893

712317

Colonel E. H. Marsden, Executive Officer March 11, 1947

Major Richard M. Brundage, MC, Chief, Medical Division

MEDICAL RESEARCH AT UNIVERSITY OF CALIFORNIA AT LOS ANGELES

SYMBOL: ARCT

1. In response to your request for assistance in the preparation of the program for Dr. Warren's work at the University of California, Los Angeles, to be used in reply to the letter from Colonel Stewart dated February 28, 1947, I have prepared certain facts available from Dr. Warren's report.

2. Dr. Warren's proposed program includes among other things the following major items:

(a) Mechanism of blood vessel injury by radiation.
(b) Bone marrow injury from radiation and its repair and treatment.
(c) Mechanism of "metal" deposition in bone and mechanism of removal from bone.
(d) Protein degradation following radiation and chemical injury.

In addition to the above mentioned specific items, certain special studies in connection with the follow-up of the Japanese bombings and the Bikini Crossroads Operation are anticipated with AEC approval. These would be coordinated through Dr. Warren's office. Reimbursement and travel of personnel involved in these studies, as well as the rendition of reports of their results, would also be correlated through Dr. Warren's office.

3. For the above mentioned program the $25,000 budget for buildings, utilities, and contingencies was requested by Dr. Warren and was approved by the interim Medical Advisory Committee in their report of 29 January 1947.

1 Attachments
Ltr. fr. Calaveras Eng. Works,
dtd. 2/24/47

EMB

Colonel E. H. Marsden, Executive Officer — March 11, 1947

Major Richard M. Brundage, MC, Chief, Medical Division

MEDICAL RESEARCH AT UNIVERSITY OF CALIFORNIA AT LOS ANGELES

SYMBOLS ABCT

1. In response to your request for assistance in the preparation of the program for Dr. Warren's work at the University of California, Los Angeles, to be used in reply to the letter from Colonel Stewart dated February 26, 1947, I have prepared certain facts available from Dr. Warren's reports.

2. Dr. Warren's proposed program includes among other things the following major items:

(a) Mechanism of blood vessel injury by radiation.
(b) Bone marrow injury from radiation and its repair and treatment.
(c) Mechanism of "metal" deposition in bone and mechanism of removal from bone.
(d) Protein degradation following radiation and chemical injury.

In addition to the above mentioned specific items, certain special studies in connection with the following of the Japanese bombings and the Bikini Crossroads Operation are anticipated with ASC approval. These would be coordinated through Dr. Warren's office. Reimbursement and travel of personnel involved in these studies, as well as the rendition of reports of their results, would also be coordinated through Dr. Warren's office.

3. For the above mentioned program the $65,000 budget for buildings, utilities, and contingencies was requested by Dr. Warren and was approved by the interim Medical Advisory Committee in their report of 29 January 1947.

1 Attachments
Ltr. fm. Calaveras Eng. Works,
dtd. 2/26/47

SAVE

SAI

ARMY SERVICE FORCES
UNITED STATES ENGINEER OFFICE
MANHATTAN DISTRICT
OAK RIDGE, TENNESSEE

IN REPLY REFER TO BIDMT-1

26 February 1946.

Subject: Future Medical Research Program.

MEMORANDUM to Brigadier General K. D. Nichols, District Engineer.

1. Biological research conducted under the auspices of the Manhattan District prior to the cessation of hostilities was primarily of a preliminary nature in order to provide immediate information on tolerances involving various types of radiation from external sources and from inhaled, injected and ingested radio-active materials. Tolerances to various chemical compounds similarly introduced into the body were also studied. In order to establish unequivocal and enduring tolerance levels, however, further work will be required which will be primarily long-term in character. Repetition in some categories of research, because of their critical nature, will be necessary.

2. Colonel Warren has pointed out that much of the data already collected is proving valuable from a medical legal point of view. It is anticipated that further research will also serve in this manner.

3. It appears now, that medical research on fissionable and other materials required in atomic energy, and not previously investigated, will need future study in much the same manner as has been done with uranium and plutonium.

4. In general, it appears necessary that a research program be continued and should be conducted under contract with various universities and medical Research Centers. The biological program should be modified to a slower pace, and fewer aspects of the biological investigations should be conducted simultaneously. The reduced rate and number of projects will provide better continuity and more secure results.

Hymer L. Friedell
per rl.

HYMER L. FRIEDELL,
Lt. Col., Medical Corps,
Exec. Off., Med. Sect.

702

1075983

ARMY SERVICE FORCES
UNITED STATES ENGINEER OFFICE
MANHATTAN DISTRICT
OAK RIDGE, TENNESSEE

IN REPLY REFER TO

EIDMT-1

26 February 1946.

Subject: Future Medical Research Program.

MEMORANDUM to Brigadire General K. D. Nichols, District Engineer.

1. Biological research conducted under the auspices of the Manhattan District prior to the cessation of hostilities was primarily of a preliminary nature in order to provide immediate information on tolerances involving various types of radiation from external sources and from inhaled, injected and ingested radio-active materials. Tolerances to various chemical compounds similarly introduced into the body were also studied. In order to establish unequivocal and enduring tolerance levels, however, further work will be required which will be primarily long-term in character. Repetition in some categories of research, because of their critical nature, will be necessary.

2. Colonel Warren has pointed out that much of the data already collected is proving valuable from a medical legal point of view. It is anticipated that further research will also serve in this manner.

3. It appears now, that medical research on fissionable and other materials required in atomic energy, and not previously investigated, will need future study in much the same manner as has been done with uranium and plutonium.

4. In general, it appears necessary that a research program be continued and should be conducted under contract with various universities and medical Research Centers. The biological program should be modified to a slower pace, and fewer aspects of the biological investigations should be conducted simultaneously. The reduced rate and number of projects will provide better continuity and more secure results.

HYMER L. FRIEDELL,
Lt. Col., Medical Corps,
Exec. Off., Med. Sect.

702

1075984

Section I -- Instrumentation
Section Head — Dr. John B. Hursh

To this section will fall the following duties. (1). Construction and maintenance of instruments for the division of radiology and biophysics. (2). General supervision and responsibility for radioactivity measurements in this division. (3). Until this duty is transferred outside the division, responsibility for the industrial monitoring program now carried out by the division of special problems.

Section 2 -- Tracer Chemistry
Section Head — Dr. Robert M. Fink

It will be the duty of this section to extend animal tracer studies already under way with polonium, radium and uranium. Cooperating with section eight, the personnel of this division will carry out the planned human tracer studies with plutonium, radium, polonium and uranium.

Also in the jurisdiction of this section will be the carrying out of any metabolic studies requested by the district or its successor in the future.

In collaboration with the instrumentation section, experimental methods will be developed for the application of radioactive isotopes produced by the Uranium Pile to basic biological and medical problems, both of the Manhattan project and of the more general fields of biology and medicine.

Section 3 — Radiation Physiology
Section Head — Dr. Thomas Noonan

In this section, it is proposed to carry out: (a) chronic and acute radiation exposures in animals including sperm studies (b) chronic and acute exposures to radioactive isotopes (c) search for therapeutic aids after radiation exposure (d) pathology (e) hematology (f) general animal care for other sections.

Section 4 — Radiation Chemistry
Section Head — Dr. Kurt Solomon

It will be the primary duty of this section to seek, primarily by chemical techniques, (1) means of detecting early damage from radiation and radioactive materials, (2) studies on the mechanism of radiation effects. It is expected that much of this work will be collaboration with section 3.

Section 5 — Spectroscopy
Section Head — Dr. Luville T. Steadman

The personnel of next year's spectroscopy section is now engaged in two problems (1) Studying the distribution of uranium in rabbits, particularly bone storage after oral and lung administration, (2) endeavoring to obtain as large uranium and lead storage in bone as possible for cooperative x-ray diffraction work with Mr. Mermagen. It is hoped with the x-ray diffraction technique to determine if these elements are stored by replacement in the apatite lattic of bone and if they are, the positions that uranium and lead atoms occupy in the mineral lattice.

It is proposed to conclude work on Problem I by September, 1946. Problem II will be continued at least until the possibility of obtaining useful information has been more thorogoughly explored.

From September throughout the remainder of the year it is proposed that the primary work of the Spectroscopy section will be in collaboration with Sections III and IV in studies o n the basic nature of the effects of radiations on biological systems. It will also endeavor to carry out analysis ofspecifically requested by the district or its successor.

The primary technique now available for this work is absorption spectra in the visible and ultra violet. Probably emission spectra techniques will be little used in this application. Also it may be advisable to acquire an infra-red absorption spectrometer to expand the ist of chemical entities for which this section wil have useful analytical techniques.

Section VI -- Radiation Mechanics
Section Head -- Francis W. Bishop.

(This section is based on the assumption that Mr. Bishop's research activites, as distinguished from shop and maintenance duties will be carried out in the Division of Radiology and Biophysics. I believe they might equally be carried out as a part of administration.)

This section will have three principal assignments: (1) By the use of tissue window techniques to study the growh of normal and abnormal tissues through the microscope and the effect of radition upon their growhh. Photography will probably be used as an important aid in this work. (2) In collaboration with Section III, to study the differential effects of extremely high intensity radiation dosage as compared with lower dosage rates (3) With the electron microscope to study appropriate problems.

Section VII -- Physics
Section Head -- Dr. E.A. Lamar (tenative)

(This section is non-existant at the present time). There are several problems in applied and basic radiation physics that such a section might undertake to solve.

Section VIII -- Metabolism
Section Head -- Dr. Samuel Bassett.

This section has engaged in collaboration withthe Division of Special Problems and a group at the Los Alamos project in human tracer studies with plutonium. This particular series is nearly complete.

Studies on human subjects requested by the District that still remain to be completed are on (1) uranium, (2) radium, and (3) probably a few on polonium. Six months is an estimate of the time necessary to carry out this work.

GENERAL RESEARCH

To the extent th t I am acquainted with it, I am impressed that as far as an animal research program can have furnished it with irradiation periods of about 1½ years, good data is available for calculation of permissable daily human exposure to x-ray and gamma radiation. Unsolved problems remaining are (1) a method of detection of radiation damage in humans such as would be produced by 10 r per day certainly a dangerous level if long continued. (2) a specific method for counter-

acting the effects of acute radiation injury. (3) knowledge is still lacking concerning the immediate mechanism by which radiation injuries are produced.

It is likely that the most profitable approach to (1) will be of biochemical nature. I propose therefore th t this shall be one of the principal research assignments of section IV with cooperation of section V.

A search for specific methods for combatting acute radiation injury ought to be one assignment for Section III. One potential method already considered by Dr. Boche and Mr. Bishop is the possibility that immediate lowering of body temperature to about 80° as can be done in human subjects, might permit greater tissue recovery before irreversible breakdown has occurred. They were planning experiments with hamsters in which a state of hibernation can be induced.

There are probably other leads to Problem 2 worthy of investigation.

Dr. Kay Fink already has work underway on the effects of irradiation on nuclear protein metabolism as indicated with radioactive phosphorus that may be considered work on Problem 3. I believe this work should be carried forward. Work also contemplated by Mr. Bishop with rabbits ear windows also bears on this problem as do the contemplated studies with high intensity radiation.

Toxic Effects of Radioactive Elements

Polonium: The toxicity of polonium has already been studied at Rochester in the case of single intravenous experiments in rats. The toxicity of plutonium and radium have been studied in Rochester and to some extent in other project areas after single administrations. However, no data has been obtained where a constant polonium level has been maintained in the body over a period of months. Thus we do not have nearly the certainty in setting a tolerance level for body polonium content (and other alpha emitting elements) that we have in setting a tolerance to external radiation.

In setting such a level for district use it has been assumed until recently that the ratio of tolerance body content of polonium to the average single lethal dose is the same as the ratio of the daily permitted dose of external radiation to the average single lethal dose.

Unfortunately a second method of making this calculation gives very different results. If we assume that the permitted polonium content of the body bears the same relation to permitted radium content as their relative toxicities measured in semi-acute experiments and that the allowable radium in the human body is 0.1 micro grams we obtain an allowed body content for polonium many times lower than calculated by the first method.

Since the polonium content of the body falls to negligible amounts a year after exposure we felt relatively safe in using the first tolerance value during the war. Now that the war is over, the tol rance value has been reduced by a factor of 8. This has made plant operation extremely difficult since in previous operation periods many personnel have exceeded this value. The district's demand for polonium is increasing rather than decreasing.

Even this new permitted value is much higher than what may be the true tolerance value in the light of human and animal radium toxicity data. It therefore appears that long term toxicity studies in this field, are from the interest of the district very much indicated. A worthwhile program on t is subject can scarcely be completed in one year. These notes are a brief summary of a complicated subject.

Radon: The districts present permitted radon in air value is ten times the pre-war value accepted by the Bureau of Standards and most state health units. This larger value is due to the impracticality of meeting the lower value in district plants/ There have been no good studies made in animals that can lead to a good estimate of

radon tolerance. Such studies are therefore probably indicated.

I believe we have found practical methods of removing radon so that chamber studies need not be a Rochester Project hazard.

Metabolic Tracer Studies: It is probably desirable to carry out the human tracer studies with U 235, radium andpolonium originally planned.

Also it is probably worthwhile to continue studies now underway in which the effect of BAL and related compounds on polonium excretion are studied.

With radio-carbon we are planning to investigate the similarities and differences of the carbonate ion as compared with bone seeking inorganic ions such as plutonium in their in vitro and in vivo behavior.

There are other problems concerned with polo nium metabolism that merit further and more conclusive studies.

We are willing, at the request of the district, to carry out other tracer work, for example with thorium, radioactive fission products and other radio-isotopes.

Instrumentation: I believe that the circuit that Dr. Enns developed for portable ionization instruments is in ruggedness, simplicity and sensitivity much the best of any now in use in the district. I hope to see several monitoring instruments designed using this circuit, somethi g Dr. Enns did not have the time to do.

I hope to see an investigation carried out by competent physicist as to the actual reliability of radon determinations on breath samples as a means of determining radium contents of the bodies of human subjects.

It seems desirable to further investigate the vane electrometer as a means of measuring smal amounts of ionization.

The following very vrief outline lists smaple problems that are of primary interest as representing applications to medical research and therapeutics of tools made available by the chain-r acting uranium pile. I propose that these be carried out to the extent of 15% of our year's budget.

1. General development of methods, physical, synthetic organic and analytical for the use of C14 and H3 in mediczl and biological research.

2. The synthesis of radioactive amino acids and other compounds, and the study of their distribution in tumor bearing animals. This work is to lay an experimental foundation for the improved treatment of cancer with radioactive isotopes.

3. To study the use of radioactive aminoacid analogues as therapeutic aids in experimental animal cancer.

4. Partly in collaboration with other groups in the medical school to use C14 and H3 as tools in the solution of medical resear h problems.

5. To study the use of radio-iodine as a therapeutic aid in thyroid disease.

Most of this work will be carried out in Secti on II, Tracer Chemistry. However it is proposed that the heads of other sections will also be able to take part in cert in of these problems as part-time co-workers.

[redacted]

Dr. Charles
Dr Stern.

I. Basis of contemplated problems in previous work. We have found (1943-6) in male mice, under chronic X-radiation, a dominant gene and chromosome mutation rate of nearly 10^{-4} per r. That is, one sperm in 10,000 undergoes a change which affects the immediate offspring, for each roentgen received.

Insofar as the Manhattan project is concerned with men rather than mice, it is important next to ask

(1) Do the same sorts of mutations occur in man?

(2) What proportion of them have significantly harmful effects?

It is to these questions that next year's research would be directed.

II. Proposed problems.

A. Morphological, embryological and cytological study of the mutants on which our rate calculations are based:

1. fertility mutations. When do the embryos die: at a harmlessly early gestational stage, or at a later period which would be at least a physiological strain of the mother and possibly dangerous? Why do the embryos die?

2. behavior mutations (circling, extreme restlessness, back somersaulting). What defects of the vestibular apparatus or central nervous system can be found? Have equivalent changes been reported in man?

3. structural mutations (situs inversus viscerum, several blood vessel anomalies, accessory adrenal cortical nodules, defective parietal suture). Are these comparable to reported sporadic conditions in man? What effects have they on length of life, general vigor, etc.?

B. Continuation of studies on

1. the frequency of chromosome rearrangements in haemopoietic cells under acute exposure of 200r or less,

2. relation of that frequency to rate of decrese in blood elements after radiation,

3. chromosome rearrangements as a possible basis of differences in species sensitivity at low exposures.

The work now under or contemplated is outlined as follows:

1. The series of control radiated ani als is being extended and the clinical, hematological and pathological alterations associated with radiation intoxication and being studied further.

2. The experimental program of marrow transplantation is to continue with emphasis on the following subdivisions.

a. Intravenous marrow transplantation
   1. Multiple marrow transplantations.
   2. Single marrow transplantations.

b. A study of vari u s means and agents to stimulate bone marrow such as nitrogen mustard, turpentine and the leukocytosis promoting factor of Menkin etc.

c. Intravenous lymphoid transplantation of spleen and lymph nodes.

3. Further studies are to be carried out to demonstrate the bone marrow reserve and its effect on radiation intoxication.

4. The tissue culture program is to be reopened and further studies made to grow fractions of the hemopoetic tissues. If this can be done studies will be instituted to study the effects of radiation on hemopoetic tissue and alterations in certain tissue sensitivity by growth in tissue culture.

5. It is planned to undertake studies on radiated animals employing folic acid and rutin to determine the effect of each of these agents on the bone marrow and the bleeding tendencies.

6. A program is being made up to study further the effects of transplantation of normal embryonal tissue, benigh tumor and malignant tumor to homologous and heterologous hosts as well as the associated cultivation of thes tissues in vitro.

[redacted]

[redacted]

Capt. Valentine
Section Head.

In broad terms we are interested in investigating the effects and the mechanism of the effects of radiation on the hemopoietic system, and, as corollary to this, in investigating proper techniques and methods for evaluating these effects and, if possible, of counteracting them. This involves, of necessity, basic hematologic research into the properties, chemical and physical, the life cycle, and physiologic functions of the various formed elements of the blood as they exist in the normal individual and in individuals with blood dyscrasias, as well as investigation of the mechanism of coagulation and hemostasis which often becomes deranged following radiation. Considerable animal work is contemplated with extension of techniques or results to human patients whenever it is possible or warranted.

At the present time the following investigations are either underway or will be started soon.

(1) Blood histamine studies have recently been initiated on animals and humans. It is desired to determine the total amount of blood histamine in normal animals, in radiated animals which are markedly leukopenic but not thrombopenic, and in radiated animals which are both severely leukopenic and thrombopenic. In this manner, it is hoped that the partition of histamine in the formed elements of the blood in both normal and radiated animals can be determined. Blood histamine studies in normal humans and in individuals with a variety of hematologic abnormalities will also be done. The partition of histamine between cells and plasma under different conditions will be investigated.

(2) Studies on the life cycle and the role of the blood platelet in hemostasis will be continued. Direct measurements of platelet utilization in thrombopenic, radiated animals have already been done by this section and are the subject of a previous report.

(3) Likewise studies on the life cycle of the leukocyte of the same type previously conducted in this laboratory will be continued.

(4) It is desired to investigate the problem of "marrow reserve," i.e. the reserve capacity of the marrow which can be called upon in time of strain. Cross circulation experiments in which a normal animal is connected in series with several depleted, radiated animals having non-functioning bone marrow are contemplated.

(5) It is anticipated that a few more cross circulation experiments of the type previously done by this section in an effort to elicit indirect radiation effects remote from the site of direct radiation can be carried out. It is desirable to follow a few animals which have been cross circulated with radiated animals for long periods of time--twenty-four to possibly seventy-two hours.

(6) An investigation of techniques and methods of evaluating coagulation defects will be begun shortly.

Other investigations in accord with the broad outline of this program as stated above will be planned in more detail when personnel of this section is brough to full strength.

RESEARCH PLANS FOR THE DIVISION OF PHARMACOLOGY 1946-47

Harold C. Hodge, Division Head.

SECTION	SECTION HEAD	RESPONSIBILITY SPHERE
I. Industrial Hygiene	Herbert Stockinger	a) Second-year continuation studies of U nitrate dust inhalation in dogs and rats. b) 30 day inhalation studies of U dust of graded particle sizes. c) Control studies for program b d) Particle size measurement laboratory e) Dust retention laboratory f) Industrial service testing lab. g) The role of phosphate containing enzymes and related substances in uranium poisoning. h) Lipis and lipid uranium complexes i) Protein uranium complexes j) Action of uranium on isolated cells
II. Biochemistry	William Neuman	a) Electrochemical studies of U salts and their complexes b) Studies on the mechanism of U fixation by bone c) Relationship between citrate formation and tolerance to uranium d) Continuation of studies on the distribution and excretion of U.
III. Ingestion	Elliott Maynard	a) Age factor in resistance to uranium poisoning b) Single oral dose of uranium c) Paired feeding in U poisoning. d) Injury cycles in U poisoning.
IV. Chemistry		a) Fluoride analyses b) Uranium analyses c) Biochemical analyses.
V. Pathology	James K. Scott	a) Extension of the studies on the use of histological examination of kidney as criterion in U poisoning. b) Studies on renal epithelium regeneration. c) Histological changes as related to acquired tolerance d) Extension of the investigations of hepatic response with high doses. e) Investigation of gastro-intestinal hemorrhages with high dietary exposure

VI. Physiology

a) Minimal concentration going through kidney to produce damage as shown by function tests.
b) Transillumination studies
c) Physical vx. functional effects on tubule.
d) Contact-concentration relationship for kidney.

Section II Biochemistry
Section Head Dr. William Neuman

A. Electrochemical studies of uranium salts and their complexes.

1. Polarographic studies on the uranium-citrate and the uranium lactate complexes.

2. Electrophoretic studies on the uranium-citrate and the uranium-lactate complexes.

3. Centrifugal studies on the state of U_4 in body fluids.

4. Solubility studies on the decomposition of uranates and uranium oxides in body fluids.

Background: Chemical studies on the behavior of uranium in the presence of complexing substances have given information of inestimable value in the understanding and interpreting the results of toxicological studies. To date, fairly extensive work has been conducted on uranium acetate and uranium bicarbonate complexes. In addition, some information is available as to the likelihood of oxidation and reduction in the animal body. Additional work is necessary on two important complexes, uranium citrate and uranium lactate. Further at the present state, it appears that little interconversion of U_6 to U_4 occurs normally. Extended studies on the state of U_4 in body fluids would be most helpful.

Purpose: To determine the conditions of formation and breakdown of uranium citrate and uranium lactate complexes. To determine the state of U_4 and other complex salts in body fluids.

Method of Attack: Study of complexes is best facilitated by the use of polarographic and electrophoretic techniques. In studying the U_4 problem, preliminary results indicate a colloidal condition which is best studied by means of a centrifuge. Solubility information will be helpful in deciding what ion forms are ultimately formed when oxides and uranates dissolve in body fluids.

B. Studies of the mecanism of uranium fixation by bone.

1. In vitro studies of the absorption mechanism.

2. In vitro studies of the exchange properties of bone salts.

3. In vivo studies of bone circulation.

4. In vivo studies of the factors affecting deposition.

Background: It appears from distribution studies that from a long-term point of view most of the uranium retained by the body is found in the bones. Our understanding of bone metabolism is very inadequate. Considerable work must be done to determine whether uranium may be mobilized or prevented from depositing and factos affecting the deposition must be elucidated. This study has been begun on a small scale and to date has yeilded very interesting and useful information.

Purpose: To determine the means by which uranium gets to and is deposited in bone and to determine the factors which affect these processes.

Method of Attack: The actual mechanism of absorption or fixation can best be studied in vitro. Absorption isotherms and chemical precipitations have already been begun and further work along this line is anticipated. Because of the lack of information on bone metabolism and bone circulation some fundamental studies will be necessary in order to elucidate how and in what state uranium reaches bone. Animal experimentation will provide information on the factors affecting deposition after the fundamental mechanism has been somewhat cleared up.

C. Studies of the relationship of citrate formation by the kidney and tolerance to U.

1. Studies of citrate excretion by ingestion and inhalation exposed animals.

2. Studies of citrate excretion as caused by other nephro-toxic agents.

3. Studies of histological origin of citrate.

Background: Work by Miss Randall has indicated a very striking coincidence between the development of tolerance and the recovery phase following kidney damage and the unphysiological excretion of large amounts of citrates in the urine. The coincidence is so striking that it seems very possible that citrate production following the administration of uranium is one of the, if not the, underlying mechanism of tolerance phenomenon.

Purpose: To determine the mechanism by which citrate is produced following tubular damage by uranium and the relationships between this excretion and the phenomenon of tolerance.

[redacted]

of citrate excretion is characteristic of uranium damage irrespective of type of exposu Accordingly ingestion and inhalation exposed animals will be examined. It also will be helpful to learn whether other nephro-toxic agents initiate citrate excretion or whether this represents a specific response to uranium. Finally, information on the exact mechanism involved in the origin of this citrate will be most valuable. It may be possible that citrate production can be induced and thus tolerance may be also induced without actually administering uranium.

D. Studies on the Distribution and Excretion of Uranium

1. Studies on the distribution and excretion of uranium in tolerant animals.

2. Studies on the absorption of uranium from the gastro-intestinal tract.

Background: Several aspects of the distribution and excretion problem have as yet not been investigated. These are: The distribution and excretion of uranium by animals previously exposed to uranium and absorption and subsequent distribution in animals receiving uranium by ingestion.

Purpose: To determine the distribution and excretion of uranium in animals previously exposed to uranium.

Method of Attack: It will be necessary to employ radioactive siotopes of uranium to learn exactly what happens to material given previously exposed animals because of the residual uranium already present in the tissues. Such an experiment was planned and conducted previously but the District provided a mixtureof isotopes which prevented

Section III Ingestion Toxicity
Section Head Elliott A. Maynard.

A. Age factor in resistance to uranium poisoning.

Background: Most of the ingestion experiments have been carried out beginning with young weanling rats. It has since been found that age markedly influences toxicity. Young animals showed a greater resistance than adults. Since the purpose of the ingestion experiments was to furnish correlative information for the inhalation program, it seems advisable to determine the influence more exactly of age on toxicity. Animals employed in the inhalation experiments varied widely in age.

Purpose: To determine the effect of age on the toxicity of ingested uranium

Method of Attack: Litter-mated animals will be placed on diets containing U nitrate at varying ages from weanlings to full-grown adults. The animals will be examined for the usual signs of toxicity. For comparative purposes some injection studies may also be conducted.

B. Single oral dose of Uranium.

1. Effect of single ingestion on growth
2. Effect of single ingestion on reproduction.

Background: All evidence at present points singularly to an initial damage to animals placed on uranium containing diets. It is possible that most of the total effect of uranium feeding is obtained on the initial first day exposure. At any rate, it is important to know to what extent that first day exposure affects the over-all picture.

Purpose: To determine the effects of a single day feeding of U nitrate.

Method of Attack: One group of animals will be given a single day's feeding of U nitrate and subsequently examined for signs of toxicity. This program will include determination of body weight changes, histological changes and biochemical analyses. A second group of animals will be given single day feeding and subsequently , the effects of this observed on reproduction.

C. Paired feeding in uranium poisoning.

Background: one of the most consistent findings in the ingestion program was that following initial exposure the animals ate little food and lost considerable weight. The question arises as to how much of the weight loss is the result of loss of appetite .

Purpose: To determine the effect of loss appeteie on the weight loss incurred following exposures.

Method off Attack: A series of animals will be exposed to the uranium diets and concurrently an equal number of controls will be placed on stock diets. The controls will have restricted access to food along the lines of the paired feeding experiment. The usual observations will be made.

D. Injury Cycles in uranium poisoning.

1. Indications of cyclic depressions of food intake.
2. Cyclic biochemical changes - NPN and body weight.

Background: One of the striking observations noted in dog feeding experiments was that the NPN showed a consistent although slight rise throughout the period. Superimposed upon this rise were cyclic peaks occurring at about 22 day intervals. It seemed that these animals showed a response , a recovery, and finally, an additional response. The three animals observed all showed a very similar pattern of response.

Purpose: To determine whether injury results from uranium exposure on a chronic basis follows a cyclic pattern.

Method of Attack: A group of rats will be placed on uranium diets and observed individually for recurrent changes in food intake and blood NPN. A program studying dogs will also be undertaken.

Section VI Physiology.

A. Minimal concentration going through kidney to produce damage as shown by function tests.

Background: It has never been determined exactly how much uranium is required to cause observable changes in kidney function.

Purpose: To determine if possible, the quantity of uranium required to affect kidney clearances.

Method of Attack: Graded doses of uranium will be administered to rats and rabbits and these administrations will be followed by clearance determinations of at least three substances: diodrast, inulin and uranium.

B. Transillumination Studies

Background: Considerable information may be obtained on the exact action of uranium in the tubule by observing tubules as they are actually fundtioning in Vivo. Certain studies along these lines have already been carriedout by Dr. Adler on the frog kidney.

Purpose: To dbserve the functinal changes resulting from uranium administration as they appear in the kidney nephron.

Method of Attack: Procedures will be standard as employed in the usual transillumination work. Dr. Orcutt has had considerable experience in Chicago in the techniques involved.

C. Physical vs functional effects on tubule.

Background: The limitations to visual changes which may take place in the glomerulus and tubule are obvious. As a complement to the transillumination studies a program designed to separate the effects of the tubular membrane from the effects of actual cell activity will be undertaken.

Purpose: To establish whether the effects ofuranium consist of the breakdown of tubule surfaces or injury to the tubular cells.

Method of Attack: The procedures would be adapted from the published studies of Bayliss.

D. Contact-concentration relationship for kidney

Background: In spite of the great amount of monitoring and sampling of urines obtai ned from industrial workers, there is little or no information on the concentration which may be considered dangerous. Much information may be obtained by sacrifice experiments in which varying quantities of uranium are administered. Subsequently, the determination of uranium concentration in the urine may be correlated with clearance tests and histological findings.

Purpose: To determine the urinary concentration of uranium: the minimal which results in observable function and histological changes.

Method of Attack: The experiments will be similar to perfusion experiments already conducted in this laboratory.

Dr Strehlgen

[redacted]

1. INDUSTRIAL HYGIENE SECTION PROGRAM

(a) Second-Year of Animal Inhalation Toxicity Studies of T-Nitrate

PURPOSE: A pilot-study for the investigation into the possibility of chronic toxicity developing from repeated daily exposures to a soluble T-dust. Do cyclical changes occur after prolonged exposure?

BACKGROUND: Dogs and rats, previously exposed for a year to a variety of soluble and insoluble T-dusts, will be culled at the termination of the present studies for continuation to a single T-dust, T-nitrate.

PROCEDURE: 23 dogs and 60 rats will be exposed daily, 6 hours a day for 1 year from the initial date, July 1, 1946. The exposure-concentration level of nitrate-dust will approximate 1 mg/m³. This level is calculated to approach the maximal, tolerated level for continued exposure.

Schedule of Exposure

Date	Animal Numbers Dog	Rat	Unit Number
July 1	8	10	3
July 3	3	10	11
July 29	3	10	12
Aug. 12	3	10	5
Aug. 26	3	10	2X
Sept. 9	3	10	7
Total	23	60	

Toxicological Studies - Unit 3 will serve as exposure-unit at start: Unit 12 from the middle of August to July 1, 1947.

Observations

1. Symptoms
2. Mortality
3. Weight - semi-monthly - all animals
4. Blood Chemistry - monthly - NPN - 10 dogs
5. Hematology - alternate months - 5 dogs, 5 rats
6. Pathology

Serial Histologic Study - 5 rats - quarterly - Oct. 1, Jan. 6, Apr. 1.
3 dogs - quarterly - Oct. 1, Jan. 6, Apr. 1.

Terminal Histologic Study - dying animals

Approx. - 15 rats
Appros. - 14 dogs

Observations - continued

7. Tissue-analysis for T - Fluoro Uric - scheduled sacrificed animals only.

Tissue: rat - lung, kidney, femoral epiphysis
Tissue: dog - " " " "

Terminal sacrifice - 4 dogs - 2o tissues
10 rats - 10 tissues

Tissues as above on remainder.

INDUSTRIAL HYGIENE

(b) Thirty-Day Inhalation Studies of T-Dusts of Graded Particle Size

PURPOSE: To determine toxic responses from inhalation of T-dust particles of graded size.

BACKGROUND: Pilot studies of intratracheally injected T-dusts of graded size have shown that particles of less than 1 micron are more toxic than those of larger size.

PROCEDURE: (a) The toxic response determined in dogs, rats, and rabbits, exposed for 30 days to particles differing in their size range will be compared to similarly graded particles of larger dimensions.

(b) Particles of graded size will be prepared in quantity by methods already developed on a pilot scale in these laboratories.

(c) Animals, ca. 5 dogs, 20 rabbits, 30 rats, will be used for each experiment.

INDUSTIAL HYGIENE

(d) Particle Size Dust Measurement Laboratory

PURPOSE: To continue routine measurement of particle size as standardized in this laboratory during the past year.

The value of this laboratory will be to supply critical information to the Industrial Hygiene section on:

(a) Projects (a), (b), and (e).

(b) Industrial surveys as required.

[redacted]

INDUSTRIAL HYGIENE

(e) Dust Retention Laboratory

PURPOSE: To continue, extend and amplify program as outlined in current project #111, "The Retention and Absorption by Alveolar Transport of Inhaled T-Dust".

(a) To determine the amount of dust retained by animals inhaling dusts of known characteristics of concentration, particle size, and solubility at given breathing rates.

(b) To locate the sites of absorption.

(c) To determine the mechanism of toxic response of inhaled soluble dusts.

(see accompanying detailed statement on relation of particle size of uranium dusts to toxicity.)

[redacted]

INDUSTRIAL HYGIENE

(e) Relationship of Particle Size of Uranium Dusts to Toxicity

Submitted by A. Rothstein

STATEMENT

Problems concerned with the relationship of particle size and solubility of uranium dusts to the toxicity resulting from their inhalation.

A. Measurement of particle sizes.
b. Differential retention of dusts in various parts of the respiratory tract.
c. Differential toxicity of various size fractions of dusts.
d. Transport of uranium across the alveolar and nasal membranes.
e. Transport of uranium by phagocytes.

BACKGROUND

Authorities on dust-hazzard agree that the particle-size of inhaled material is of primary importance as a factor in the toxicological response. It has been possible to make some predictions from a theoretical point of view, of the effect of particle-size on various aspects of inhalation-toxicity, but a careful examination of the literature reveals a startling paucity of experimental data. Even the methods used for measuring particle size are open to severe criticism.

It is proposed to attempt to fill in some of the gaps in our knowledge of the particle size factor by a continuation and extension of work that has been in progress during the past year. Adequate techniques have been worked out for (1) measuring particle sizes, (2) fractionating dusts into narrow size fractions, (3) maintaining dust-clouds of constant concentration, (4) measuring retention of inhaled dusts, (5) determining toxicity of size-fractions placed in the lung, and also for (6) determining the rate of transport of material out of the lung. Techniques are available for determining phagocytic activity and nasal absorption of material.

It is felt that the whole field of particle size as a factor in toxicology is ripe for exploration and that it will yield interesting results. A survey of plant conditions is strongly indicated also.

PROGRAM - PRESENT STATUS AND PROJECTED EXPERIMENTS

A. Measurement of Particle Size - Development and standardization of specialized methods for particle size determinations will soon be completed. These will be available for routine sampling of dusts both in experimental chambers and in industrial plants. The methods include use of a high resolution optical microscope and the electron microscope for size-measurements, impactor and thermal precipitator for sampling-procedures.

b. Preparation of Size-Fractions - Methods for separation of dusts in narrow size-fractions have been evolved. Preparation of size-fractions of different ranges is projected for various experiments.

c. Sonic Dust Feed - a sonic type dust feed has been constructed together with its amplifier and sound-proof box. Previous results have indicated that this type of feed gives very constant concentrations of dust.

d. Differential Retention in the Respiratory Tract of Various Particle Sizes. Calibration of the retention apparatus is almost complete and experiments will soon be in progress. It is intended to determine retention of different size-fractions of 3 uranium compounds (UO_2, UO_3 and UO_2F_2) of different solubilities.

e. Differential Toxicity of Various Size-Fractions of Dusts - Experiments have been completed indicating that for UO_2, small particles are much more toxic than large, when the UO_2 is placed in the lungs intratracheally. Similar experiments with UO_3 are projected.

f. Transport of Uranium across Alveolar and Nasal Membranes. Phagocytic Transport of Uranium. Some preliminary data have been obtained on UO_2 using the intratracheal technique. With this compound it appears that two routes of excretion from the lungs are present, one favoring large particles, involving transfer through the blood stream. Only the latter transfer is assumed to involve a toxic response.

With soluble compounds nasal absorption is probably an important factor in evaluating relatively larger particles, nasal absorption may be as important as lung retention of smaller particles.

EQUIPMENT

Special equipment necessary to the project is already available. Barring unforseen circumstances, only standard laboratory equipment will be required in the future.

(f) Industrial Service Testing Laboratory

Purpose: To determine the toxicity of materials of industrial concern as requested.

Procedure Inhalation units and/or injection and percutaneous absorption will be established as required.

Tests will be made according to methods already standardized by these laboratories.

This project is anticipated to be self-supporting.

(g) The Role of Phosphate-Containing Enzymes and Related Substances in T-Poisoning.

BACKGROUND: Indications from past work have pointed to the phosphate group of biological materials as playing an important role in T-toxicity. Although past work in vitro on the effect of T on enzymes in the animal organism has as yet failed to demonstrate any specific activity of T on this group of substances, it has been demonstrated in the intact animal that T exerts its action on precisely these substances. In the kidney complete and persistant absence of normallyoccurring phosphatase has been demonstrated by staining techniques following T-exposure.

PURPOSE: To study the effect of T on enzymes and related substances having to do with phosphate. The first attack on the problem will be the study of

(a) phosphorylated coenzymes
(b) nucleic acids
(c) nucleoproteins
(d) phosphoproteins.

(h) Lipids and Lipid T-Complexes

A detailed discussion of the background and method of approach is included.

The start of the attack on the problem will be made on the reactions of pure lipid substances with T.

(h) The Role of Lipids in the Mechanism of T-Poisoning.

Charles Spiegl.

BACKGROUND

A Close chemical relationship between lipid and T is indicated from several sources in our past experience. Thus, from analogy with other metallic salts it may safely be predicted that T-fatty acid compounds can easily be formed at least in vitro. Other suggestions such as the adsoprtion of T-salts by oils, the solubility of $TO_2(NO_3)_2$ in ether, and some evidence for the development of fatty livers in T- poisoning all point in the direction of T-lipid interaction. The importance of investigating the formation of such a complex is readily apparent in view of the fact that quantities of lipids are found in the blood, that the cell membrane probably contains a large amount of lipid or lipo-protein surface, and that proteins usually are combined with lipids. Consequently, the lipids may enter into the transport, adsoption on the cell membrane, permeability into the cell of T, and reaction of the element with protein substances. None of these important factors in the mechanism of T-toxicity have been investigated.

THE Problem

It is desired to show:

1. The role of lipis in adsoprition, desorption, and permeability of cell membranes to T with particular emphasis on the cels of the kidney.

2. The function of lipids in the distribution of T throughout the body.

3. The nature of the lipid-T complex.

Satisfactory solution of the problem would give results of immediate and of long-range value for:

1. A better understanding of the mechanism of T-action on cells, particularly in the kidney.

2. A more quantitative mathematical interpretation from adsoprtion calculations of the relationship between dosage and the rate of adsoption on cells, of adsorption into the cell, and elimination from the body. It may be possible to relate these factors to the amount of tissue destruction.

3. Valuable contributions would also be made to the field of fundamental science. Development of a theory of in vivo cell adsorption and membrane equilibrium would be a worth-while contribution to cellular physiology. Incidental to the main problem, the preparation and characterization of new compounds of the T- fatty acid type would further the organic chemistry of fats and oils. The possibility of discovering a specific organic complexer with T would not be overlooked.

METHOD OF APPROACH

The problem outlined lends itself to study in 2 phases, (1) the preparation and characterization of pure chemical compounds similar to those likely to be encountered in vivo and tje determination of the kinetics of compound or complex formation with these pure materials, (2) physiological studies of the interactions between lipids and T in the body. The simultaneous approach along these 2 lines of reasoning would enable more papid progress in the outlined program.

I. Reactions of pure chemical compounds.

A. Fatty acids and T.

1. Preparation of T-fatty acid salts for standards and characterization of these by as many of the following constants as feasible: melting point, X-ray crystallography, iodine values, colorimetric absorption curves, and refractive index.

a. Determination of the relationship between complex formation of T and fatty acids with the lipid chain-length and unsaturation.

b. Determinati on of the relationship between complex formation of a typical fatty acid and hexa or tetra-valent T.

B. Phospholipids and T

1. Preparation of T-cephalins and lecithins. Investigation of the relationship between the complexing power and the valence-state of the T.

C. Fats and T. Determination of the complexing power of T with (1) a synthetic mixture of fatty acids or glycerides, (2) commonly available purified fats, (3) normal blood lipids, and (4) blood lipids from T-poisoned animals.

D. Preparation of radio-active T-lipids compounds for in vivo studies

E. Investigation of the lipid-protein interactions.

II. Physiological reactions of the lipids.

A. Determinations of total lipid changes in T-toxicity. The quantitative investigation of alterations in total fat, individual fatty acids, and the phospholipids in (1) the kidney, (2) total body carcass, (3) blood cells and plasma, and (4) other organs such as the lung, liver, and thyroid if indicated.

This type of study may well suggest the particular lipid fraction active in the toxicology of T and show further whether the cels of the plasma are the trasporting agents in the blodd.

B. Mechanism of cellular lipid reaction. Fundamental studies in this field can best be made first by in vitro followed by invivo studies on normal and on T-poisoned animals. The study of the effects of T on the lipids of t e body may wel be complemented by a study of the effects of adding lipids in T-poisoning.

1. Studies of the cell membrane surface.

a. Determination of the amount and character of lipid present.

b. Complexing (adsorption and desorption) of T from the cell surface.

c. Isopopic investigation of the kinetic nature of T o n a membrane.

2. Study of the transport of T across a membrane.
   a. Rate and influence of various factors such as blood acidity on the rate.
   b. The type of compound carrying T across a membrane.
   c. Cellular lipid-protein interactions, both in the absence and in the presence of T.
3. A possible ingestigation of the change in T-lipid reactions induced by chemical means in the body. This tenative section may have a bearing on the future treatment of T-poisoning.

(i) Protein T-complexes.

BACKGROUND: Past work relating to the microanalytical determination of uranium has demonstrated the specific combination of proteins with uranium.

PURPOSE: The purpose of this investigation is to extend these preliminary findings in order to evolve the mechanism of the toxic action of uranium. The cel surface has been shown to be a complex mixture of protein and lipid. This study should supplement study (g) in enzymes and (h) in lipids.

INDUSTRIAL HYGIENE

(j) The Effect of Uranium on the Cell Surface.

(A physiological investigation in the mechanism of uranium action.)

by Rothstein and Laskin.

BACKGROUND

Previous research on the mechanism of uranium-poisoning has been largely concerned with the whole animal: the kidney or other organs and with isolated components of living tissues such as the enzyme systems and the protein constituents. Little direct research has been done at the cellular level of organization.

Present evidence on the effect of uranium on the whole animal indicates that the kidney is the most susceptible organ and that a particular group of cells making up the proximal convoluted tubules are involved in the primary injury. The tubules suggest two possibilities for the mechanism of uranium poisoning. The first is that the specific group of cells have some special properties or components different from those of other tissues enabling a reaction with uranium to occur. The second possibility is that optimal environmental conditions (extracellular) are present only in the locality of these cell groups.

Present evidence also indicates that no specific enzyme system is inhibited, but rather that the cel surface of the cels constituting the proximal convoluted tubules is injured resulting in a general cellular debilitation and destruction. The general nature of the results of previous research indicates that extension of the uranium toxicity problem to the cellular level of organization would be most profitable in revealing the mechanism of uranium poisoning. Techniques are available by which the effect of uranium on the cell surface can be elucidated as a function of environmental conditions.

PERSONAL BACKGROUND

Both Dr. Rothstein and Mr. Laskin have had considerable previous experience in the field of cellular physiology specifically in relation to problems dealing with permeability and the cell surface. They already have available (a) an extensive bibliography on the references in this field, (b) detailed notes on methods and results in the field, (c) a review article covering the previous work in the field written by Mr. Laskin. (d) several earlier studies on the toxicity of various materials to cells.

PROGRAM

Properties of the cel surface can be directly measured in terms of permeability and electrical properties of the cell. Indirectly the properties of the surface may also be studied intterms of cellular activity involving such functions as movement, excretion and metabolic activities.

If the cells of the proximal convoluted tubules have some unique inherent property which causes them to be particularly susceptible to uranium then only these cell types could be used for the studies. Tissue slice and tissue culture techniquew are available which would readily lend themselves to the problem. It is, however, more probably that the apparent specificity of uranium for the tubulary cells is due to extracelular conditions (such as pH) which exist only in the specific region of the kidney from which the cel types originate. If the extracelular conditions were duplicated for other cells, similar effects of uranium may

be observed. If this proves to be the case, then the effects of uranium on the cell surface could be studied with simple techniques on isolated cells such as erythrocytes, phagocytes and unicellular organisms.

Another method of approach which might yield valuable information is the use of model systems. This would involve sutdies on simple protein and lipid monolayers and deposited interfacial membranes of proteins and other cellular components.

In summary, the program would attempt to answer the following questions:

1. Is the susceptibility to uranium inherent in the cels of the renal proximal convoluted tubules or is the susceptibility due to extracellular conditions in the proximity of these cells?
2. Are other cells susceptible to uranium under similar extracellular conditions?
3. How does uranium affect the various cell functions?
4. How does uranium affect the cell surface in terms of permeability and electrical properties?
5. Can the phenomenon of uranium toxicity be duplicated in a model system on protein and lipid monolayers? If positive results are obtained, can the mechanism of uranium action be specifically traced?

In a relatively virgin field such as this, it is difficult to predict the most fruitful approach until some exploratory work has been attempted. The methods suggested in the previous section are merely an indication of the type of approach to be used.

(k) Investigation of the Nature of Fluoride in Blood

BACKGROUND

Earlier work (principally German) on the biochemistry of fluorine has led to the following observations:

1) There is considerable uncertainty as to the base level of blood fluorides.
2) An antagonism appears to exist between fluorine compounds and thyroxin.
3) Organic fluorine compounds appear to be more toxic than the fluoride io n.
4) Fluorine apparently exists in the blood in an oragnic and an inorganic state somewhat analogous to blood iodine.
5) In hemophilia the blood fluorine often rises to very high levels.
6)3-fluorotyrosine has given excellent results in the treatment of Basedow's disease (toxic hyperthyroidism).

These observations have suggested the following broad outline for a research program on blo d fluorine.

PROBLEM

Preliminary experiments in this laboratory have tended to confirm the observation that blo d fluorides exist in both an "organic" and"inorganic"form. The purpose of this problem is to investigate the nature of the compounds of fluorine existing in the blood, devoting special attention to the so-called "organic"fraction.

METHOD OF ATTACK

Reference to the literature indicates that the normal range of F. in the blood is rather wide; however, the methods used have alwyas included operations which in our experience have resulted in loss of fluorine. Accordingly, it will first be necessary to determine the range of normal vlues as measured by our technique.

The approach to the main problem might well procede according to the following lines:

a) Determine the fluorine content for whole blood, cells and plasma.
b) Prepare hemoglobin, protein and lipid fractions and determine the fluorine content of each.
c) Investigate the complexing of fluorine with hemoglobin and other proteins.
d) Investigate the relation between fluorine and non-diffusable (protein bound) blood calcium.

In addition to the main problem of elucidating the nature of blood fluorides, the following secondary problems are of considerable interest;

a) An investigation of the possible relations between fluorides, iodide and calcium levels and the thyroid gland.
b) the effect of fluorine upon enzyme systems of the blood, particularly by means of an in vivo experiment.
c) The solubility of fluorides salts and complexes in plasma.
d) How high can the blood fluoride level be raised before ill effects are observed in animals.

The use of radioactive fluorine in attacking these problems may be of value and should not be overlooked.

SIGNIFICANCE OF THE PROBLEM

These experiments are intended to give fundamental information regarding the mode of action and metabolism of fluorine in the system. This information would appear to be of value for the following reasons:

1) Fluorides are used in the treatment of disease. Blood levels appear to show significant variations in certain pathological conditions

2) Exposure to fluoride is of industrial significance, particularly since the advent of atomic energy programs.

3) Exposure to fluoride may occur through consumption of foods heavily contaminated with fluoride containing fertilizers.

4) The determination of base levels is of immediate practical value in the impending litigation between the duPont Co. and residents of New Jersey areas.