period; consequently, a group of 50 rats have been injected with 100 mg/kg of powdered beryllium metal and are being observed for mortality, hematology, and organ weights at death.

Beryllium Distribution:

The radioisotope, Be_7, has been produced by bombardment of lithium in the cyclotron of the University of Rochester Physics Department. Only a few samples have been studied; however, it appears that beryllium adminstered intravenously appears promptly in the urine, and is deposited in the kidney, the liver, the spleen, the bone marrow, and perhaps in the bone mineral.

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

Section Code: 3210, 3230, 3260

Agents Affecting Beryllium Action:

A group of 5 rats given beryllium metal intratracheally 3 months previously were administered peanut oil by the same route. The severe leukocytoses and the tendency to eosinophilia, which were observed in a similar preliminary test, have again been observed.

The following substances have been observed to produce local ulceration or minor reaction in the rabbit and guinea pig when introduced intracutaneously: beryllium metal, beryllium lactate, and ammonium beryllium fluoride complex. An injurious reaction is obtained with doses varying from 0.07 to 0.67 mg of either the soluble or the insoluble preparations. A similar but less severe inflammation has been obtained in the rat. A leukocytosis in all cases has accompanied local inflammation. The administration of 0.05 mg beryllium fluoride complex appears to be free of local effects. Beryllium sulfate at 0.07 mg produces only a mild inflammation as does 0.07 beryllium lactate; the latter 2 substances will be further tested at a somewhat lower level.

Effects of Beryllium on Cells and Tissues:

Beryllium has no pronounced effects on glucose oxidation or fermentation by yeast.

On the basis of a possible competition of beryllium for Ca-systems, work was undertaken on frog and then on turtle heart. In one preliminary but crude frog-heart experiment, marked effects were observed. These could not be duplicated on a simple turtle heart preparation (total removal of the heart). No effect was observed on a turtle heart in situ when beryllium was placed in the pericardial cavity. Finally in a more complicated preparation, in which a vein and artery were cannulated beryllium saline was perfused through the heart at normal pressure in such a way that the coronary circulation was supplied. Two turtles were used and in neither were any abnormalities in heart rate, output, or beat characteristics observed.

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

Section Code: 3532 (Elective Research)

Infectious Agents Affecting Beryllium Action:

The simultaneous administration of the hyaluronidase with a suspension of pneumococcus type XV, given intratracheally, induced a marked leukocytosis in rats compared with an absence of reaction in the control animals. When hyaluronidase was given with pneumococcus type III, 11 (100%) of the hyaluronidase rats succombed with a severe pneumonia and pleuritic reaction, whereas only 1 (20%) of the control pneumococcus types VI, VIII, and XXII are now being undertaken under identical conditions.

The organisms isolated from a rat dying with beryllium poisoning in May of this year is being introduced intratracheally into 12 rats, 4 of which will receive simultaneously hyaluronidase and 3 a dose of beryllium metal.

Eighty guinea pigs with suitable controls have been injected with the tubercle bacillus and exposed weekly to beryllium sulfate mist in an attempt to determine the interplay of these two stresses on the animal body. At the present time, after three months of such treatment, slightly greater enlargement of the lymph nodes was noticed in the animals receiving both agents although the difference was not remarkable. Animals subjected to beryllium only have suffered a greater weight loss than those with tuberculosis and beryllium.

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

Section Code: 3260

Lung Function Tests in Animals:

An attempt was made to use the ear oximeter to determine O_2 saturation

in blood of rabbits, in the hope of applying such measurements to the determination of cardio-respiratory reserve. The oximeter earpiece circuit was borrowed from the Physiology Department. The electrical measuring circuit was built by ourselves. A rabbit cage was constructed in which the animals could be exposed to any desired O_2 tension. When the animals were at equilibrium with the desired O_2 tension, blood samples were drawn from the ear or from the femoral artery for VanSlyke determination of O_2 saturation. These analyses were compared with the oximeter readings. Our results can be summarized as follows:

a. Rabbits like dogs can withstand very low O_2 saturation of the blood, considerably lower than man.

b. When the oximeter is mounted on the ear of albino rabbits, the readings are relatively unstable due to marked changes in circulation in that organ.

c. When the oximeter is mounted on folds of shaved skin on the belly or back, the readings are stable. However, when the O_2 tension is reduced so that the O_2 saturation drops from 95 to 45%, the oximeter may only drop from 95 to 80 or 75%. The oximeter indicates qualitative but not quantitative changes in O_2 saturation of the blood. The reason for the discrepancy is not apparent at the present time.

PROGRAM Th.

THORIUM

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

Section Code: 3210, 3220

Method Development:

Preliminary studies of the polarographic behavior of soluble thorium salts indicates that this method will probably not be useful for estimating thorium. A catalytic wave with nitrate ion has been found, but its usefulness is doubtful.

A number of procedures have been tried; for example, the dithiazone and the oxalate; and have been discarded. Sufficient preliminary testing has been carried out using about 30 dyes to be able to discard all but one of them; this one has possible usefulness in the colorimetric method.

Sampling and Measurement:

A visit was made to the Maywood Chemical Works on September 24 for the purpose of becoming better acquainted with the Industrial Hygiene problems of the thorium industry. It was noted that the only dust hazard in the production of the thorium nitrate was in the handling of monazite ore and in stirring out the thorium nitrate at the final step of the process. The company officials reported they had had no industrial compensation claims presented during the course of their 40 years of thorium production. This company has only a small number of employees.

Problem Code: Th.2 (Toxic Effects)

Section Code: 3230, 3250

Feeding Experiments:

Groups of weanling and 6 month old rats are being fed diets containing 3 levels of a typical soluble and a typical insoluble thorium compound. The dietary levels are 0.05, 1, and 10%. Body weights are recorded. Ten per cent of thorium nitrate in the diet is sufficient to cause death after a protracted period of 2 months or more.

Problem Code: Th.3 (Toxic Limits)

Section Code: 3210, 3220, 3230, 3250

Intratracheal Toxicity:

The 14-day MLD for thorium nitrate in rats has been established for the intratracheal route to be 75 mg/kg; for thorium dioxide the MLD was greater than 1,000 mg/kg -- the maximal dose possible by injection. After a period of two months, thorium dioxide animals have suffered no observable injury from this amount of oxide in the lungs.

Inhalation Experiments:

To monitor the inhalation experiments, it has been decided to employ an alpha counter for thoron to make sure that Project personnel are not exposed. This counter has been built.

Problem Code: Th.4 (Fate)

Section Code: 3220

Distribution of Thorium:

When thorium marked with UX1 is administered to rats, rabbits, and guinea pigs, a characteristic pattern of distribution has been found depending on the route of administration. When thorium is administered intramuscularly, there is no evidence of mobilization from the site of implantation. When it is administered by mouth, there is no absorption from the gastro-intestinal tract; or perhaps it would be better to say that the absorption is so slight as to be undetected by our experimental procedures (less than 0.1%). When it is administered intravenously, it is deposited in the liver, spleen, and bone marrow. Certainly none (<0.5%) appears in the urine. There is a variable, but significant fecal excretion.

PROGRAM F.

FLUORIDE

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

Section Code: 3210

Sampling and Measurement:

Beryllium has been found to interfere with the titration of fluoride by the thorium nitrate-alizarin back titration procedure, recoveries being seriously lowered when the ratio of mols. of beryllium to mols. of fluoride exceeds one; beryllium does not interfere with the separation of fluoride (as sodium fluoride) by the Willard and Winter distillation. Alkaline fusion, followed by perchloric acid distillation, is required for the determination of the fluoride content of beryllium fluoride; beryllium oxyfluoride may be analyzed for fluorine by the usual perchloric acid distillation, by alkaline fusion and subsequent distillation, or by direct titration of an aqueous solution of the salt. A satisfactory procedure was not devised for the analysis of beryllium ores for fluoride content, owing to the fact that the treatment with hydrofluoric acid is required for complete solution of the samples. In spite of the fact that known amounts of fluoride could be satisfactorily analyzed by a procedure applicable to Cascade Impactor samples, in practice such samples could not be satisfactorily handled owing to difficulty in neutralizing aliquots and to poor colors for matching the end points.

The Harshaw Chemical Company has forwarded through the Service Division (Division of Medical Services) approximately 50 atmospheric samples and 41 urine samples for fluoride analysis; one sample of human tissue (lung) has also been received. One hundred forty-two exposure chamber samples have been received from the engineers for fluoride analysis. Thirty-seven osseous tissues from Mr. Sprague's two-year chronic uranium exposure have been analyzed for fluoride content.

Problem Code: F.4 (Fate)

Section Code: 3210

Determination of Fluoride in Blood:

A satisfactory method has been developed for the analysis of blood for fluoride content, and the method has been written up for inclusion in the second volume of the Pharmacology monograph. To date samples of lamb,

beef, dog, rabbit and human blood have been analyzed; a series of thirty bloods from individuals living in Rochester has been analyzed, and the results are now being correlated with the intake of fluoride in drinking

PROGRAM S.M.

SPECIAL MATERIALS

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

Section Code: 3210

Halogenated Hydrocarbons, Hexachlorpropylene (HCP), and Trichloracrylyl Chloride (TCAC):

HCP and its oxidation product, TCAC, are both highly toxic substances. The LD50 for rats exposed for 30 minutes to the vapors of these substances is approximately 460 and 100 ppm, respectively. Whereas the unpleasant odor of HCP may serve as a warning when even a few ppm are present in the air, TCAC is more insidious, and it appears probable that a dangerous concentration may be built up in the atmosphere without sensory warning.

HCP upon inhalation by animals produces excitement; continued inspiration of higher concentrations resulting in the excitement stage of anesthesia; TCAC under similar conditions gives some degree of sedation. Both vapors in man may produce eye irritation, redness of the nasal membrane, unproductive cough, and nasal discharge, and reddening of the pharyngeal mucous membranes; lymph glands in some cases become slightly enlarged and tender; some loss of appetite is also noted. The production of sensitization appears to be a characteristic of the compounds. The above symptoms are produced by extremely mild exposures to TCAC that result from the penetration through the Army Assault Canister.

Treatment would consist of removal from the toxic atmosphere and continued rest. The Army Assault Canister offers protection against HCP but not against TCAC. For TCAC the use of a supplied air type of mask is advocated.

PROGRAM I.S.

ISOTOPES

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

Section Code: 3120

The Application of Filter Paper Chromatography to the Qualitative Analysis of Volatile and Non-Volatile Organic Acids:

Background: The use of filter paper chromatography for the qualitative analysis of mixtures of organic compounds depends upon the difference in the partition ratios between the water phase and an organic solvent phase for the organic compounds investigated. This difference is utilized by setting up experimental conditions as follows: A small drop of the organic compound mixture is placed near one end of a long strip of filter paper (the water phase) and that end (not including the spot) dipped in the selected organic solvent. This brings about a movement of the organic solvent boundary and a following movement of the various organic compounds at a rate depending on their respective partition coefficients. After a suitable interval of time, the process is terminated by removing the end of the filter paper from the solvent; the solvent boundary is marked and the paper is dried and sprayed with some dye to give an identifying color reaction. The various organic compounds are found to be distributed along the strip as more or less narrow bands and can be identified by calibration experiments performed with the single pure compounds.[1] The R_F value is defined as:

$$\frac{\text{Movement of spot}}{\text{Movement of advancing front of organic solvent}}$$

In the application of filter paper chromatography to the qualitative analysis of mixtures of organic acids, certain difficulties are encountered as follows: (1) streaking of many acids at the concentrations required for detection by color reaction; (2) spreading or evaporation from the paper of the volatile members of the group; and (3) too rapid migration, essentially with the solvent boundary, of the compounds of low water solubility.

Of a number of organic acids salts and derivatives investigated, the potassium hydroxamate was found to be most satisfactory in overcoming some of these difficulties. Synthesis of the hydroxamates enabled the

1. Gordon, A. H., Martin, A. J. P., and Synge, R. L. M., Biochem., 37, Proc. xiii (1943).

separation of most of the common organic acids with chain lengths of about eight carbon atoms or less.

Method: The potassium hydroxamate derivatives of the organic acids were prepared by reacting the methyl ester with about a two-fold excess of a mixture of potassium hydroxide and hydroxylamine in methyl alcohol. The hydroxamate derivatives obtained from about 10^{-6} mole of each of the organic esters were applied to the filter paper. The chromatogram was developed for each of a number of suitable organic solvents. In order to make the bands visible the dried filter paper was sprayed with ferric chloride solution. This displayed the organic derivatives as purple spots on a yellow background.

Results: The R_F values were determined for a number of hydroxamate derivatives and for a variety of organic solvents. These data are given in Tables I and II (Pages 60 and 61).

Discussion: Converting the organic acids to their hydroxamate derivatives increases the polarity, decreases the volatility and makes possible the use of a convenient and reasonably sensitive color reaction for development of the chromatogram without increasing the molecular weight to such a degree that solubility differences between members of a homologous series are seriously diminished. In general, the color reaction is sufficiently sensitive to detect on the finished chromatogram a spot containing approximately 10^{-7} mole of acid, assuming a quantitative conversion of the ester to the hydroxamate in the preparative procedure, while use of mixture containing more than about a milligram of any one component is likely to lead to spreading or streaking due to the overloading of the paper. With proper controls, a rough quantitative estimation may be made of the amount of acid in a spot by judging from the size of the spot and the intensity of the color.

Disadvantages in the use of the hydroxamate derivatives include the manipulations involved in their preparation and the multiple spots obtained from the acids with multiple functional groups. The multiple spots are presumably due in general to failure to carry the preparative reactions to completion, although the possibility of complicating impurities in some of the stock organic acids employed has not been entirely ruled out.

The colors in the developed chromatogram are relatively stable, although continued exposure to strong light causes a loss of contrast, principally by action on the ferric chloride background. Intense spots of the ferric hydroxamates tend to spread to other papers in contact with them, so that in filing it is well to insert blank sheets of paper between the chromatograms.

TABLE I

R_F Values of Hydroxamate Derivatives of Organic Acids in Various Solvents on Whatman No. 1 Filter Paper.

Hydroxamate	Solvent						
	n-Hexyl alcohol	n-Amyl alcohol	n-Butyl alcohol	sec-Butyl alcohol	Methyl ethyl ketone	Iso-butyric acid	Phenol
Formic	0.06	0.12	0.40	0.54	0.22	0.45	0.57
Acetic	0.23	0.35	0.51	0.63	0.40	0.57	0.70
Propionic	0.43	0.56	0.68	0.78	0.61	0.68	0.78
Butyric	0.63	0.71	0.79	0.87	0.75	0.74	0.80
Valeric	0.73	0.78	0.86	0.90	0.84	0.83	0.84
Caprylic	0.86	0.88	0.90	0.91	0.91	0.87	0.90
Pelargonic	0.84	0.85	0.91	0.90	0.91	0.88	0.90
Capric	0.89	0.89	0.92	0.90	0.90	0.92	0.95
Benzoic	0.69	0.73	0.82	0.86	0.83	0.79	0.85
Phenylacetic	0.73	0.76	0.83	0.83	0.84	0.75	0.86
Lactic	0.14	0.23	0.42	0.53	0.28	0.50	0.66

TABLE II

Rf Values of Hydroxamate Derivatives of Organic Acids in Phenol and in Isobutyric Acid on Whatman No. 1 Filter Paper

Hydroxamate	Solvent	
	Phenol	Isobutyric Acid
Oxalic	0.14, 0.40	0.23, 0.28, 0.32
Malonic	0.11, 0.23	0.19, 0.32
Succinic	0.40, 0.72 orange	0.45, 0.52 orange
Glutaric	0.47	0.37, 0.52
Adipic	0.54, 0.67	0.44, 0.60
Pimelic	0.60, 0.73	0.52, 0.69
Azelaic	0.63, 0.74	0.66 streaked
Sebacic	0.89	0.74, 0.89
Citric	0.09, 0.23	0.20, 0.29
Tartaric	0.10	0.19
Pyruvic	0.59, 0.86	0.54, 0.62 orange, 0.73

Radiocarbon and Filter Paper Partition Chromatography:

Background: Due to the important role that carbon compounds play in biological systems, it was thought useful to study the degree to which C^{14} containing products of intermediary metabolism as separated by partition chromatography could be identified by radioautographic exposures.

Method: For the purposes of this study one milligram of Chlorella suspended in 0.1 ml. of distilled water was exposed to light in the presence of 2 ml. of air containing a few micrograms (one microcurie) of radioactive carbon dioxide for a period of four hours, and the tube containing the Chlorella was then connected with a tube containing solid KOH for an additional hour. The Chlorella suspension was divided into two equal portions, one of which was hydrolyzed in 0.1 ml. of 1 N HCl for 24 hours, and the other centrifuged and extracted with 25 microliters of hot 80 per cent alcohol for 30 minutes. A two-dimensional chromatogram of each of the solutions (plus added amino acid markers) was developed with phenol and collidine* and after the filter papers were dry, they were pressed directly against sensitive X-ray film (E.K. "No Screen") for three days.

Results: The alcohol extract gave an excellent chromatogram, the radioautograph of which showed exposed areas as follows:

1. A rather poorly defined very dark spot or spots due to relatively fat-soluble radioactive substances, possibly organic acids of low volatility, which had travelled essentially with the boundary in both phenol and collidine.

2. A dark spot in approximately the position taken by glucose in routine chromatograms (R_F values of 0.42 and 0.41 in phenol and collidine, respectively).

3. A moderately dark spot coinciding in position with the glycine, alanine, arginine, valine, and proline added as markers.

4. Light spots tentatively identified as aspartic acid, serine, and threonine; two light spots near glycine and two near alanine which could be sugars or peptides; and an unidentified light spot with R_F values of 0.98 and 0.06.

There was no visible exposure in the area occupied by the phenylalanine added as a marker or in the positions ordinarily taken by the other amino acids not listed in the preceding paragraphs.

A chromatogram prepared from a 80 per cent alcohol extract of

*The mixture referred to as collidine in this description was actually the organic phase from a mixture of 1 part, 2, 4, 6 collidine, 1 part 2, 4 lutidine, and 2 parts H_2O as recommended to us by Dr. C. E. Dent.

200 mg. of non-radioactive Chlorella caused no "chemical exposure" of an x-ray film, and when treated with ninhydrin showed spots tentatively identified as aspartic acid, glutamic acid, serine, glycine, threonine, alanine, histidine, and arginine, and an unidentified spot with R_F values of 0.10 and 0.05.

Due to a marked temperature change during development, the chromatogram prepared from the Chlorella hydrolysate was skewed to such an extent that detailed identifications could not be made, but insofar as it could be interpreted, it appeared qualitatively similar to the alcoholic extract chromatogram.

Discussion: These experiments appear to be convincing evidence that an extremely valuable tool for intermediary metabolism studies with radioisotopes is at hand. With the possibility of preparing filter paper chromatograms of moderately water-soluble compounds of low molecular weight, such as amino acids, sugars, organic acids, purines, peptides, and a variety of other organic and inorganic substances, a considerable amount of data concerning the metabolic fate of radioactive compounds may be obtained by a very simple procedure. A particularly valuable feature of the chromatographic technic is its ability, without any deviation from the routine procedure, to isolate and draw attention to unexpected or even unknown compounds involved in a metabolic process under study, and in addition to furnish considerable aid in their identification.

The lower limit to the quantity of any given compound which may be handled successfully by partition chromatography seems to be determined, in general, by the sensitivity of the procedure employed for detecting the spots. With most good visual color reactions this lower limit is in the order of a microgram (and frequently embarrassingly close to the upper limit), but with radioactive compounds of high specific activity quantities many orders of magnitude smaller may be easily detected, identified, and isolated in a small volume of solvent for further tests.

Use of Radioactive Reagents in Filter Paper Partition Chromatography:

Background: One of the principal limitations in the application of filter paper chromatography to biological studies is the rather narrow range of concentrations over which the usual technique affords reliable results. The smallest amount of a substance which may be detected by color reactions is of the order of 10^{-6} to 10^{-5} grams whereas the largest amount which may be handled without overloading the paper and causing streaking and spreading is of the order of 10^{-4} to 10^{-3} grams. This is a particularly serious limitation in the analysis of mixtures of organic compounds derived from biological material since the concentration of individual compounds may vary by several orders of magnitude.

A method for the extension of the useful concentration range suggested itself from experiments in which radioautographs of filter paper chromatograms were prepared in order to determine the metabolic transformations of an injected radioactive compound. Excellent chromatograms could be prepared

with quantities of materials many orders of magnitude less than those required for visible color reactions. Therefore, it seemed reasonable to investigate the use of radioactive reagents, rather than color reagents, for the detection of the various compounds on filter paper. Two methods were tried for incorporation of the radioactive isotope into the compounds to be separated.

Method and Results:

1. Suspension of the latent chromatogram in a closed vessel filled with a radioactive gas which was able to react with the class of compounds under investigation.

a. Various quantities (10^{-11} to 10^{-8} mole) of silver nitrate and ethylamine hydrochloride were placed as individual spots on a strip of filter paper. The paper was allowed to dry and then exposed to the vapors of hydriodic acid containing I^{131}. After an hour's exposure, the filter paper was prepared for a radioautograph by pressing it against an x-ray film with a sheet of cellophane intervening. The radioautograph clearly showed the spots, and, in general, the density of the spots varied directly with the amount of material added.

b. A second experiment with non-radioactive hydrogen sulfide as the reagent acting on spots containing microgram quantities of various metal ions has indicated that radioactive hydrogen sulfide may be a valuable reagent as a chromatographic indicator.

2. Addition of the radioactive reagent to the compound mixture prior to its placement on the filter paper.

a. A mixture of amino acids and radioactive hydriodic acid was tested and the resulting radioautograph of the chromatogram showed a single spot for the hydriodic acid demonstrating that the amino acids and the hydriodic acid went their separate ways. A mixture of radio-potassium and mono-carboxylic acids showed the same unsuccessful result.

b. On the other hand, preliminary tests with simple amino hydroiodides have shown that amines carry the radio-hydriodic acid along even when little or no hydriodic acid was present in the system.

Discussion: The methods presented above represent only a few of the simpler procedures for introducing a radioactive reagent. The success obtained in these experiments warrants further study of methods whereby the sensitivity of radioautographic techniques can be utilized in extending the range of usefulness of filter paper chromatography.

PROGRAM O.S.

OUTSIDE SERVICES

Problem Code: None

Section Code: 3110

Analyses for Special Material Plants:

The following analyses were made for the special material plants:

a. 82 air samples were analyzed for radon content.

Five Geiger tubes of local design have had mica windows cemented on, been filled, and tested as a service to the University of California Atomic Energy Project, Los Angeles, California.

Spectrochemical Analyses:

As a service to outside organizations, the following spectrochemical analyses were performed for the period July 1 to September 30, 1947:

12 samples including soil, paint, and foliage for uranium

10 samples of soil for uranium and vanadium

10 samples of soil for uranium

6 samples human autopsy material for uranium and beryllium

9 samples human autopsy material for uranium and lead

4 samples human autopsy material for uranium

1 sample human liver biopsy for U, Th, Be, Va, Pb, Ni, Cr, and Cu.

In addition, about 25 miscellaneous analyses mostly for Be were done for units within the Project.

Electron Micrographs:

A series of 20 electron micrographs of BeO dusts were made as a service to Clifton Products Co.

Problem Code: None

Section: 3310, 3311

Measurements of Industrial Samples:

During the months of July, August, and September, 1947 the following measurements were made of industrial samples:

a. 7133 industrial film badges, processed and reported.

b. 89 radon gas samples (analyzed by Dr. John Hursh)

c. 30 breath samples for body Hg content (analyzed by Dr. Robley D. Evans, M. I. T.)

d. 278 fingerprint impressions (analyzed by Dr. Roger Harvey, University of Illinois College of Medicine).

e. 5 dust tubes analyzed.

Analyses of Miscellaneous Materials:

In addition to the above-named routine work, these sections were used extensively in consulting capacities, and as a centralized medium for the collection of samples, expediting the analysis by other sections, and reporting results relative to the following:

a. 1 analysis of artificial teeth for radioactive and toxicological hazard.

b. 45 soil samples received (for uranium content), 15 analyzed and reported.

c. 57 urines (analyzed for uranium content).

d. 45 urines analyzed for fluoride.

e. 166 smear samples counted on alpha counter.

f. 256 dust samples for uranium content received, 67 analyzed and reported.

g. 9 five-gallon river water samples, not yet analyzed.

Film Research:

Additional film research was carried on regarding the effect of

heat to density of various exposures; the film effect of exposure to radioactive iodine; a comparison of the sensitivity of Eastman Kodak Type K film with the DuPont film; and a study (still going on) of the method of counting proton recoil tracks versus the cadium and lead strips on film for determining neutron exposure. Much time has also been spent in considering features for a new badge design.

PROGRAM P.H.

PROJECT HEALTH

Problem Code: None

Section Code: 3330

Project Medical Service:

1. The medical office has on file 38 physical records which were completed during the months of July, August, and September, 1947.

2. During these months 43 chest reports were completed and filed in the medical office.

3. There were no lost-time accidents due to injuries received on the Project. Of the injury cases reported to the medical office, one was referred to Emergency at Strong Memorial Hospital, one to X-ray at Strong Memorial Hospital, one to an outside physician, and two returned to the medical office for second treatment. The usual compensation forms have been submitted on these cases.

4. The results of the tuberculin tests that were given during the month of July to the employees of this Project are as follows:

Test with First Strength:

	No. of Reactions
Negative	113
+	4
1+	3
2+	1

Check x-rays have been taken on personnel whose reactions were positive.

5. The report of the blood counts being done in the medical office for the months of July, August, and September, 1947 is as follows:

a.	Initial complete blood counts	42
b.	Routine complete blood counts	203
c.	Sedimentation rates	47
d.	Wasserman tests	46
e.	Special complete blood counts	1
f.	Incomplete blood counts	4
g.	Termination complete blood counts	30

6. For the above months, 90 repeat urinalyses were completed with 43 initial urinalyses, 1 special urinalysis, 3 orthostatic albumen tests, and 29 termination urinalyses.

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

Back-Scatter from Silver Foil Samples:

Background: One method used in this laboratory to prepare beta samples for counting involves the distribution of the radioactive solution on a silver foil in the form of small droplets disposed over a specified central area. These droplets are then evaporated with gentle heat from an infra-red lamp. When dry, the foil is placed on a brass sample and counted under a thin window, bell-type counting tube.

In order to obtain absolute activity measurements the geometry and absorption of the counting tube was determined in the usual manner. The standard used was a silver foil on which 1 mg. of uranium had been plated at least two years previously. Since after this interval U_{238}, UX_1, UX_2, and U_{234} would be present in equilibrium concentrations, we can predict the number of hard beta disintegrations per unit time by counting the alpha emission in an alpha parallel plate counter. The uranium foil was placed on the beta counter sample mount and counts were made with an increasing number of 2 mil. aluminum filter discs between the foil and the counter window. The hard beta per minute count was extrapolated back to zero window thickness and divided by the total number of hard beta disintegrations per minute as inferred from the α count to give a figure for the tube geometry.

Similar filter experiments were conducted with the beta isotope foil sample, the activity of which we desired to determine. The counts were extrapolated back to a no-window count so that absorption in the mica window could be allowed for.

This method of calibration did not take into account the possibility of a difference in the back-scatter of the uranium betas as compared to the back-scatter of the beta emission to be measured. The preliminary experiments to be described are an attempt to evaluate back-scatter as a source of error in such calibration measurements.

Method: For use in these experiments a thin-walled cylindrical lucite cup was made. This cup is illustrated in Figure 1 (Page 71). It consists of a bottom portion with a base designed to fit in our sample holder support. The top outside lucite ring is designed to be forced down

flush around the top inside lucite ring so as to stretch smoothly a sheet of varnished cellophane in a fashion similar to the wooden embroidery frame technique. The top inside ring may then be put in place on the base forming a cellophane covered lucite cup of 5 cm. inside depth, of 4.5 cm. inside diameter, and of 0.3 cm. wall thickness. The cup was designed to that when in place on the sample holder support of our shielded counting apparatus, the cellophane surface was at a window distance equivalent to the position of the silver foil in our usual counting technique.

A brass plug was machined out so that it would just fill the air space inside the cup. A 3 mil. silver foil was soldered to the upper surface of the plug. When the plug was in place in the cup, the foil surface approximated the lower surface of the cellophane cover.

The experimental procedure employed was to remove the cellophane covered top of the cup and evaporate small droplets of the beta isotope solution distributed within a central circular area on the under surface of the cellophane. The circular area chosen (3 cm. in diameter) corresponded to the silver foil area used for deposit.

When the droplets had completely evaporated, the top was replaced on the cup after having first removed the brass plug. Counts were now made with a series of aluminum filter thicknesses from zero thickness to 14 mils. in steps of 2 mils. The same procedure was repeated with the brass plug in place in the cup. These two sets of data were plotted on semi-log paper giving usually straight line plots representing conditions of no back-scatter and back-scatter respectively.

The beta emitting isotopes so far studied are UX_2, Phosphorus 32, Iodine 131, and Iron 59.

Results: The data were in all cases except Fe 59* extrapolated back to zero window thickness and the ratio of metal backed count to air backed count was calculated giving values as follows:

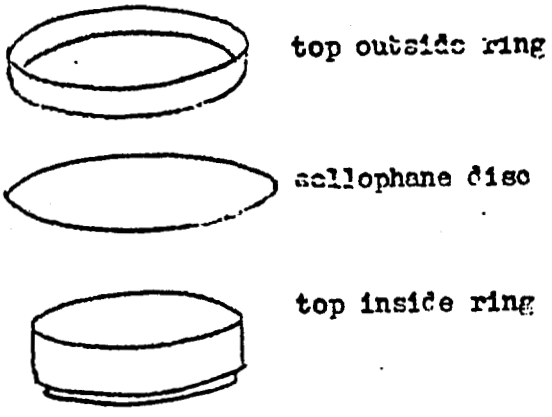
Isotope	Max. Beta Energy M.E.V.	Metal-backed Count / Air-backed Count
UX_2	2.32	1.7
P_{32}	1.72	1.7
I_{131}	.60	1.4

Discussion: It would, therefore, appear that backscatter under the conditions of the above measurements is less for soft betas than for hard betas and that, therefore, the betas from UX_2 are not a suitable reference standard for absolute measurements of I_{131} beta activity unless a correction is applied.

The second point of interest in this data is the magnitude of the back-scatter. Theoretical considerations dealing with this point will be reported at a later time.

*The iron isotope contained beta particles of 2 maximum energies and, therefore, the data did not plot as a simple straight line on semi-log paper.

Figure 1



LUCITE SAMPLE MOUNT

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

Section Code: 3160

Use of the Electron Microscope for Dust and Fume Studies:

Background: The study of plant dusts and fumes has been facilitated by use of the electron microscope for the measurement of particle size and shape. The specific techniques under study are:

Method: a. The preparation of dust and fume samples to be studied by electron micrography. These include BeO, UO_2, UO_4 as bulk materials and metallic fumes such as Al, Zn, Mg, etc.

b. The detection and identification of some of these materials by electron diffraction patterns.

c. The use of replica methods for those materials that clump in the suspension ordinarily used in preparation of electron micrography specimen.

Results: a. Successful methods of preparation have been developed for BeO, UO_2, and UO_4 as well as for the common fumes. Several hundred electron micrographs have been made of a large and varied assortment of dusts and fumes in order to catalog their characteristic appearance and their idiosyncrasies in the precipitators.

b. The modification of one of the electron microscopes for the production of electron diffraction patterns is just getting started and no results will be reported at this time.

c. The use of replica methods for size measurement of dust particles that clump in the suspension medium has proved disappointing. The materials frequently transfer from the glass slide on which they were initially deposited through the polystyrene step to the silica film which is used in the electron microscope. It was difficult to account for this behavior since the material appeared to be flat on the primary glass slide and also on the electron micrograph. To determine how much of a third dimension these particles actually had, gold shadow castings and more particularly stereoscopic micrographs were made. This study revealed that instead of a flat field, that which appeared to be a single particle would often be a superimposition of two or more particles and the formation of irregular chains extending into space. In view of these findings, it becomes obvious that a method of replica production that involves pressure would break down these chains and distort particle size measurements. The direct transfer of some materials instead of the cast of these materials was explained. The comparatively large third dimension and the orientation of these particles that have been noted in the stereoscopic study indicates that caution must be exercised in the measurement of the particle size and that a large number of particles must be measured to arrive at a true value.

Electron Micrographs of Single Whole Cells:

Background: Because of the desire to secure electron micrographs which included a single whole cell, the RCA microscope had to be modified.

Method: In order to reduce the magnification by the order of several hundred times, the objective pole pieces were removed. The objective aperture is an integral part of the pole piece and its removal results in a considerable loss in contrast. The RCA microscope has no provision for an objective aperture at these magnifications. Therefore, a non-magnetic pole piece was constructed in the shop with provisions for mounting the objective aperture.

Results: The resulting pictures show a remarkable increase in detail and contrast and this modification should pay dividends in any work involving magnifications in this range.

Further work has been done toward autoradiography using electrostatic magnification of the images formed. Useful beam currents have been obtained, but because of the instability of the metals used as an active cathode, sufficiently long exposures cannot be made at present. Investigation of other cathode materials is necessary.

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