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SUMMARY

MEDICAL RESEARCH PROGRAM

1943-1946

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The following series of abstracts was prepared by the undersigned from completed research reports, partially completed and analyzed data and personal communications during the period [illegible] 1946 to 1 July 1946. Since that time much of the work has been finished and issued in the form of program or final reports. However much is being continued and the results as yet unpublished.

It is possible that some of the conclusions arrived at may have become altered by the collection of additional data. However, in most instances, the individual authors or project directors have approved the abstract. Certain small problems may have been unintentionally omitted in the search. Except for problem assignments where the research was specific and limited, the plan of abstracting has been to group numbers of small problems of related nature into general reports with the elimination of the author's name.

In general this series of abstracts provides a clear concept of the current status of results in the overall project and as such furnishes a large amount of usable information in abbreviated form. With the appearance of the complete series of final reports and the Manhattan District Technical Series, this compilation will be recalled.

Joe W Howland
JOE W. HOWLAND
Major, M C
Chief, Research Branch
Medical Division

Comment on Radiobiological Techniques

It is obviously impossible to distinct subjects of this type because inclusion of specific detail is necessary. However the inclusion of the index was felt to be warranted inasmuch as it clearly indicates the scope of the work on this type of problem.

[redacted]

UNIVERSITY OF ROCHESTER

A.H. DOWDY, DIRECTOR

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INTRODUCTION:

Uranium compounds have been utilized industrially, in a limited fashion, in the ceramic industry for many years. The Manhattan Project, however, was the first industrial project to use the uranium compounds on a vast scale.

The Manhattan Project was to employ thousands of people who would work with uranium compounds. These people in the plants, at work, would inhale these compounds as dusts and fumes, they would inadvertantly ingest these substances; their eyes and skin would come into daily contact with these materials. What, if any, toxic effects would these uranium compounds have on the human body?

Animal experimental work has been done with the uranium compounds, but there is only one brief report[1] in the scientific literature on the toxicological effects of these compounds, as used in industry, upon the human body. This report is fragmentary, but it does indicate that the uranium compounds may be potentially toxic and this point was verified by many investigators in animal experimentation. This potential toxicity is rather unusual because the source is twofold. One source is the possibility of heavy metal effect on the body, comparable with that of lead, mercury, and arsenic; and the other, more remote, is the possible latent toxic effect on body tissue by the uranium compounds because of their inherent property of radioactivity.

The medical authorities of the Manhattan Project were keenly aware of these potential hazards, but the successful execution of the war effort made it imperative that the industrial plants of the Manhattan Project start immediate operation. This was accomplished in spite of insufficient pharmacological and toxicological data on the uranium compounds. A medical research program, however, was also initiated for the specific purpose of accumulating sufficient reliable data as rapidly as possible so that a maximum factor of safety to the employees exposed to these substances could be established. This data, it was hoped, would also help prophylactically and therapeutically in combatting the possible acute and chronic toxic effects of the uranium compounds.

Since the plants started operation before sufficient data could be accumulated to establish a maximum allowable air concentration of the dusts of the uranium compounds in the plants, such a safe level had to be set arbitrarily as a temporary empirical standard. The consensus of opinion among the medical authorities of the Manhattan Project was that it was safe to adopt the same standard as set for lead by the American Standards Association, i.e., 150 micrograms per cubic meter of plant air as the maximum allowable dust concentration.

In order to determine as rapidly as possible the overall comparative toxicity of the various uranium compounds, thirty day inhalation and ingestion experiments were initiated in which a variety of animal species were exposed to these compounds under conditions which simulated as nearly as possible the conditions prevalent in the industrial plants. In addition

[1] De Leat, Maurice, Ing. Chem., V. 9, pg. 247, 1926.

to the above, inhalation experiments were carried out [illegible] many of which were entirely new and other which were used for the first time on such a large scale. Information was obviously needed about the toxic properties of these substances also to insure a maximum factor of safety to the employees of the Manhattan Project.

I. Inhalation experiments:

The specific purpose of the inhalation experiments was fourfold:

1. Development of safe standards for exposure to uranium and other compounds.

2. Testing of respiratory protective devices for industrial use (masks).

3. Measurement of approximate toxicity of certain special materials.

4. Methods for recognition and control of poisoning.

Dates of Work -- November 1943 to present.

Protocol of Experiments:

Substances tested: UO_2F_2, UNO_3, UCl_4, UO_3, UO_4, UF_4, U_3O_8, UO_2, $Na_2U_2O_7$, $(NH_4)_2U_2O_7$, "hi-grade" ore.
OF_2, F_2, HF.
B, BF_3, BF_3 -- dimethyl ether complex.
BCl_3.
C_7F_{16}, C_8F_{16}, $C_{21}F_{44}$ (Fluorocarbons)
C_2F_3Cl.

Method: Dogs, rats, rabbits, mice, cats, guinea pigs and hamsters (four or more species) are placed in inclosed chambers for prescribed periods of time from thirty days to one year. The number of animals is sufficiently large to permit statistical evaluation. The atmosphere in such chambers is controlled as to the uranium content, temperature, humidity and particle size and concentration of the uranium compounds. Representative animals in each group are studied for the following criteria of poisoning:

1. Mortality.
2. Hematological change.
3. Chemical changes of blood and urine.
4. Physiological changes (weight changes).
5. Distribution of uranium in the tissues.

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Control animals are studied in a similar chamber in which all variables are controlled but no uranium dusts are added.

Table I summarizes the results of the thirty days inhalation experiments. The overall mortality includes the summarized mortality of all the species studied. The per cent mortality is derived by dividing the number of animals (numerator) who died by the total number of animals studied (denominator.)

In Table II, the representative larger species (dog, rat, rabbit) are grouped and the toxic effects noted, i.e., mortality, pathological findings at autopsy, loss of weight, abnormal blood chemistry, urinary findings and hematological effects. The reaction severe indicates a marked degree of pathological change in all of the criteria noted above. The reaction classed as moderate implies a moderate degree of damage in most of these criteria and the reaction noted as slight indicates some change away from the normal in some of the criteria.

Table III represents a summary in which the high, mid and low concentrations of uranium compounds used in the chambers are compared in their effects upon four animal species (dogs, rabbits, rats and mice), with relation to abnormal changes found in the lungs and kidneys at autopsy, abnormal findings in the blood and urine chemistry, abnormalities in the hematopoietic system and effect upon weight.

This table clearly shows that the slight untoward effects noted at the low concentrations were negligible*, yet these low concentrations were all well above the temporarily established arbitrary tolerance figure, i.e., 150 micrograms of uranium dust per cubic meter of air.

These experiments indicated that the tolerance figure of 150 micrograms of uranium compound per cubic meter of air certainly insured a maximum figure of safety, for at least thirty days of constant exposure. The next question was whether this same figure would still be a safe level if the exposure were longer than thirty days. Since the plants of the Manhattan Project anticipated operation for the duration of the war and possibly longer, there was a real need for information about the possible toxicity of the various uranium compounds as a result of chronic exposure.

The same procedure was followed in the chronic as in the acute thirty days inhalation experiments. The following four uranium compounds were selected: UF_6, UO_2, UCl_4, and UF_4. These compounds were chosen because the

*It will be noted that there is a slight mortality among the animal species in the low concentrations but if reference is made to Table I, it will be noted that there was a slight mortality even among the control animals.

greatest number of employees were exposed to these substances. $UO_2(NO_3)_2$ was also added to the above list because of the necessity of a base line of comparison with data available in the scientific literature. (Most of the animal experimental work done prior to the Manhattan Project was done with $UO_2(NO_3)_2$). Two concentrations of dusts were to be used in the experimental chambers. One dust level was selected because it was thought that it would produce no toxic effects and the other level (approximately ten times greater) was chosen because it would probably cause minimal toxic effects. In this manner, the probable maximum safe level of dust concentration would be established.

Table IV summarizes the data available to date. The experimental chambers have been in operation from four to eight months. Four animal species are represented in the table (dogs, rabbits, rats and mice). Again, it is to be noted that only minimal toxic effects resulted at the higher dust concentration, and no toxic effects have been observed at the low concentration. This low concentration is still well above the established arbitrary tolerance level of 150 micrograms of uranium compound dust per cubic meter of air.

In addition to these animal experiments, a program was carried out to study the effectiveness of various face masks. The purpose of these experiments was to give added protection to employees in case of accidental respiratory exposure to high concentrations of the uranium compounds.

Animal inhalation experiments were also carried out on special compounds. Some of these compounds were entirely new and other were compounds which were used on a large industrial scale for the first time. The potential toxicities of these compounds were thus investigated and the degree of hazard was approximated. The results obtained in these experiments helped to direct the precautionary and prophylactic measures instituted at the plants where these substances were used.

Outline of Experimental Results on Inhalation of Special Compounds:

High Concentrations of F_2 (Acute): Tested at a concentration time level of 10,000 ppm for five minutes to five hours with the mortality percentage at 30-100 in three species. Death was caused by lung and kidney damage.

Chronic Exposure to F_2:

1. On a thirty day experiment at 4 ppm a low mortality observed. Growth was unchecked in all species except rabbits. Some pulmonary inflammation was observed.

2. At 0.5 ppm for thirty days no effect was observed.

Exposure to C_2F_3Cl (P539):

1. No mortality at 100 ppm for fourteen hours.

2. Very low mortality at 500 ppm for fourteen hours except in guinea pigs.

Exposure to C2144 (highly polymerized flurocarbon): No mortality at 5000 ppm for four hours.

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Exposures to Boron and its Compounds:

1. Methods of analysis developed.

2. Very low mortality at 20 ppm for 211 hours. Death due to lung damage.

3. Boron trichloride showed no mortality at 50 ppm and a slight mortality at 100 ppm when in the dry state. In the wet state, and containing decomposition products, the mortality was appreciable at 20 ppm.

4. Boron trichloride-dimethyl ether complex shows no mortality at 20 ppm in a 30 day experiment. Mortality was high at 100 ppm.

5. An eleven hour experiment at 135 ppm showed no mortality.

Fluorocarbons:

C_7F_{16} foreshot showed no mortality at 100 ppm at fourteen hours;
Crude showed low mortality at 300 ppm at fourteen hours;
Refined showed no mortality at 750 ppm for fourteen hours and 4600 ppm for five hours.

C_8F_{16} crude showed low mortality at 8 - 100 ppm for fourteen hours;
Foreshot showed low mortality at 100-130 ppm for fourteen hours;
First fraction showed no mortality at 272 ppm and low mortality at 373 ppm for fourteen hours;
Second fraction showed no mortality at 1000 ppm for fourteen hours.

II. Ingestion Experiments:

Since the people in the plants would inadvertently ingest the uranium compounds, it was imperative to carry out animal experimental work to determine the oral sensitivity of animals to uranium compounds in order to:

1. Check the sensitivity of each compound as determined by inhalation;

2. Determine the probable role of ingestion in the inhalation experiments;

3. It was hoped that the information thus gained could be extrapolated to the human species with some degree of accuracy.

Protocal of Experiments:

Rats and dogs were fed various uranium compounds over periods of

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time extending from 30 days to two years. The rats were given specified percentages of each compound in their diets. The dogs were given amounts of these compounds graduated according to the body weight of the animal.

Observations on the growth, weight change, pathology and distribution of uranium in the tissues were made at specified intervals.

Control animals were tested under identical conditions except that uranium compounds were not present in their diet.

It was necessary to use the number of compounds and the various dust concentrations reported in Table V in order to discover the relative toxicities of the uranium compounds. When this was learned, five uranium compounds were selected and were fed for either one or two years to a number of animal species. The purpose of this was to see if there were any toxic effects as a result of chronic ingestion of these compounds. The reasons for the selection of the five uranium compounds which were used for the chronic ingestion experiments have been enumerated in the section on inhalation.

At the completion of the ingestion experiments it was possible to group the various uranium compounds into graded toxicity table. (See Table VII). This was obviously useful as a means of orientation in the industrial plants where these compounds were in use. This served as a guide in directing the institution of prophylactic measures, and in guiding the emphasis into proper channels on unusual precautionary steps in the industrial plants.

As was mentioned previously, the workers in the plants would inadvertently get the dust from these compounds in their eyes, and their skin, of course, would come into contact with these substances. Information was therefore needed about the topical toxicity as well as the potential eye irritant action of the uranium compounds. Tables VIII and IX summarize the results of experiments on eye and skin sensitivity to these compounds.

It is of interest to note that a number of the compounds were absorbed through the skin or conjunctiva in sufficient amount to produce systemic changes which resulted in death. At autopsy, evidence of renal damage was found.

At the completion of the ingestion and inhalation experiments and the accumulated data on the topical and eye application experiments, it was possible to evaluate the degree of toxicity of the various uranium compounds. This information is summarized in Table X.

It was not only necessary to learn about the gross toxic effects of the dust of the various uranium compounds on the human body, but it was also imperative to learn about the physical characteristics of these dusts. It was important to learn more about the effects of the uranium compounds on the various bio-chemical systems and metabolism of the animal body, and it was essential to study the degree of tolerance which animals showed to these compounds. It was obviously necessary to learn about the distribution, absorption and excretion of the uranium compounds once they are introduced into the animal body, and lastly it was important to learn about the intricate behaviour mechanism of the uranium compounds in the animal body. All of this work would be of the utmost importance in helping to discover early indices of uranium intoxication and to find possible prophylactic and therapeutic measures for uranium poisoning.

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The following is a brief resume of the work that was accomplished in those fields:

1. Retention Studies and Particle Size:

Methods: Accurate methods have been developed for the measurement of the particle size of uranium compounds in air. These techniques are used in the control of the concentration of the uranium compounds in the experimental inhalation chambers, and in the monitoring of the concentrations of such compounds in industrial areas where humans are exposed.

Techniques for the fractionation of the particles of uranium compounds into lots of uniform size have been developed. In this way exposure of experimental animals to particles of known size can be determined. Preliminary data indicates that the small particles are most toxic.

Methods for the uniform suspension of such particles in experimental chambers have also been developed.

A method for the measurement of the total dust inhaled by an experimental animal is almost completed, and preliminary tests are satisfactory.

2. Pharmacology:

Lipids in Uranium Poisoning (Haven '148): Following exposure to toxic amounts of uranium compounds, the total lipid of the liver and kidney tend to be less than normal due to the increased water content. Fatty livers tend to be found after exposures to uranium nitrate and oxyfluoride. The phospholipid fatty acids of the liver are more unsaturated than normal; those of the blood, more unsaturated; and those of the kidney, less unsaturated than normal. In general, there is no effect on the total cholesterol. In the liver there is an increase in ester cholesterol and a decrease in free cholesterol. In the kidney there is an increase in ester cholesterol and no change in the free form. Adrenal cholesterol is decreased to one-half its normal value. It is not known where the changes in the unsaturation of the phospholipid take place.

The Effect of Various Agents on Uranium Nitrate and Oxyfluoride Toxicity (150): The mortality after toxic doses (5 mg/kg) of these substances was not changed by choline, neutralized choline, disodium hydrogen phosphate, lactic acid sodium lactate, calcium lactate and essential fatty acid administration.

Renal extracts delayed but did not decrease the mortality from lethal doses of uranium nitrate.

Adrenal cortical hormones also caused a delay in the mortality but no reduction in it.

Oral sodium bicarbonate and sodium citrate reduced the mortality by an appreciable percentage in nitrate poisoning.

The Effect of Diet on Uranium Toxicity ('38) Neuman): The addi-

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tion of 0.5% sodium bicarbonate to the diet reduced the mortality from 80% to 20-30%. This is less beneficial if given twenty-four hours after the toxic dose is administered. The salts of metabolized acids (citrate, malate or lactate) on injection give similar results to those obtained with the bicarbonate. Acid in the form of ammonium chloride increased the mortality to 80 to 95%.

Histological Study of the Pancreas after Uranium Poisoning (33) (Haven): No evidence of any abnormality of the pancreas was found after uranium poisoning.

Therapeutic Use and Metabolic Role of Citrates in Uranium Poisoning (#128) (Haven): Sodium citrate, fumarate and succinate are as effective as bicarbonate in the treatment of uranium poisoning. A three to four fold increase in the citric acid excretion is noted after uranium poisoning. There is no relation between the citric acid and uranium contents of bone.

The Effect of External Temperature on Survival from Uranium Poisoning: Normal temperature is optimum for survival from uranium poisoning.

The Effect of Fluid Intake on Survival from Uranium Poisoning:

Restricted fluids raised the mortality in uranium poisoning.

The Effect of Uranium Poisoning on Phosphorus Turnover: There is no evidence, as shown by radiophosphorus studies, of an enzymatic block in phosphorylation caused by the administration of uranium. A slightly lower rate of equilibration in bone is noted.

3. Tolerance Studies (Haven) (#129):

Tolerance can be divided and studied in the classifications of natural and acquired tolerance.

Natural Tolerance:

a. Species difference as illustrated by the fact that white mice will tolerate three times the acute dose for Wistar Rats (5 mg/kg); C_3H mice will tolerate more than ten times the acute dose for rats. The following table shows the order of susceptibility of various animals to uranium nitrate both by injection (percutaneous) and by ingestion.

Uranium Nitrate by Injection LD/50(ratio)		Uranium Nitrate by Ingestion gm/kg 2% UO_3	
rabbit	1	rabbit	0.1
rats	8	dogs	0.2
guinea pig	36	rats	1.0
white mice	128	hamsters	5.0

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b. Dogs are more sensitive to oral uranium compounds than rats, as illustrated by the following table.

Compound	Maximum Amount Tolerated for 30 days	Maximum Carried for 1 year Rats (% in diet)	30 days
UO_2F_2	.02	0.5	
UCl_4	0.1	1.0	
UNO_3	0.5	2.0	
UF_4	10.0	20.0	
UO_2	20.0	20.0 plus	

c. Males are more tolerant than females.

d. Interference in growth of rats by the following uranium compounds as an index of toxicity.

30 day experiment

20% of UO_2, U_3O_8, in diets causes no growth interference.
20% of UF_4 in diets causes slight growth interference.
0.5 to 1.0% of UCl_4, UO_3, UNO_3, U acetate causes some growth interference.

0.1 to 0.2% of UO_4, UO_2F_2 in diet causes some growth interference.

One year experiment

UO_2 and UF_4 almost non-toxic over period of two years at 20% of diet.
UNO_3 at 0.5% of diet causes detectable growth interference after two years of feeding.
UO_2F_2 at 0.25% of diet causes growth interference after two years of feeding.

Acquired Tolerance:

a. Rats acquire tolerance for lethal doses of uranium nitrate and chloride by the repeated administration of small doses from day to day.

b. Sex differences are also apparent after repeated doses.

c. Increasingly larger doses in rats show

1. A ten day interval of dosage is better than twenty day interval.

2. A ten day interval shows no appreciable mortality occurred until 16 mg/kg dose was given (three times lethal dose).

3. With twenty day interval the mortality started at 4 mg/kg.

4. Sex differences are apparent, the male being more resistant than female.

d. The small[redacted] be administered so that tolerance is acquired is 0.33 mg/kg [redacted] --- 0.1 mg/kg gives no tolerance.

e. The repeat dosages are best tolerated when the urinary citric acid excretion is at its peak.

f. Uranium is not excreted more rapidly in tolerant animals than in intolerant animals.

Studies in Phenols in Blood and Urine in Uranium Poisoning (Mavon 73):

a. A rise in free and total phenol excretion follows administration of uranium nitrate in doses of 0.22 to 2.5 mg/kg. This reaches a maximum in four days.

b. The increase in phenol increases with the dosage administered.

c. No change is observed in the conjugation of phenols.

4. Distribution and Excretion of Uranium (Stable and Unstable Isotopes) Neuman (132)

December 1944 to present.

Method: Ashing of tissues, isolation of the uranium from a protein complex and analysis by the fluroescent method.

Current Results:

A. Hexavalent uranium - one dose (i.v. $UO_2(NO_3)_2$, (rats) - analyses from a period of forty-five minutes after injection to forty days after injection shows that:

a. Fifty percent of hexavalent uranium is excreted in the urine in twenty-four hours and small quantities are excreted up to forty days. Total excretion amounted to 65-75%. No fecal excretion was noted.

b. Blood concentrations dropped to less than 0.1 mg/100 cc in four hours.

c. Initially, the kidney contains 20% of the dose. This falls to 1% at forty days.

d. Bone contains 30% initially with a gradual fall to 10% at forty days.

e. Soft tissues at all periods except the forty-five minute period show less than 2% of total dose.

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B. Tetravalent urani[redacted] (SO_4 i.v., rats) - analyses from a period forty five minutes after injection to forty days show that:

a. 10% is excreted in twenty-four hours with small amount up to a total of 30% in forty days. Fecal excretion initially low up to a total of 20% at forty days.

b. Blood drops more slowly than hexavalent uranium, but shows a concentration of less than 0.1 mg at twelve hours.

c. Kidney initially contains 15% of the dose, falling to 1% at forty days.

d. All soft tissues except liver and spleen show less than 2% of the dose.

e. Liver and spleen initially contain 35% of the dose, falling to 5% at forty days.

C. Acute dose with radioactive isotope (U 232) (rats). Results are the same as the above with the exception that less retention or rapid removal occurs in the kidney.

D. Analyses on animals exposed by inhalation (chronic exposure).

Soluble compounds show no retention by the liver or lung with a gradual rise in the bone and kidney concentration. At the present time the approximate levels to which this concentration will rise have not been reached. Current observations indicate that the curves are still rising and have not leveled off. Concentrations in the bone in no way approach those felt to be toxic from a radiation standpoint.

In the insoluble compounds the lung continues to show an increasing concentration of uranium while the kidney and bone contain very small (slightly more than trace) amounts.

E. Analyses of Animals exposed to uranium by chronic ingestion.

The bone in chronic animals runs three to four times the observed concentration in the kidney. Levels in bone up to 450 micrograms per gram of bone have been observed. No placental transfer of uranium occurs.

F. Mechanism of Deposition in Bone.

a. In vitro - the bone absorbs the uranium from plasma ultrafiltrate and from a bicarbonate buffer. This removal is very rapid, reaching a steady state in twenty-four hours. Concentration ratios of bone to buffer are more than 1000 to one.

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b. In vivo - the amount of deposition in a given bone is directly proportional to the circulation of the bone, the vertebrae being higher than femur which itself is higher than skull. Young animals deposit more than adults. Thus, less uranium reaches the kidney in young animals which makes them more tolerant than adults. In older rats a sex difference is observed and can be explained on the basis that females mature more quickly and therefore deposit less uranium in bone and more in the kidney.

5. Metabolism Tests:

Blood Clotting: Three different tests were selected, namely the prothrombin time, the blood fibrinogen level and the bromsulfalein retention, as measures of testing the presence or absence of liver damage due to uranium compounds.

Short exposures to uranium hexafluoride concentrations of 2.0 mg per cubic meter (six hours) showed marked increases in the prothrombin time and fibrinogen levels which lasted from one week to one month before returning to normal. No change in bromsulfale in retention was noted. Following chronic exposures to lower levels some fluctuation in these determinations was noted with a tendency toward the reduction of the fibrinogen levels.

Uranium tetrachloride caused 25 to 30% retention of bromsulfalein after single acute exposures. The levels returned to normal in one month. The kidney function in these animals was normal.

Uranium nitrate showed some fluctuation in the above prothrombin and fibrinogen levels after five to six months of exposure at 0.25 and 0.45 per cubic meter.

Phosphatase Stains: The normal kidney tubular cells stain with phosphatase stain. Following uranium nephrosis of the tubules, these stained areas disappear in approximately fourteen days. Although the epithelium regenerates to normal appearance histologically, the phosphatase stain does not return. This indicates an alteration of the physiological properties of the tubular cell. This observation is confirmed in the absence of phosphatase in the urine following repeated uranium exposures to animals. (See Dounce in Mechanism section).

Carbohydrate Metabolism: Following toxic exposures to uranium nitrate a moderate decrease in the glucose tolerance of both dogs and rats is observed. This is accompanied by small decreases in the ability to form muscle glycogen. No change, however, is found in the liver glycogen. The glucose tolerance curves of these animals show progressive flattening of the curves up to eighteen days. The absence of typical diabetes is suggested by fasting normal blood sugars. Some slight elevations of blood lactic and pyruvic acids were noted immediately following exposure.

Blood potassium and Carbon Dioxide: No changes were observed in the blood potassium and blood protein levels following exposure to toxic con-

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centrations of uranium nitrate (3 mg/m^3)

The blood carbon dioxide drops following exposure and reaches minimal levels between the first and seventh day. Following this time there is a return to normal levels. The minimal values are less than one-half of the control values.

Renal Clearances: Following exposures to uranium nitrate at 3.0 mg/m^3 the inulin clearance is decreased reaching its maximum during the third week of exposure with a return to normal at the fifth week. The diodrast clearance falls abruptly to from 30 to 75% of its normal value at one week. Normal values are observed again in approximately thirty-five days. There is a maintained increase in the chloride clearance. No change is observed in the NPN. Proteinuria is noted during the first three weeks of the experiment. The urine catalase levels reach their maximum at the lowest point of the diodrast clearance.

The conclusions drawn from such observations indicate that uranium causes a decreased tubular function which compensates as the animals recover.

6. Mechanism Section:

a. The toxicity of uranium for the kidney tubules depends on the release of the uranyl ion by the breakdown of the uranyl-bicarbonate complex at a low pH. The mechanism by which the kidney is injured is as follows: The uranyl-bicarbonate complex passes through the glomerulus into the tubule at which point the pH falls below a level of 6.5; this causes a breakdown in the complex with the HCO_3 radicle being resorbed and the free uranyl ion now able to attack the tubular cells.

b. Tetravalent uranium combines with plasma proteins and does not pass through the glomerular filter unless protein also is passing.

c. The reduction of hexavalent to tetravalent uranium is dynamically possible in the animal body although such a reaction is undoubtedly very slow and occurs to only a small extent. The reduction of tetravalent to trivalent is impossible. Pentavalent uranium is too unstable to exist.

d. No polymerization of uranium occurs under conditions compatible with life.

e. The excretion of hexavalent uranium depends to a large extent on the acidity or alkalinity of the urine; the latter promoting increased excretion, the former depressing it.

f. A method for the measurement of urinary catalase excretion has been developed and standardized for use in the detection of heavy metal injury to the kidney.

g. It has been detected that the urinary protein present after uranium poisoning is plasma albumin.

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h. The diffu[redacted]anium complex through a filterable membrane is an increasing function of the carbon dioxide content of the medium. Uranium carbonate and bicarbonate complexes have been shown to be identical.

i. It is thought that bicarbonate is the most important carrier of hexavalent uranium. Sodium citrate and bicarbonate increase uranium excretion. It is conceived that the hexavalent ion is deposited or shows an affinity for epithelium and the tetravalent ion for endothelium.

j. The effect of the uranyl ion on renal function is as follows: 1) the small dose shows no effect on substances which filter through the glomeruli (creatinine, xylose, inulin); 2) There is an increase clearance of substances reabsorbed in the distal half of the proximal convoluted tubule (chlorides, amino acid nitrogen); 3) There may be an increase in substances partially reabsorbed in the proximal convoluted tubule (glucose, urea); and decrease clearance of secreted substances (phenol red, diodrast, hippuran); 4) Larger doses reduce apparent filtration in glomeruli by the blockade of tubules and diffusion of water soluble material across denuded tubular wall and thus reducing all clearances; 5) In catalase excretion in the rabbit, this substance is normally not filtered or secreted by the kidney. The amount of catalase at one and a half to two hours after exposure is a linear function of the amount of hexavalent uranium given. The hexavalent ion is at least four times as effective as the tetravalent in causing the appearance of catalase in the urine.

III. General Conclusions:

In conclusion, the purposes of this entire research program was fourfold:

1. To supply information which would be helpful in the establishment of a maximum factor of safety for the employees exposed to the uranium compounds.

2. To furnish data which would establish early indices of uranium toxicity.

3. To find helpful prophylactic and therapeutic measures to combat possible acute and chronic effects of these substances.

4. To furnish evidence to the Manhattan Engineering District which would be helpful in the event of law suits by employees for injuries to health attributable to these compounds.

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TABLE [redacted]

Compound	Concentration mgm uranium per cubic meter of air	Overall Mortality	% Mortality	Pathology: Renal	Pathology: Pulmonary	Blood Chemistry: NPN	Blood Chemistry: Urea Nitrogen	Urine Chemistry: Albuminuria	Urine Chemistry: Catalasuria	Hematology	Weight Response
UF_6	13.5	56/75	74.7	Severe renal damage in all dying animals	Moderately severe to moderate damage	marked increase	marked increase	marked increase	marked increase	Moderate to minor changes	Marked loss
	2.0	34/98	34.0	Severe renal damage in all dying animals	Moderately severe to	moderate increase	moderate increase	------	------	Drop in Hgb and RBC	Mod. loss to slight gain
	0.2	2/91	2.1	Mild renal changes	no damage	------*	------*	Occasional increase	Occasional increase	------	Slight gain (below normal)
UO_3	10.6	18/124	14.5	Severe damage	" "	increase	increase	-----	-----	Rise in WBC & platelets	usually loss
	2.4	25/209	12.0	Mod. to severe	" "	slight to marked inc.	slight to marked inc.	slight to marked		no changes	Slight gain to loss
	1.37	14/42	33.3	Mild damage	" "	-----	------	-----	-----	-----	Gain
	0.64	0/4	0.0	Mild damage	" "	disturbed glucose tolerance		increase	increase	-----	Slight loss
	0.28	10/126	7.9	Slight damage	" "	elevation	-----	increase	increase	-----	Slight gain
UO_4	11.0	70/97	72.1	Severe damage	" "	marked inc.	marked inc.	marked	marked	Incr. WBC	Marked loss
	2.0	17/116	14.7	Mod. damage	" "	increase	increase	increase	increase	Decr. RBC Hgb	Incr. or decr.
	1.1	0/30	0/0	Slight damage	" "	slight inc.	slight inc.	slight increase	slight increase	-----	Gain
	0.26	7/96	7.3	Mild	" "	no effect	no effect	none	none	Sl. change	Gain
	0.11	0/78	0/0	Very slight	" "	no effect	no effect	slight increase	slight increase	-----	Gain
UF_4	16.7	12/65	18.5	Mod. damage	" "	occasional increase	occasional increase	none	none	No changes	Loss
	3.6	2/94	2.0	Very mild	" "	----	----	----	----	-----	Sl. loss to gain
	0.5	1/112	1.0	None	" "	No effect	No effect	none	none	-----	Gain
Ore	22.0	12/90	13	Mod. to severe	" "	increase	increase	----	----	Minor ?	Loss
	3.0	9/127	7.1	Slight or absent	" "	no effect	no effect	----	----	-----	Gain
	0.6	9/129	6.3	Absent	" "	" "	" "	----	----	[illegible]	Gain
UO_2	19	12/85	14	Mod. to severe	" "	increase	increase	sl. increase	sl. incr.	[illegible]	slight loss
	9	3/130	2	Moderate	" "	? change		no change	no change		
	2	1/123	1	None	none	none	none	none	no		

*No Observation

SECRET

115819

mpound	Concentration mgm uranium per cubic meter of air	Overall Mortality	% Mortality	Pathology Renal	Pathology Pulmonary	NPN	Blood Chemistry Urea Nitrogen	Urine Chemistry Albuminuria	Urine Chemistry Catalasuria	Hematology	Weight Response
ntrol	0	1/25	4.0	No changes	No damage	none	none	none	none	no changes	gain
	0	4/85	4.7	No change	"	"	"	"	"	"	"
	0	10/105	10.5	No change	"	"	"	"	"	"	"
	0	1/95									
	0	0/12									
	0	16/178	8.9	No change	"	"	"	"	"	"	"

1151820

Compound	Concentration mgm uranium per cubic meter of air	Dog	Rat	Rabbit
UF_6	13.5	Severe	Severe to Moderate	Severe
	2.0	Severe	Moderate to Severe	Mod. to severe
	0.2	Slight	None	Slight
$UO_2(NO_3)_2$	10.6	Severe	Moderate to Severe	Severe
	2.4	Severe	Moderate	Severe
	1.37	Moderate	Slight to Moderate	---
	0.84	Moderate	---	---
	0.28	---	Slight	Slight
UCl_4	11.0	---	---	Severe
	2.0	---	Moderate	Severe
	1.1	Moderate	Absent	Slight
	0.26	---	Slight	Slight
	0.11	---	Absent	Slight
UF_4	30	Moderate	Moderate	Moderate
	16.7	---	Slight	Slight
	3.6	Mod.toSlight	Absent	---
	0.5	---	Absent	Absent
UO_2	19	---	Slight	Moderate
	9	Slight	Absent	Absent
	2	---	Absent	Absent
Ore	22	---	Moderate	Mod. to severe
	3	Sl. or absent	Absent	Absent
	0.6	Absent	Absent	Absent

RELATION OF GROUND CONCENTRATIONS TO TOXIC EFFECTS

Compound	Concentration mgm uranium per cubic meter of air	Overall Mortality	Pathology		Abnormal Changes: Blood	Urine	Peripheral Blood (Alb & cat.)*	Weight Response
			Renal	Pulmonary				
HIGH CONCENTRATION								
UF_6	13.5	75	Severe	Severe	Marked	Marked	Moderate	Marked loss
UCl_4	11.0	73	Severe	Severe	Marked	Marked	Moderate	Marked loss
UNO_3	10.6	14.5	Severe	Severe	Mod.	Mod.	Mod.	Loss to gain
UO_2	19	14	Moderate	None	NPN rise in	--**	Slight	Sl. loss
U_3O_8	14.2	18	Slight	None	NPN rise in	—	Normal	Gain
UF_4	17	19	Moderate	None	NPN rise in	--	--	Loss
Ore	22	13	Moderate Severe	Moderate Severe	NPN rise in	--	Slight	Loss
MID CONCENTRATION								
UF_3	2.0	35	Mod to mod severe	---	NPN	Marked	Moderate	Loss to gain
UCl	2.0	15	Moderate	---	NPN	Marked	Slight	Loss or gain
UNO_3	2.4	11.5	Mod to mod severe	---	Sl. to severe	Marked	No change	Loss or gain
UO_2	9	2	Slight	---	None	---	---	Gain
UF_4	3.6	2	Slight	---	---	---	---	Plus/minus
Ore	3	7	---	Normal	---	---	---	Gain
LOW CONCENTRATION								
UF_6	0.2	2	Slight	---	None	Slight	None	Gain
UCl_4	0.11-0.26	7	Slight	---	None	Slight	None	Gain
UNO_3	0.28-0.69	2.6(?)	Slight	---	NPN	Slight	None	Gain
UO_2	2	1	None	---	None	Slight	None	Gain
UF_4	0.5	1	None	---	None	None	None	Gain
Ore	0.6	7	None	---	None	None	None	Gain

** No observation
* Albuminuria & Catalasuria

TABLE IV

CHRONIC INHALATION SUMMARY

Compound	Concentration mgm uranium per cubic meter of air	Duration (months)	Evaluation	Results
UF_6	0.20	6	±	Slight renal damage
	0.05	4	0	No toxic effect
UCl_4	0.20	7	±	No toxic effects
	0.05	5	0	No toxic effects
UNO_3	1.3	8	+	Slight renal damage with subsequent recovery
	0.45	6	±	No toxic effects
	0.15	6	±	No toxic effects
	0.05	6	0	No toxic effects
UF_4	3.0	5	±	Slight renal damage
	0.50	5	0	No toxic effects
UO_2	10.0	6	0	No toxic effects
	1.0	6	0	No toxic effects
Control	0.0009	6	0	No toxic effects

1151823

TABLE V

30 DAY EXPERIMENTS (ACUTE)

Compound	Animal	Concentrations	
UO_2F_2	Rats	.05, 0.1, 0.25, 0.5, 1.0, 2.0, 20.0	% of diet
UCl_4	Rats	0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 20.	% of diet
UF_4	Rats	0.5, 2.0, 20	% of diet
	Dogs	0.5, 10.0, 20.0	mg/kg/day
UO_2	Rats	0.5, 2.0, 20.0	% of diet
	Dogs	5.0, 20	mg/kg/day
UO_3	Rats	0.5, 2.0, 20	% of diet
U_3O_8	Rats	0.5, 2.0, 20	% of diet
UO_4	Rats	0.1, 0.25, 0.5, 1.0, 2.0, 20	% of diet
U acetate	Rats	0.5, 2.0, 20	% of diet
High Grade Ore	Dogs	5.0, 10	mg/kg/day

1151824

TABLE VI

CHRONIC EXPERIMENTS

Compound	Animal
UO_3	Rats at 2.0, 1.0, 0.5, 0.1, 0.05 and 0.01 % of diet for 2 years Dogs at 0.02, 0.1, 0.2, 0.5, 2.0 and 10 mg/kg/day for one year
UO_2F_2	Rats at 0.01, 0.05, 0.1, 0.15, 0.25, 0.5 % of diet for 2 years Dogs at 0.0001, 0.0002, 0.001, 0.0025, 0.005, 0.02, 0.1, 0.5 and 5.0 for 12 months
UCl_4	Dogs at 0.002, 0.01, 0.02, 0.05, 0.1, 0.5 and 5.0 mg/kg/day for 12 months
UF_4	Rats at 0.5, 2.0 and 20.0 % for two years Dogs at 5.0, 10.0 and 20.0 mg/kg/day for one year

TABLE VII

CURRENT RESULTS OF FEEDING EXPERIMENTS ON U. COMPOUNDS

	Rats	Dogs
Non-toxic or slightly toxic	UO_2 U_3O_8 UF_4	UO_2 U_3O_8 UF_4 Ore
Moderately toxic	UCl_4, UO_3 UNO_3 U acetate	UNO_3, UO_3 $Na_2U_2O_7(NH_4)_2$, U_2O_7
Highly toxic	UO_2F_2, UO_4	UO_2F_2, UCl_4, UO_4

TABLE VIII

EYE [redacted]

Compound	Dose	Vehicle	Damage	Duration of Damage	Mortality	Findings at Autopsy
UCl_5	0.001	none	4+	long (3-4 weeks)	2/4	Renal Damage
UNO_3	0.350	water	3+	long (3-4 weeks)	3/4	" "
UCl_4	0.250	water	2+	long (3-4 weeks)	3/4	" "
$Na_2U_2O_7$	0.1	none	3+	short (4 days)	1/4	" "
$(NH_4)_2U_2O_7$	0.1	none	2+	short (4 days)	3/4	" "
UO_2F_2	0.2	water	2+	short (4 days)	3/6	" "
UF_4	0.1	none	1+	short (4 days)	3/6	" "
UO_3	0.1	none	0	0	4/4	" "
	0.75	lanolin	0	0		
UO_4	0.1	dry	0	0	0/4	------
	0.75	lanolin	0	0	0/4	------
U_3O_8	0.1	dry	0	0	0/4	------
	0.75	lanolin	0	0	0/4	------
UO_2	0.1	dry	0	0	0/4	------
	0.75	lanolin	0	0	0/4	------

1151826

TABLE IX

[redacted]

SKIN ABSORPTION

Compound	Number of Rabbits	Dose (gm)	Mortality	Irritation	Remarks
UO_2	6	1.0	0/6	0	non toxic
U_3O_8	6	1.0	0/6	0	non toxic
UF_4	6	1.0	0/6	0	non toxic
UO_3	6	1.0 ether	6/6	1 plus	Renal damage at autopsy
UCl_4	6	2.0 lanolin	6/6	2 plus	" " " "
UO_2F_2	6	1.0 lanolin	6/6	0	" " " "
	6	1.0 water	5/6	0	" " " "
UO_3	6	1.0 lanolin	4/6	0	" " " "
$Na_2U_2O_7$	6	1.0 lanolin	4/6	0	" " " "
UO_4	6	1.0	0/6	0	non toxic
UF_4	6	1.0	0/6	0	non toxic
[illegible]	6	40 lbs.	0/6	4 plus	———
UCl_3	6		0/6	0	———
BF_3 dimethyl ether	6		0/6	0	———

[redacted]

1151827

SUMMARY OF TOXICITY DATA

On the basis of mortality, weight loss, histopathological changes, chemical changes, the uranium compounds can be grouped for their ability to produce acute uranium poisoning as:

1. Highly toxic UO_2F_2, $UO_2(NO_3)_2$, UCl_4 and UF_6
2. Moderately toxic UO_4
3. Slightly toxic U_3O_8, UO_2, UF_4 and ore

RADIATION SECTION

Film Monitoring Studies for determination of radiation exposure.

For the purpose of indicating the intensity of radiation to which personnel are exposed, film has the advantage of being subject to fewer accidents of the type giving gross errors in reading of indicated exposures. Primary disadvantage is the fact that blackening to equally biologically hazardous amounts of radiation is dependant upon the nature and wave length of the radiation. In practice this effect has been largely overcome. Type K Eastman Kodak x-ray film made up in the size of dental packets is used because of its great sensitivity. Lead cross is attached to film, making possible by differential density readings of developed film both outside of and covered by the lead cross, to measure the relative amounts of incident beta and gamma radiation and their quality as well as penetrating power. With proper calibration readings are made in r units or their equivalent.

Suitable film holders were developed as identification badges for plant personnel. With each batch of films are developed calibrating strips of the same film which has been exposed to known graduated amounts of gamma radiation. Use of these calibrating strips makes it possible to compensate for unavoidable variations in development techniques as well as batch developed variation in film sensitivity.

The results of this standardized study have been used in monitoring personnel in all parts of the Manhattan District, and a service division has been set up in the laboratory here to supervise and interpret the results so obtained.

Development of Neutron Sensitizers for Film Work.

A photographic film packet has been developed surrounded by a foil of cobalt, rhodium or silver. These metals are sensitive to slow neutrons and emit beta and gamma radiation when exposed to neutrons. If these badges are worn by suitable personnel, the human body with its high hydrogen content slows down the fast neutrons to slow neutrons which in turn act upon the metal of the foil. Thus beta and gamma rays are emitted which blacken the enclosed film. For the monitoring of locations these special film packets are attached to the sides of water containers which also serve as a means of slowing the fast neutrons.

Development of Instruments for the Measurement of Radiation.

The following instruments have been designed, fabricated, and placed in operation by this section:

1. An all purpose (vane) meter for the measurement of beta and gamma radiation.
2. A surface alpha meter.
3. A built-in alpha meter for measurement of hand exposure.
4. Alpha electrometers.
5. Alpha counters.

6. Radon air measuring apparatus.
7. Radon breath measuring apparatus.
8. Thin window Geiger counters and dipping Geiger counters.

BIOLOGICAL RESEARCH SECTION

1. Metabolism of Polonium

A. Animal Experimentation: Following intravenous administration, the urinary excretion in both high and low dosages amounts to 0.02 to 0.1 per cent of the dose per day except for the first day when the highest dose shows three to ten times the rate of excretion. The fecal excretion is approximately twenty times the urinary rate on the first day, rising to seventy times on the tenth day and six times on the fiftieth day. The retention equals twenty-five per cent of the dose in ten days, forty five per cent on the fiftieth day, seven per cent at 130 days and 0.7 per cent at 300 days. (This includes radioactive decay on the 140 day half-life basis). The distribution of polonium in the tissues shows the spleen, lymph nodes, kidney, bone marrow, and blood cells with the highest concentration in 10 days. At 50 days, the blood cells, testes, and seminal vesicles show approximately the same concentration as the ten-day studies.

Following subcutaneous injection, the excretion rates, in terms of the amount absorbed, are similar to those after intravenous administration with the exception of a higher urinary excretion during the first two days. About 83 per cent of the dose remains in the body at ten days, of which about 40 per cent is retained in the injection site.

Oral studies show that 3 per cent to 5 per cent of the dose is absorbed from the gastro-intestinal canal. Fifty per cent of the absorbed material is excreted in the urine in ten days. The fecal excretion is approximately the same per cent of the absorbed dose as in the intravenous animal.

Skin absorption in the human subject indicates that no absorption is detectable in four days of exposure. The feet of mice show less than 1 per cent of total dose absorbed over a two-day experimental period.

By inhalation a large proportion (probably over 30 per cent, and in some cases approximating 100 per cent) of the vaporized polonium inhaled by a rat is retained in his lungs. Most of the polonium captured by the lungs is absorbed into the blood stream giving essentially the effect of an intravenous injection. Sufficient polonium may be retained in the lungs (11 per cent of the dose at 10 days) for a prolonged period to produce direct effects on the alveolar epithelium (see M-1811).

B. Human Subjects: Following intravenous injection into human subjects (four cases) in amounts from 0.18 to 0.3 microcuries per kilogram, the twenty-four hour urine showed 0.07 to 0.8 per cent of the dose. The average daily urinary output in the first week was 0.06 to 0.24 per cent of the dose; in the second week, 0.04 to 0.08 per cent; and at 70 days, 0.02 per cent of the dose per day. The fecal daily excretion during the first week amounted to 0.56 to 2.03 per cent of the dose; during the second week, 0.73 per cent; and at 70 days, 0.25 per cent of the dose. A very

rapid disappearance of the injected polonium from the blood stream was observed as evidenced by only 1.7 per cent of the total dose remaining in 15 minutes.

In oral administration of 0.19 microcuries per kilogram, the blood and urine values were approximately one-tenth of the dose found after intravenous injection of a similar dose. This indicated absorption of less than 10 per cent of the dose. The fecal excretion amounted to 77 per cent of the dose by three days. At the end of 230 days less than 0.6 microcuries remained in the body of a total of 18.5 microcuries given.

C. Effect of Various Agents on Polonium Excretion: Of a large number of substances tested, only BAL (British Anti-Lewisite) caused a transitory rise in the fecal excretion several weeks after injection. This increased output was balanced by a later decreased output for a few days. In the first 24 hours after intravenous injection of polonium, BAL produced a urinary excretion of 5 per cent of the dose as contrasted with a normal of 1 per cent of the dose. Following this, the excretion dropped to normal levels. BAL treatment immediately after subcutaneous injection caused no significant differences in the excretion rates. Tissue analyses showed essentially similar concentrations, except in the spleen whose activity was considerably lower after BAL treatment.

2. Distribution and Excretion of Radium:

Rats were injected with 11.5, 16.0, 17.2, and 1000 microcuries per kilogram of radium as the chloride and followed for one, ten, and fifty days. The urinary excretion on the first day amounted to 9.3 to 21.6 per cent of the dose; the fecal excretion, 0.18 to 25.2 per cent of the dose. The total excretion observed was from 14.0 to 44.0 per cent of the dose on the first day; a fall to 1 per cent on the sixth day; 0.5 per cent at 10 days and 0.1 per cent at 50 days. At the ten day period, 40 to 60 per cent of the dose remained within the animal; at 50 days, 32 per cent of the dose. An additional observation indicated that the excretion rates were not influenced by the size of the dose. One animal carried for 236 days still retained 40 per cent of the injected dose.

Distribution studies showed that at 24 hours, 83 per cent of the amount remaining in the body was deposited in bone; at 10 days, 99 per cent; and at 50 days, 96 per cent. The highest activity found in the bone was present in the trabeculae. The gastrointestinal tract showed 8.2 per cent of the total dose at one day, falling to a small figure at 10 and 50 days. Other soft tissues showed 8.8 per cent at one day, also dropping at the 10 and 50 day periods. The blood showed 0.005 per cent at 10 days, and 0.002 per cent at 50 days. Oral administration studies are in progress.

3. Comparative Lethal Dose Studies of Radium, Polonium, and Plutonium:

Using dosages expressed as microcuries per kilogram, the short term lethal dose studies indicate that polonium is three times as toxic as plutonium,

and that plutonium is at least thirty times as toxic as radium.

On the basis of relative energies of the alpha particles of these three substances, it is apparent that for the radium and its daughters, plutonium, the energies per microcuries are approximately equal, the amount of radium lost from excretion being compensated for by the retention of its daughter products. On the same basis, the polonium/plutonium comparison indicates that the former is approximately twice as toxic per unit of alpha ray energy dissipated in the body for 10 days, five times at 20 days, and as high as 10 times at the thirty day period.

Biological explanation of the differences may be explained on the distribution of these elements, the radium burying itself deep in the bone, the plutonium in the endosteal layers near the marrow, and the polonium in the hematopoetic and lymphatic tissues themselves.

The long time (six to eight month) studies of the comparative lethal dose tend to corroborate the above findings.

Pathological findings indicate that radium shows an affinity for the hematopoetic system, vascular system, liver, kidney, bowel, bone, and testes with damage proportional to dose and time. Polonium shows an affinity for the hematopoetic system, bowel and testes only with damage again probably proportional. Plutonium shows its affinity for the hematopoetic system, liver, kidney, bowel, bone, and testes. The changes observed with radium, although more widely distributed, are in general not as severe as polonium. An unusual finding is the development of far advanced arteriosclerosis of the aorta and larger arteries including the coronaries. Plutonium tends to show its most severe development in the hematopoetic system, liver, and bone.

4. Distribution and Excretion of Radioactive Materials in Non-toxic Doses.

Studies are being conducted on the injection of non-toxic amounts of plutonium, polonium, radium, uranium, and possibly lead into human subjects in order to determine the excretion rates in the urine and feces of these materials. These studies are not ready to report at the present time.

The information found from such studies will be used in determining accurately the allowable excretions of these substances in the careful monitoring of plant personnel working with them. The urinary quotient will indicate the amount of harmful material present in the body at that unit time.

5. Suggested studies:

a. Physiological experimental work with C_{14} and H_3, specifically on protein metabolism such as in wound healing, repair, aging, cancer and the like.
   1. How substituted amino acids behave in the body.
   2. Investigation of bone metabolism with an idea to therapy, etc.
b. Chronic effects of alpha emitters over a ten-year period. For us in chronic tolerance studies.
c. Additional studies on radioactive materials, metabolism in general particularly with regard to the items mentioned in (a) above.

1151832

REPORT OF INVESTIGATIVE WORK BY THE SURGICAL SECTION

The work carried out and to be reported by the surgical department can be divided into the following 3 divisions:

1) The transplantation of bone marrow.
2) The culture of bone marrow by tissue culture methods.
3) The transplantation of a kidney.

In as much as some of the data is still in the process of examination and analysis and experiments are still under study, this report at the best must be considered to be of a provisional nature and subject to change contingent upon further analysis of the data and experimentation.

I <u>Transplantation of bone marrow</u>:

In undertaking such a problem as marrow transplantation it was first necessary to establish certain base line data on the potential recipient. This included selection of a type of radiation that would produce damage of the proper extent or degree. Total body radiation of 300 r acute dosage, from 2 250 KV machine was finally selected since it produced approximately an L.D. of 50 or slightly greater.

Employing this dosage of radiation studies were then carried out to determine the type and extent of damage to various tissues and organs of the dog with especial reference to the hematopoetic tissue and the included bone marrow. In order to obtain such information, old and new methods of study had to be tested and then used. Radiation damage to the host was then estimated by correlation of clinical, hematological and pathological findings. Radiation damage to the bone marrow at a given period of time could be determined more precisely by direct examination of the marrow from the living host by removing such a specimen as a rib.

The experiments on the transplantation of bone marrow are divided into the following groups:

A. Control Studies
B. Transplant Studies

A. Control Studies

1. <u>Pilot, Radiated</u>

A group of 7 normal dogs received 300 r total body radiation, underwent rib resections 3 days preradiation, 4, 7, 14, 21, 28, and 39 days post-radiation and was studies as controls to determine the pattern and extent of derangement of the clinical course of the peripheral blood and bone marrow picture. Hematological and pathological studies were made as the time of unexpected death or sacrifice. The peripheral blood was examined at hourly intervals for the first 48 hours post-radiation in 4 of the animals.

Three of the 7 radiated dogs died during the course of study. All

of the animals manifested dehiscence and necrosis of their surgical wounds. Bone marrow studies showed conspicuous signs of degeneration beginning 4 days post-radiation and maximal damage between 7-14 days. This degeneration applied to the myeloid, erythroid and megakaryocyte elements. At 21 days post-radiation early signs of regeneration could be detected which was more evident at 28 days. At 39 days post-ratiation and thereafter the marrow was hyperplastic but with small areas of debris.

2. Pilot, Non-Radiated

A group consisting of 4 normal dogs underwent similar procedures and study as group 1 except that no radiation was administered. Normal findings were noted in these animals.

3. Control, Radiated

Another group consisting of 8 normal dogs was studied as controls to determine or obtain the normal pattern and extent of derangement of the clinical course, the peripheral blood picture, as well as the pathological and hematological pattern at time of unexpected death or sacrifice 80 days post-radiation.

Six of the 8 dogs died of radiation intoxication i.e., purpura, sepsis, oral ulceration, depression of the hematopoeitic system and the associated panhematopenia. The surviving animals showed hyperplastic changes in the hematopoeitic tissues.

4. Control, Radiated-Operated

A group of 4 normal dogs received 300 r total body radiation, underwent rib resections 3 days pre-radiation, 2, 7, and 39 days post-radiation, had pseudo marrow transplantation procedures 2 days post-radiation and was studied as controls to determine the pattern and extent of derangement of the clinical course, the peripheral blood and the bone marrow picture. Hematological and pathological studies of the marrow were made at the time of each rib resection and unexpected death or at sacrifice, 60 days post-radiation.

Three of the 4 dogs died of radiation intoxication with findings as noted above. In addition wound dehiscence and necrosis was common. Degenerative changes in the bone marrow were conspicuous at two days post-radiation and considerably more marked at 7 days post-radiation. At 39 days post-radiation the marrow was hyperplastic but areas of debris were still present. This hyperplasia applied to erythroid, myeloid and megakaryocytic elements.

The data from the preceeding groups provided the base line information upon which to compare the results from the transplanted groups as noted following.

B. Transplant Studies

1. Marrow Suspension Transplant

A 5th group consisting of 5 normal dogs received 300 r total body radiation, underwent rib resections 3 days pre-radiation, 4, 7, and 39 days post-radiation, received a single normal marrow suspension transplantation into the distal end of the shaft of right femur, the right humerus, and the anterior chamber of each eye, and was studied to determine the pattern and extent of derangement of the clinical course, the peripheral blood and the bone marrow picture. Hematological and pathological studies were carried out at the time of unexpected death or sacrifice, 30 or 60 days post-radiation.

Four of the 5 dogs died from radiation intoxication with the transplant sites of the long bones showing considerable evidence of old hemorrhage, organization but very little evidence of new marrow growth. Residual fat deposits with connective tissue reaction were the findings from the marrow transplants to the anterior chamber of the eyes.

In the one dog that survived comparative studies of the long bones did not indicate that the transplanted sites were more hyperplastic than the non-transplanted sites.

2. Bone and Marrow Transplantation

A 6th group consisting of 4 normal dogs received 300 r total body radiation, underwent rib resection 2 days pre-radiation and 2, 4, 7 days post-radiation, received a transplantation of normal bone marrow and accompanying bone cortex and endosteum to the shaft of the right femur and a transplantation of a segment of rib to rib, 2 days post-radiation. A marrow suspension transplant was placed in the anterior chamber of each eye. In addition this group was studies to determine the pattern and extent of derangement of the clinical course, the peripheral blood and the bone marrow picture. Hematological and pathological studies were carried out at the time of unexpected death or sacrifice, 30 or 60 days post-radiation.

Three of the 6 dogs died of radiation intoxication with the transplant sites showing old hemorrhage, connective tissue reaction and new bone growth but very little evidence of new marrow growth. Residual fat deposits with connective tissue reaction were noted in the anterior chamber.

In the three dogs that survived various degrees of regenerating or hyperplastic marrow was found but the transplanted sites always appeared less active than the non-transplanted sites.

3. Marrow Transplantation to heavily radiated long bones

A group of 4 normal dogs received radiation of 2500 r to both entire lower extremities simultaneously. Two days post-radiation transplantation of a normal bone marrow suspension was carried out. Standard studies were carried out to determine the pattern and extent of derangement of the clinical course and the peripheral blood. Hematological and pathological studies were carried out at the time of unexpected death or sacrifice.

All of these dogs survived these procedures. This dosage of radiation was not of sufficient magnitude to destroy all of the marrow in the irradiated extremities. Comparative studies indicated that less active marrow was found in the transplanted sites.

The impression gained from the transplantation of marrow to long bones was that such procedures were associated with a good deal of bleeding into the marrow cavity and no evidence that the marrow transplantation was successful.

Accordingly the next group of experiments were directed at determining the potential effectiveness of introducing extramedullary myelopoesis.

4. Marrow Transplants to Spleen - Non-Radiated

A group of 4 normal dogs received a transplantation of normal bone marrow suspension into the spleen. Standard studies were carried out to determine the pattern and extent of derangement of the clinical course and the peripheral blood. Hematological and pathological studies were carried out at the time of unexpected death or sacrifice.

All of the animals survived the procedures. Residual fat deposits and connective tissue response without evidence of extramedullary myelopoesis were found at the sites of transplantation.

5. Marrow Transplants to Spleen - Total Body Radiation

A group of 4 normal dogs received total body radiation of 300 r and a transplantation of normal bone marrow suspension into the spleen 2 days post-radiation. Standard studies were carried out to determine the pattern and extent of derangement of the clinical course and the peripheral blood. Hematological and pathological studies were carried out at the time of unexpected death or sacrifice.

Three of the 4 dogs died of radiation intoxication. The sites of transplantation were as noted above.

6. Marrow Transplants to Spleen - Spleenic Radiation

A group of 4 normal dogs received radiation of 2500 r to the spleenic area and a transplantation of normal bone marrow suspension into the spleen 2 days post-radiation. Standard studies were carried out to determine the pattern and extent of derangement of the clinical course and the peripheral blood. Hematological and pathological studies were carried out at the time of unexpected death or sacrifice.

All of the animals survived these procedures. The spleens were small and fibrotic with residual deposits of fat and no evidence of extramedullary myelopoesis at the transplant sites.

As the result of these marrow transplants to the spleen it did not appear desirable to attempt to induce further extramedullary myelopoesis into the liver or retroperitoneal tissues by the direct trans-

plantation of homologous bone marrow.

7. Marrow Transplantation Intravenously

A group consisting of 4 normal dogs received 300 r total body radiation and intravenous injections of refined bone marrow suspension 2, 4, 7, and 9 days post-radiation. Studies were carried out to determine the pattern and extent of derangement of the clinical course, and the peripheral blood. Hematological and pathological studies were carried out at the time of unexpected death or sacrifice.

Two of the 4 dogs died of radiation intoxication. However all animals showed periods of elevation of the total WBC count that were not noted in the control radiated group. At these periods of elevation atypical types of cells were noted frequently. These elevated periods and atypical cells were not noted immediately after transplanation, however, nor could such findings be expained on the basis of the injection of a particulate matter such as india ink.

Pathological studies showed miliary bone deposits within small veins throughout the lungs.

Because of these findings this phase of the experiment is being extended.

II Culture of Bone Marrow by Tissue Culture Methods:

After due consideration it was deemed desirable to study the cultivation of bone marrow in vitro in order to provide a means (1) whereby the marrow could be studied in more detail and more accurately with especial emphasis on the effects of radiation on certain cytological alterations (2) to consider the feasibility of establishing a marrow bank if marrow could be cultivated successfully and if marrow could be transplanted successfully.

Such techniques as the roller tube, the Carrel flask, and the sitting drop slide technique have been employed. Each has their advantages and disadvantages but a combination of the slide and flask technique appears to provide the optimum conditions for growth and study.

With these techniques the following studies were carried out:

1. the effect of varied media on the growth of bone marrow.
2. the effect of varied gas tensions on the growth of bone marrow.
3. the effect of varied stimuli to bone marrow and its growth is standard media.
4. the comparative studies on the growth of normal and radiated bone marrow.
5. the preparation of marrow for transplantation.

The results of these studies are now in the process of tabulation and interpretation.

Further studies will probably be resumed when the current report has been completed.

[redacted]

III Transplantation of a Kidney

In the process of working with and the incidental exposure to uranium and uranium compounds, it was believed that a significant degree of renal damage would result. Because of this anticipated damage it was felt desirable to direct some attention to measures which would effect some benefi to the damaged organ and the associated derangements to the host. One of the first procedures considered was the addition of a normal kidney with the ultimate hope that a method could be worked out eventually whereby this transplanted kidney could function for at least a short period of time until the damaged kidneys had recovered sufficiently to carry on their function again. During the course of study of the toxicity of uranium and its compounds it was found that the incidence and degree of renal damage encountered in industrial and laboratory personnel was a great deal less than expected and accordingly less attention need be directed to therapeutic measures.

This experiment concerned itself with the initial studies carried out to determine a surgical technique that could be employed for the transplantation of a homogenous kidney.

The results of these experiments indicated that the cannulae technique for anastomosis of blood vessels and the transplantation of a kidney to a neck was more desirable. When the kidney of one dog was transplanted into the neck of another dog there was an increased initial blood flow through the artery secondary to the denervation. This increased blood flow may last for several days and was associated with excretion of abnormally large quantities of urine, providing the venous return was adequate. Some of the transplanted kidneys functioned for only 24 hours, others for as long as 5 days. Eventually kidney function failed leaving a large, swollen, soft dead kidney. Inasmuch as autogenous transplanted kidneys may survive for as long as 9 months it appears that the failure of renal homotransplants to function indefinitely cannot be ascribed to surgical errors but to a factor or factors of incompatibility.

[redacted]

1151838

I. ACUTE EXPERIMENTS - Single Dose.

A. Median Lethal Dose Experiments. The median lethal dose (that amount required to produce death in half of the animals) for x-radiation was determined in several species of animals, the results being listed in the following table. For the purpose of comparison of species, the studies on rabbits carried out at the Metallurgical Laboratory at the University of Chicago are included.

Rabbit	790 r
Rat	600 r
Monkey	5550-600 r
Dog	Work not yet completed.

It must be commented that the sensitivity of the above species for chronic x-radiation is in the same order.

The rat is equally sensitive at seven weeks and at seven months of age. Males and females show equal sensitivity with regard to acute lethal effects

If the dosage is adjusted by increasing the time of exposure at constant intervals, the mean survival time plotted against the log of the dose is a straight line. If the dosage is adjusted by varying the intensity at constant time, the mortality plotted against the dosage squared is a straight line. Evidence has been gained which indicates that the injury to radiation is a "two hit" phenomenon, or in other words, two single hits by x-ray photons have been made on a single element in a single cells, i.e., chromosome, spindle, etc.

Studies on those factors which influence the median lethal dose have been made, but are not yet ready to report. These include studies as to the rate of dose, metabolic effects, and the effect of various drugs on x-radiation injury.

B. Pathology of Acute Radiation Damage: Tissue changes in sacrificed rats at 28 days were different from those found in animals dying from irradiation injury within this period. The three tissues primarily affected were the bowel, testis, and hematopoetic system. The sacrificed animals, at least for dosage values of 500 r or greater, represented a group which had been injured and except for testicular changes, had repaired itself. Degenerate testes were present at the 250 r level. Significant changes in the hematopoetic system first appeared at 400 r. These changes were only a little more severe at 1000 r than at 400 r. This same gradient was noted in the spleen and lymph nodes. The marrow, however, was severely depleted at every dosage level at which the animals died.

Tissue changes in monkeys were found in the hematopoetic system and bowel. Testicular findings were difficult to evaluate because all animals were immature. The extent of injury was most severe at the higher dosage levels, the minimal injurious dosage occurring between 375 and 450 r.

1151839

Specific details of the pathological changes and their occurrence will be found in the complete reports. The changes observed in the [illegible] are in preparation and not yet ready to report. It must be commented that no findings of marked differences were observed in the review of the dog pathology.

II. CHRONIC RADIATION PROGRAM

Mice, monkeys, rats, and dogs were exposed to chronic radiation in the form of x-radiation from 250 and 1000 KVP machines. Mice, monkeys, and rats were exposed to 0.1, 0.5, 1.0, and 10.0 r per day six days per week for two years. Dogs were exposed to 0.1, 0.5, 1.0, 3.0, 6.0, and 10.0 r per day six days per weeks for two years. Suitable controls were carried with each series and received identical treatment except for the irradiation. The results will be reported according to the species.

The experiment on mice gave little result because of an epidemic of mouse typhoid which occurred during the experiment. Such results as were obtained are almost impossible to analyze.

The experiment with monkeys is likewise faulty for several reasons. Endemic tuberculosis was present in the colony and interfered with mortality figures as well as with survival times. One notable observation was that after 1½ years of radiation at a level of 0.1 r per day, a normal offspring was conceived and delivered.

The rat experiment gave the following observations which for the purpose of clarity can be listed.

1) The survival is significantly decreased at the 10 r per day level but not at the lower levels.
2) Males and females are equally sensitive to a given dose of radiation.
3) The biological effect of x-rays whether given with the 250 or 1 million volt machine is indistinguishable.
4) Levels of 1 r per day and below show no influence on sperm production. Definite effects are noted at the 10 r level.
5) The number of leukemias appearing during the course of the experiment showed a total of 12 in the 400 animals studied. The frequency is proportional to the dose of x-radiation given. This is illustrated in the accompanying table.

Dosage	Deaths	Total Number of Animals
0.0	none	50 animals
0.1 r	1	100 animals
0.5 r	2	100 animals
1.0 r	3	100 animals
10.0 r	6	50 animals

The 10 r level is appreciably higher than shown, inasmuch as it is necessary to correct for the increase in mortality in this group which in a two year experiment [illegible] considerable.

6) Studies of the weight changes on these animals indicates that weight observations are of little value in predicting the outcome of the experiment or as a possible clinical test for exposure. The weight loss occurs only in the period just before death, at which time other observable changes are well established.

7) Survival time studies are still in progress.

8) Tumors - preliminary studies indicate that some types, particularly mammary tumors, are induced while others are spontaneous in type.

9) Hematology - not included.

The dog experiment was carried out according to the following plan: Animals receiving 0.0, 0.1, 0.5, and 1.0 per day six days weekly were carried for 622 treatments (two years) and then sacrificed. The animals receiving 3.0, 6.0, and 1000 r six days weekly were given 500 treatments and all sacrificed. The plan of sacrifice for the lower groups is as follows: at the end of the period of exposure, one-third of the animals were sacrificed; the remaining two-thirds are carried for an additional three months for recovery studies at the end of which time the females are sacrificed. The males are kept on for additional recovery studies on sperm formation.

Mortality figures at the end of the first 500 treatments (data available) are as follows:

Level	Mortality
0.0 r	0
0.1 r	0
0.5 r	0
1.0 r	0
3.0 r	20%
6.0 r	40%
10.0 r	80%

Mortality figures at the end of 622 treatments are as follows:

Level	Mortality
0.0 r	0
0.1 r	5%
0.5 r	0
1.0 r	15%

At the present time only one animal has died of leukemia. That animal received the course of 10 r per day treatments.

As in the rat experiment, observations made on weight changes have little value in predicting the outcome of an experiment.

Sperm formation is influenced at all levels above 0.1 r per day and questionably at the 0.1 r level. Whether this change in the 0.1 r per day level is real will be shown by the recovery study now in progress. It requires 270 to 300 r to sterilize 50 per cent of the dogs at the 0.5 r per day level or the 1.0 r per day level. For sterilization at the 10.0 r per day level more than thirty treatments (300 r) are required. From this observation it is deduced that a time factor is involved in the maturation of sperm.

Some animals accumulated over 5000 r before dying, while others died at 1100 r of chronic irradiation. This illustrates the variability in the species themselves.

A. Pathology - Chronic Irradiation. Pathological examination of rats following exposures to chronic x-radiation for two years formulates the opinion that there is no good histological criterion of chronic x-radiation damage when administered in dosages of 10 r per day and less. Changes in the hematopoetic system were not impressive. Testicular damage was noted with dosages of 10 r per day but not below. It is suggested that the leukemias and mammary tumors arising in these animals may have been produced by the irradiation. There is no evidence of injury related to irradiation in the heart, lung, liver, kidney, bowel, pancreas, thyroid, trachea, skin, bone, ovary, or bone marrow.

Rabbits which have received similar dosages of x-radiation over a period of one year only, show similar changes to those noted above.

Dogs exposed to 10 r and 6 r per day for 500 treatments are most apt to die with evidences of hemorrhage presumably secondary to leucopenia, thrombocytopenia, and bone marrow hypoplasia. Testes are completely depleted of germinal cells. Lymph nodes and spleen show cellular depletion and some hemorrhage. Signs of infection superimposed on reduced bone marrow activity are common. At levels of 3.0 r, 1.0 r the testis is depleted of spermatogenic elements.

Following pilot experiments carried out in a laboratory elsewhere (see report from Columbia project) a program was instituted for the controlled chronic exposure of rats and dogs to fast neutrons. The dosages selected were the equivalents to the previously selected experimental x-radiation dosages, namely 0.1, 0.5, 1.0, and 10.0 r per day six days weekly. The equivalents in n dosages are 0.017, 0.085, 0.17, and 1.7 n per day six days weekly. The neutron experiments were carried for a one-year period instead of the two-year period used for the x-rays.

Findings were significant at the highest dosage level (1.7 n per day) in both the rats and dogs. At this dosage the effect on survival was much more marked than the supposed equivalent of 10 r per day. Mortality figures in the rats are difficult to interpret inasmuch as death in many instances could not be attributed to radiation damage. At the 12½ month period in dogs, however, all five of the animals at the high level had succumbed with typical changes.

No significant mortality was observed in either species at the lower dosage levels.

A total of nine leukemias was observed in the group of 50 rats at the 1.7 n per day level. No leukemias were observed in dogs.

Cataracts were formed in many of the rats at the 1.7 n per day level. No cataracts were observed in the dogs. No effects at the lower dosage levels.

Graying of the hair occurred in the dogs at approximately the six to seven-month period. No effect was observed at the lower dosage levels.

At the 1.7 n per day level the sperm were eliminated apparently more effectively than at the equivalent 10 r per day dosage of x-radiation. At the lower dosage levels the effects on the sperm formation were not as striking as those following x-radiation.

As in the case of the chronic x-ray experiments, weight changes were found to be of little value in predicting outcome.

B. Pathology Following Neutron Irradiation - Dr. Metcalf* The observed date suggest that after irradiation of rats over a seven-month period at 1.7 n per day, the lack of ovarian follicles may be a good criterion of neutron irradiation. Changes in the hematopoetic system are not impressive except for atrophic changes in the spleen at the 1.7 n level. The testis is damaged at the 1.7 n level but not at any other dosage used. Leukemias were diagnosed as stated earlier.

Dogs which have been given 1.7 n per day for 12½ months show in most instances changes in the hematopoetic system, testis, and skin. These changes are in the nature of hemorrhage, lymphoid hypoplasia, testicular damage and bone marrow hypoplasia as well as degenerative changes around the hair follicles. Animals exposed at lower levels show no changes typical of irradiation damage.

C. Comparative Study of Chronic X-radiation and Chronic Neutron Radiation. Summary

1. Dosage for dosage, the changes which one might associate with irradiation were more pronounced in the neutron exposed animals than in the x-irradiated animals.
2. Except for the ovarian changes in neutron exposed rats, and the possibly significant hair follicle changes in the neutron exposed dogs, the tissue changes were qualitatively the same in both x-radiation and neutron radiated animals.
3. The frequency of leukemias is related to the dosage of radiation in both the x-ray and neutron groups. It is possible that neutron irradiation may cause them to appear sooner than x-radiation.

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D. The Effect of Ch[redacted]iation on the Chemical Constituents of the Blood. Dr. Kathryn Fink et al.

The following constituents of the blood were determined at frequent intervals in the dogs exposed to chronic (daily) exposures of x-radiation at 0.1, 0.5, 1.0, and 10 r per day six days per week: serum protein, serum chloride, serum alkaline phosphatase, serum cholesterol, serum acid phosphatase, plasma fibrinogen, and whole blood non-protein-nitrogen.

Although final analysis of the data has not been made, it appears that these components of the blood are not significantly altered in animals exposed to 622 treatments of x-ray at levels of 0.1, 0.5, 1.0, and 10 r per day.

Because of similarities in the reaction of dogs to radiation and to callicrein, a non-dialyzable constitutent of urine, a study of callicrein was made in dogs and rats. No clear cut relation to radiation damage was found.

E. Attempts to Determine the Presence of a Circulating Substance Present in Blood following Irradiation. Dr. John S. Lawrence and Captain William Valentine.

The general aim of the investigations here reported has been to obtain evidence for or against the presence of certain "indirect" irradiation effects. Twenty-six successful cross circulation experiments (carotid artery to carotid artery anastamosis in cats) have been performed between normal and irradiated animals. Cross circulation was established in most instances at some specified time interval after the radiation of one partner. All intervals up to 82 hours after irradiation were covered.

In seven experiments, cross circulation was established and then one animal irradiated while the other was shielded. These were considered the most critical experiments of the group. Detailed data on a leucocyte and lymphocyte counts in the normal animal obtained during an approximately 28 day period of follow-up does not support the thesis of indirect effects peculiar to radiation. A trend toward a slightly lowered absolute lymphocyte count in normal animals after cross circulation was not considered significant. In no instance did leucopenia develop in the normal animal.

F. The Rate of Utilization of Blood Platelets in the Thrombopenic Cat. Dr. John S. Lawrence and Captain William Valentine.

The rate of utilization of blood platelets in radiated, thrombopenic cats has been measured directly. Thrombopenic animals, incapable of significant platelet regeneration were cross circulated via carotid to carotid anastomoses with normal animals. After return to independent circulation, the rate of disappearance of cross circulated platelets was measured by periodic counts.

1151844

By this method it was possible to elevate the platelet count anywhere from 100,000 to around 400,000 platelets per cubic millimeter. The highest count obtained followed cross circulation with a splenectomized animal. In most instances the platelet level attained was within the physiologic range of that found for normal cats by the same method.

The cross circulated platelets gradually disappeared from the circulation over a two to a slightly more than four-day period. Under the conditions of this experiment the entire platelet mass would have to be replaced therefore every two to five days. The same figures probably apply within a narrow limit to the normal cat.

The average rate of platelet utilization was approximately 2500 per cubic millimeter per hour. In seven of eight experiments, the rate of disappearance varied from about 1600 cubic millimeters per hour to about 2800 per cubic millimeter per hour. In the experiment using a splenectomized donor, the rate of disappearance was about double the average for the rest of the group.

Attention is called to possible therapeutic implications of these findings in the treatment of idiopathic thrombopenic purpura.

G. <u>Further Studies on the Rate of Utilization of Cross Circulated Leucocytes in the Leucopenic Cat</u>. Dr. John S. Lawrence, and Captain William Valentine.

Studies are in progress and are not yet ready to report.

EXPERIMENTS TO TEST THE VALIDITY OF THE LINEAR r-DOSE/MUTATION RATE RELATION AT LOW DOSAGES.

I. ACUTE EXPOSURE

On the basis of 200,801 test cultures for lethals in the X-chromosome of the fruit-fly-Drosophila melanogaster, of which 73,901 from flies irradiated at 25 r, 31,560 at 50 r, 23,195 at 150 r, and smaller numbers at 500 r, 1000 r, 2000 r, 3000r, and 4000 r, it has been shown that the lethal mutation rate is directly proportional to the r-dosage even for the lowest dosages used. We are forced, on the basis of these findings, to conclude that there is no tolerance dose of radiation below which mutation does not occur. Our data indicate that a dosage of about 35 r actually doubles the natural lethal mutation rate in flies. Less extensive data on visible mutations seem to show the same relationship. If these findings can be shown to have transfer value to the effect of radiation on the human race through their corroboration by experiments on the mouse, a mammal, then it becomes clear that radiation in dosages which may be tolerated by the body of man may have dire effects upon the human germ-plasm. In terms of society and the human race the risks can only be stated when a statistic on the proportion of individuals of reproductive age exposed to low dosage radiation is introduced. For the individual exposed and his descendants, the risk is obviously much greater. These facts should be carefully considered in any proposed use of atomic energy on a large scale.

II. CHRONIC EXPOSURE

Experiments were carried out to test the lethal mutation rate in the X-chromosome of Drosophila under the influence of low dosages of gamma rays spread over a considerable time. Flies were irradiated with radium for three weeks at a dosage of 2½ r. units per day. The total dose was 52½ r. units.

On the basis of 48,648 tested X-chromosomes from irradiated flies and of 52,182 tested X-chromosomes from control flies, no significant difference in the lethal mutation rate between experimentals and controls was found. These results seem to be different from those expected according to the work of Spencer. The difference may be due to differences in temperature, in aging of the sperm, between the actions of X and gamma rays, or to differences between the activity of chronic versus acute exposure at low doses.

Before it can be concluded that chronic exposure to minute doses of radiation is safe from a genetic point of view, it will be necessary to exclude the other possibilities indicated above.

1151846

REPORT OF GENETICS DIVISION

General Background

1. A human sperm contains 24 chromosomes. Each chromosome contains a "string" of some hundreds of genes.

2. A human egg contains a similar set of chromosomes and genes.

3. So in the nucleus of a fertilized egg there are 48 chromosomes altogether - 24 hinds, each in duplicate - and perhaps 10,000 kinds of genes also each in duplicate.

4. Each gene has two main types of action:

   a. Each catalyses some particular chemical process, given the proper materials.
   b. Each reduplicates itself at, or some time before, each cell division. (In this latter action the bonds between genes in the same chromosome also reduplicate.)

5. So each new call of a developing embryo is equipped with 24 pairs of chromosomes with their associated genes.

6. Some genes come into action at or shortly after fertilization. They operate in a "field" of limited complexity, i.e., the cytoplasmic differences already existing in the fertilized egg. Their activity probably increases the regional differences and permits other genes in turn to come into play.

7. In general, different batteries of genes come into action at different times, in different cells of tissues, and for varying intervals determined by when and where the materials for their operation become available. Some genes perhaps remain inactive until long after birth.

8. Most, if not all, genes are very slightly unstable. Among for example a million descendants by reduplication from a single original gene perhaps one or a few will have a somewhat different structure.

9. Once a gene has undergone such a change (mutation) it reduplicates the changes structure thereafter, except for the occurrence of further mutation.

10. Mutation probably decreases the catalytic activity of genes. So a mutant gene M' acts in the same process as the normal gene M from which it arose, but less effectively.

11. A chile who inherits a mutant M' from one parent and the normal gene M from the other parent, may or may not show effects of the mutant.

    a. If MM cells are able to do more of the specific M-catalysis than is necessary, MM' cells may be able to do enough so that the presence

of M' is not indicated by any deviation from normal structure or function. The mutant M' is said to be recessive.

b. If MM cells do just enough of the M-catalysis, MM' will not. Some embryonic process will deviate from its normal course and some detail of structure or function will become abnormal, though not necessarily harmful. A mutant of this sort is said to be dominant.

12. Radiation-induced mutants, like the spontaneous, may be either recessive or dominant.

13. In man, the potentially dangerous mutants are the dominants, and the recessives in that one pair of chromosomes which determines sex.

14. A third class of potentially dangerous inheritable changes can be induced by radiation: chromosome mutations. A piece may be broken off one chromosome and attached to another. A section may be inverted in situ. Chromosomes may exchange sections.

15. Thus, in a sperm which has been exposed to radiation any one or more of many thousands of genetic changes may occur; any of the thousands of genes may mutate and chromosomes may break and re-attach at any of the thousands of bonds between genes.

16. Since any one of these changes has a very low probability at moderate exposure, the majority of exposed sperm will not undergo any mutation. In the minority which are affected one gene will mutate in one sperm, a different gene in another sperm, in still a third cell there will be a chromosome mutation, and so on.

Experimental Procedure

1. The general plan of the work is indicated by the following table.

Pure Strains (mice) bred in Jackson Laboratory

"dba" "057" "Bagg albino" x "Swiss albino"

albino hybrids

Crosses made at Rochester

dba 0 x 4 057 00
++

(rayed daily)

F_1 oo + F_1 oo albino hybrids
++

(autopsied not bred) (bred four times, then autopsied)

"F_2" litters testing fertility, and genetic significance of any abnormalities, of the F_1 ordinarily [redacted]rded a few days after birth.

2. Gene and chromosome mutations which occurred in the sperm of exposed and control males were detected as follows:

If the sperm contained a	The effect in an offspring formed from that sperm was
dominant gene mutation	deviation from normal structure or function
recessive gene mutation in the sex chromosome (transmitted to $F_1 \varnothing\varnothing$ not $F_1 oo$)	none in F_1: but half of the sons of $F_1 \varnothing\varnothing$ are affected.
chromosome mutation (transmitted to $F_1 oo$ or $\varnothing\varnothing$: studied only in $\varnothing\varnothing$)	reduced fertility

3. In brief, the procedure was to hunt among the offspring of exposed and control male mice for

   a. Sons and daughters with some abnormality of structure or function;
   b. daughters producing definitely fewer than normal offspring in four litters;
   c. daughters producing only half as many sons as daughters (i.e., daughters receiving sex-chromosome recessive mutants with lethal effects.)

4. The procedure was complicated by two main factors:

   a. all of the effects of mutant genes or chromosomes may be duplicated by developmental and post-natal accidents quite independent of heredity;
   b. the effects of mutant genes range from large and conspicuous down t very slight and difficult to detect.

5. So the procedure involved a double sifting: first, each F_1 mouse had to be classified as normal or abnormal (with respect to the three criteria in item 3 above:) second, those classified as abnormal had to be tested to determine whether the abnormality was hereditary or not. The latter discrimination is possible because the hereditary abnormalities are <u>transmitted</u> by the individual carrying them to half of its offspring.

Results

1. The essential results are shown in Graph 1. The leftmost point shows the proportion of sperm, from control males in which there was a mutant gene or chromosome. The next point to the right shows the proportion of mutant bearing sperm from males receiving 0.1 r/day. This point is plotted above that point on the dose scale which represents the average total roentgens received by those sperm from which the offspring of 0.1 r. males had been formed. The remaining points represent corresponding data for sperm of males exposed to 0.5, 1.0 and 10.0 r/day.

2. The slope of Graph 1 is the probability per roentgen that a mutation (of the magnitude and types we studied) will occur; the value is 6.4×10^{-5} per r. Thus, of a million sperm exposed to 30 r. about 1900 undergo mutation in one or another gene or chromosome. If one of the unaffected sperm happens to be used in fertilization, no harm has been done; if one of the affected, the result will be one of the abnormalities listed above.

3. The mutation hazard indicated by Graph 1 is lower than the true value because of defects in our procedure. Abnormalities found in F_1 oo were not tested by breeding, nor were all defects in $F_{1\uparrow\uparrow}$oo, because of space and time limitations. Among the untested abnormalities should be some due to dominant gene mutations. Since any particular mutation is not likely to have occurred more than a few times in the experiment, the missed mutants should be among those aberrations which were not found more than a few times altogether. So in Graph 2, are plotted, as in Graph 1, the proportions of sperm from which were formed $F_{1\uparrow\uparrow}$oo mice showing some type of structural abnormality that appeared altogether four or fewer times among the 11,000 F_1 mice examined. The increase in proportion with increasing dose can only mean genetic effects of the radiation at a rate of about 4.2×10^{-5} per roentgen.

4. Adding the two values together gives an overall mutation hazard of 1.1×10^{-4} per r. This value still <u>excludes</u> recessives and dominants or chromosome mutations with small effects.

5. The total mutation rate is distributed roughly as follows among the several classes of mutations:

dominants with structural effects	30%
recessive lethals in sex chromosome	20%
chromosome mutations	50%

6. Two major uncertainties remain:

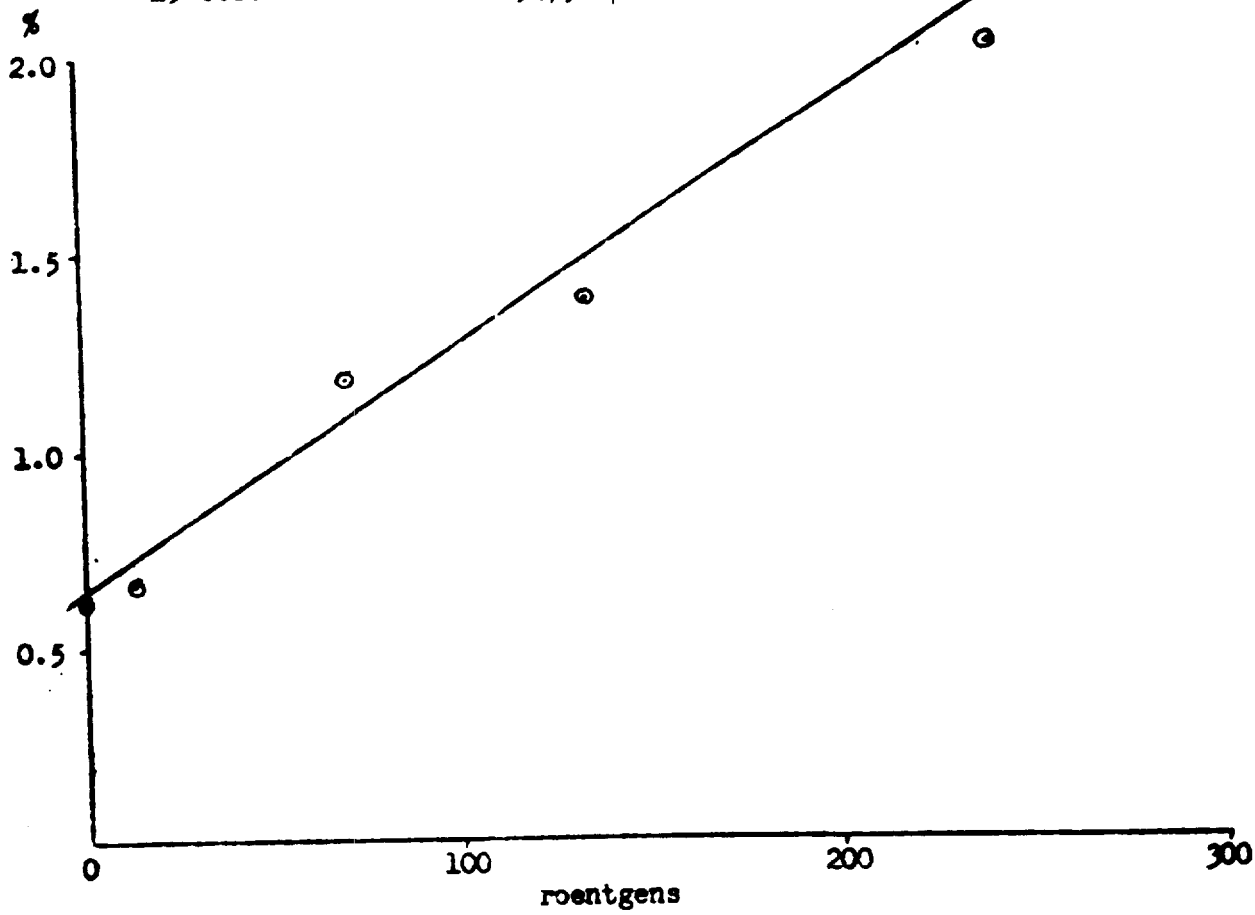
   a. Is the X-ray mutation rate in man as high as in mice?
   b. What proportion of the mutants we have found would be serious if they occurred in man?

   Obviously, neither question can be answered. With respect to the first we might better work on the conservative assumption that the mutation rate will not be lower in man than in mouse. On the second, it might be wise to take a similar attitude. Certainly, in the creature whose genetics we know best, Drosophila, nearly every mutant gene has deleterious effects. We may first notice the presence of a mutant by an alteration of some minor morphological detail, like eye color, but on further study we find that superficial change to be accompanied by a greater or less reduction of general vigor or of life span.

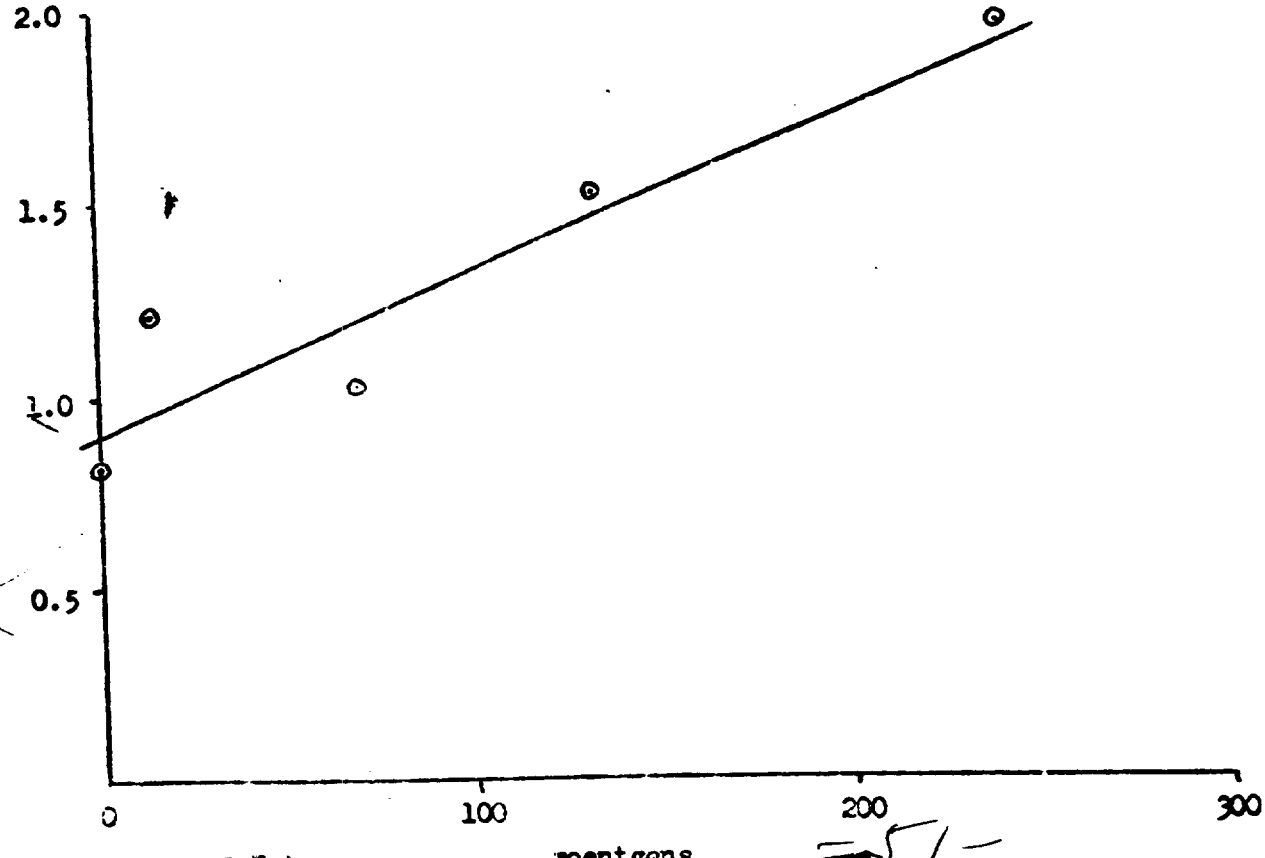
7. If we take this view, we are forced to wonder whether a human exposure of 0.1 r/day is acceptable. It means an increase of 1% in the hazard of transmitting a new mutant gene for each 100 r a parent has received at time of conception.

1151850

45 tested mutations in 5499 sperm



118 rare anomalies in 11,412 sperm



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CHICAGO - CLINTON LABORATORIES

PROGRAM

1943 - 1946

R.S. STONE, DIRECTOR

[redacted] 2 -

1151852

A. Methods of Exposure of Animals to Fast Neutrons and to X-rays. Hagen, Zirkle.

B. Use of the Clinton Pile for Radiobiological Exposures to Fast Neutrons, Slow Neutrons and Gamma Rays. Zirkle and Raper.

C. Technique of External Irradiation with Beta Rays. Raper, Zirkle, and Barnes.

D. Production of Radioactive Xenon for Animal Exposures. Abrams, Reilman and Morris.

E. Techniques of Animal Exposures to Radio[illegible] Gases. Abrams.

F. Production of Radio[illegible] Aerosols. Abrams, Rosen

G. R[illegible] Methods. [illegible]

H. [illegible] of Dosage Due to Internal Emitters. Cohn.

I. Calculations of Dosage Due to Non-uniformly Distributed Internal Emitters. Cole.

J. Photographic Measurement of Dosage Due to Internal Emitters. Cole, Moon and Spiale.

K. Measurement of Radium by Beta Counting. Tompkins and Morris.

L. Maintenance of Animals Containing Dangerous Amounts of Radioactive Substances. Prosser.

M. Special Histological Techniques. Richmond.

N. Apparatus for Injection of Animals with Dangerous Amounts of Gamma Emitters. Anthony and Morris.

O. Apparatus to Maintain Constant Rate of Injection. Snyder.

P. Methods of Insuring Localization of Injected Materials. Brues and Tompkins.

Q. Administration of Radioactive Materials by Tracheal Intubation. Seibert.

R. Quantitative Estimation of Beta Activity. Toresi.

S. Preparation of Isotopes Used in the Biological Program. Broido.

T. Analytical Methods. Broido.

U. A Modified Procedure for the Quantitative Determination of the Urinary Coproporphyrin Isomers I and III. Schwartz, Cohen and Watson.

V. The Preparation of Morphologically Intact Leucocytes from Peripheral Blood.
   I. The Use of Saponin as a Lytic Agent. Schwartz, DeGrazia, Cheney, Hasenfus and Cohen.
   II. The Combined Use of Gramicidin and Lysolecithin as Lytic Agents. Singer, Silverbach and Schwartz.

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Effects of Single Doses of X-rays on Rabbits.
1. Effects of Survival, Weight and Food Intake. Hagen and Sacher.
2. Histopathological Changes. Murray, Block, Rhoades and Heller.
3. Changes in Peripheral Blood. Jacobson.
4. Statistical Analysis of Hematological Data. Sacher.

B. Lethal Action of Single and Paired Doses of X-rays on Mice. Hagen, Simmons and Sacher.

C. Acute Histopathological Changes Due to Single and Periodic Doses of X-rays on Mice. Block, DeBruyn and Murray.

D. Effects of X-rays on Rats.
1. Lethal Action of Single, Paired and Periodic Doses. Hagen and Sacher.
2. Effects of Single and Periodic Doses on Weight. Sacher and Pearlman.
3. Acute Histopathological Effects of Single and Paired Doses. DeBruyn, Heller and Block.

E. Physiological and Clinical Effects of Single X-ray Doses on Dogs. Painter, Presser, Barron, Schwartz and Jacobson.

F. Physiological and Clinical Effects of Daily X-ray Doses on Dogs. Presser, Moore, Barron and Schwartz.

G. The Course of Mortality Among Mice, Rats and Rabbits Given Single Doses of X-rays. Sacher

H. The Course of Mortality Among Mice and Drosophila Images receiving Periodic Doses of X-rays. Sacher.

I.&J. Acute Histopathological Effects of Single Doses of X-rays on Chicks. Bloom, Heller, Rhoades and Jacobson.

K. Acute Lethal Action of X-rays on Guinea Pigs and Birds. Hagen.

L. Therapy for and Prophylaxis Against X-ray Injuries. Simmons, Presser, Sacher, Pearlman and Jacobson.

M. Effect of Age of Mice on the Median Lethal Dose of Gamma Rays. Curtis and Zirkle.

N. Effects of X-rays on Phenylhydrazine Induced Anemia in Rabbits. Jacobson, Marks and Simmons.

O. Effects of X-rays on Anemia in Rabbits Produced by Bleeding. Jacobson and Marks.

P. Effect of X-rays on the Life Span of the Reticulocyte in Vitro. Jacobson and Marks.

Q. Effects of Periodic Gamma Irradiation on Mice and Guinea Pigs. Lorenz, Heston, Eschenbrenner and Derringer.

S. Effects of Daily Eight-hour Gamma Ray Doses on Guinea Pigs. [redacted]
1. Hematological Changes. Jacobson, Sacher and Lorenz.
2. Histopathological Changes. Heller, Block and Lorenz.

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T. Effects of Daily Sub-lethal Gamma Ray Doses on Mice.
   1. Hematological Changes. Jacobson, Sacher and Lorenz.
   2. Histopathological Changes. Murray, Rhoades and Lorenz.

U. Effects of Radiation on Hemoglobin Metabolism. Schwartz, Tinsley and Katz.

V. Effects of Radiation on Porphyrin Excretion. Schwartz, Zagaria and Watson.

W. Effects of Radiation on Liver Function. Schwartz, Tinsley, Katz and Wallace.

X. Effects of Radiation on White Blood Cell Metabolism. Schwartz, Wattenberg, Cohen Singer, DeGrazia, Edwards, Flox and Boone.

Y. Effects of Radiation on the Excretion of Urorosein and Related Compounds. Schwartz, Hagedorn, and Oliver.

Z. Spectrophotometric Studies on Urine of Irradiated Animals. Schwartz, Wattenberg and Madigan.

AA. Tumor Induction and Other Late Effects of Single Doses of X-rays on Rats and Mice. Brues and Lisco.

BB. Effects of X-rays on Weights of Individual Organs. Sacher and Brues.

CC. Effects of X-rays on Enzymes and Tissue Metabolism. Barrow, et al.

DD. Physiological and Clinical Effects of X-rays on Rabbits. Prosser, Painter, Barron, Schwartz and Jacobson.

EE. Hyperheparinemia. Allen.

FF. Effect of Radiation on Lung Tumor Incidence in CF 1 Mice, by P. S. Henshaw, E. F. Riley, G. E. Stapleton, and M. B. Cupp.

GG. An Attempt to Detect Small Amounts of Irradiation Injury, by Cupp and Henshaw.

II-A. The Effect of Single Doses of X-radiation on Rabbits. by Hagen and Sacher.

Rabbits were exposed to 200 kVP x-rays in single doses of 500, 600, 700, 800, 900, 1000, 1100, and 1500r. The LD_{50} at the 30-day period was found to be approximately 800r. Analysis of the over-all mortality indicated that during the first three days, 15 per cent of the animals succumbed, while the remainder died between the 4th and 20th day.

Weight of the animals appeared to be an important factor in that regardless of sex, animals of 2 kG or over were definitely less sensitive with respect to both 1-day and 10-day killings.

Males appeared to be more sensitive than females during the first three days. During the next 17 days (up to 20 days total time) the females tended to die faster so that at the end of this period there was little difference in the mortality of the sexes.

The mean survival time decreased slightly as dosages from 700 to 900 were given. Between 900 and 1100 r, the shortening of the life of the animals with the increased dose of radiation was significant. Heavy animals tended to survive longer than light animals.

Some of the animals which had survived a previous single dose of x-radiation were given a second treatment of 800 r, 100 days after the first was given. Results of this study indicated that neither resistance or sensitivity were induced by the primary dose of x-radiation.

Histopathological studies are in preparation.

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A. Changes in the Peripheral Blood by Jacobson.

Animals receiving an exposure of 600r (less often below) develop a normochromic anemia at the 14-day period. Reticulocytes are reduced by dosages as low as 100 r. Lymphocytes are sensitive to 25r and with doses of 100 r and above, the blood levels reach their minimum between a 24 and 48 hour period. Recovery of a normal circulating lymphocyte level required more than sixty days in doses of 300 r and higher. Heterophyl values observed at the 24-hour period were markedly elevated. This was followed by a depression reaching a maximum level in 96 hours. There is a tendency toward an abortive rise in both lymphocyte and heterophyl levels between the 4th and 9th days. The platelets show minimum levels between the 5th and 7th days at dosages of 500 r and above.

Statistical analyses of the hematological data are not yet completed (Sacher).

B. Lethal Action of Single and Paired Doses of X-radiation on Mice by Scaher and Hagen

In single doses, the LD50 for 30 days for CF-1 female mice is approximately 515 r. ABC mice which were exposed before April, 1943 had an LD 50 of 450 r. However, mice of the same strain received later than the above date showed an LD 50 of 500 r without change in technique. This would indicate that there is some variation even in a certain strain of animals. No sex differences were noted in the ABC mice.

Following a paired dose of x-radiation in which the second dosage of radiation was administered after a predetermined recovery or

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conditioning period had been allowed, there was some evidence that mice did develop an increased resistance to x-radiation occurring approximately one week after the first exposure. The half time for recovery from the first dose is approximately two to three days.

C. Acute Histopathological Changes Due to Single and Periodic Doses of X-radiation on Mice. By Bloom, DeBruyn, and Murray.

The data on the pathology of single and periodic dosages of x-radiation is not completed. A complete account of the histopathological changes observed is given.

D. The Effect of X-radiation on Rats Following Single Doses of X-radiation, by Sacher, Hagen, and Fearman.

The median lethal dose (LD 50) for Sprague-Dawley rats at 200 gm. weight was found to be 600 r. The mean survival time ranged from 11.3 days at 500 r; 4 days at 1500r; and 3 days at 25,000 r. No conclusive evidence was obtained that weight, sex, age, or stage of estrus had any influence on the survival or mortality in this study.

In the studies on paired doses of x-radiation, male animals received an initial dose of 300 r of radiation followed by a determination of the median lethal dose (LD 50) at one, two, and three weeks after the first dose. A plotting of the results showed that the residual effects after the first dose decreased exponentially at a rate of 8.2 per cent per day. This rate was not maintained beyond the three week period.

In the experiment on periodic doses of x- radiation, animals were given daily doses of 12.5, 25, 50, 100 (two doses), 150 (two doses),

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200 (two doses), and 500 r. At doses above 50 r per day, the mean survival time fell within that range observed after single doses, and could be explained on the basis of the recovery studies carried out with the paired dose studies. At doses below the 50 r per day level, the mean survival time was considerably extended.

Weight studies are not yet analyzed for this report.

E. Physiological and Clinical Effects of Single X-ray Doses on Dogs, by Prosser, Painter, Barron, Schwartz, and Jacobson.

Dogs were given graded doses of 200 kVP x-rays ranging from 20 r to 800 r over the total body. The 200 to 800 r doses killed all animals in three to twenty-one days; the 300 to 350 r killed seven of eleven animals in 14 to 20 days while all animals receiving 20 to 250 r are living six months after the dose was given. From the above information the median lethal dose (LD 50) is probably around 300 r. The animals survive shorter times after the doses of 500 to 800 r than they do after doses of 300 to 400 r.

Physiological and clinical observations were made on the heart rate, temperature, blood pressure, hematology, blood and plasma volumes, weight, food and water exchange, nitrogen exchange, kidney function, plasma protein changes, histamine changes, electrocardiographic changes, gross clinical findings and pathology. A few pertinent comments only can be made of the findings in this report.

In general there is a terminal rise in the heart rate, temperature and a fall in blood pressure as a shock-like state develops. (This should be distinguished from the primary shock that develops in certain species exposed to x-radiation). The blood volume shows a fall

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in red cell elements with an increase in plasma volume. Hematological changes are typical of those of lethal x-radiation in other species. Histamine studies are equivocal and impossible to interpret. Weight changes and metabolism variations are not completely interpreted at this time but indicate a loss in weight slightly greater than in the fasting animal with the development of a negative nitrogen balance. There is a decrease in the amount of metabolites cleared by the kidney. Electrophoretic patterns of the plasma proteins show a fall in the albumin values and a rise in the beta globulin fraction.

This paper will be completed in about one and one-half months.

F. <u>The Clinical Physiology of Dogs Given Daily Total Body X-radiation</u>, by Prosser, Moore, Barron, and Schwartz.

Dogs were exposed to 12.5 r, 25 r, 40 r, and 50 r daily. The dogs which received 40 r and 50 r daily died in about the same time as animals receiving a single lethal dose. At the 50 r level (average total of 917 r received) the survival was 18 to 20 days. At the 40 r level (average total of 920 r received) the animals died in 24 to 32 days. At the 25 r level (average total of 928 r received), the animals died in 27 to 43 days, and at the 12.5 r level (average total of 1278 r) the survival time was from 73 to 209 days. All animals except the 215 r daily ones died acute deaths with leucopenia, lymphopenia, and thrombocytopenia typical of acute x-radiation death from single lethal doses. At the 12.5 r level (plus one animal at 25r) death was due to the development of an aplastic anemia (the red marrow also being involved). Young animals were more susceptible than mature animals.

The hematological findings showed an initial rapid decrease of the white blood cells with the heterophils following this general pattern.

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This was followed by a slow decrease with a sudden drop shortly before death, The lymphocytes showed early reduction followed by a slow fall. The red blood cells showed a slow decline in the first few weeks and a more rapid fall afterwards in all but those animals exposed to 12.5 r. In the latter the decline appeared after two months of exposure. The reticulocytes showed an initial reduction followed by a return to normal values. A fall was noted again several weeks before death. Clotting times increased immediately before death. Sedimentation rates were increased immediately before the onset of the terminal period.

The blood volume determinations except for the 12.5 r group showed a decrease in the total red cell volume. The plasma volume except the 12.5 r remained constant. The 12.5 r animals showed no change for about four months after which the above described changes were noted.

Weight changes were similar to the acute experiments in doses greater than 25 r daily. In the 25 r and 12.5 r groups, the weight changes occurred after approximately 65 to 80 per cent of the dose had been accumulated.

Complete studies as outlined in the preceeding report on the physiological and clinical effects of single x-ray doses were carried out, but aside from the above account are not yet sufficiently completed for analysis.

I & J. <u>The Acute Effects of 200 KVP X-ray Treatment on Chickens,</u> by Bloom, Heller, [illegible].

Chickens were treated with single doses of whole body x-radiation in doses ranging from 2 r to 1200 r. The median lethal dose

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(LD 50) appeared to be between 400 and 800 r. Histopathological changes in the 11th week chicken paralleled those reported for comparable maturity with the exception of damage to the enmuilenna sheath cells in the chickens. The three week chicken suffered additional damage to immature structures, but the threshold of observable damage to the lymphatic tissues (25 r) was comparable to that reported for mammals.

By application of a slight modification of Meyes' method for peripheral blood counting of fowl, dramatic decreases in blood constituents, paralleling those reported elsewhere for mammals, were seen in three week and five week chickens after treatment with 600 r and 800 r respectively. Numerous degenerating cells were observed in dry smears made during the first weeks after irradiation, in contrast to the scarcity of such findings in smears of mammalian blood at the same period. Although the normal values for leukocytes of chicken blood were found to be several times those of mammalian blood, the difference could account only in part for the greater degeneration observed in the chickens.

K. <u>Acute Lethal Action of X-rays on Guinea Pigs and Birds,</u> by Hagen.

Acute lethal dose studies were carried out on guinea pigs and chickens with the special purpose of establishing the relative sensitivity of species.

The median lethal dose (LD 50) for guinea pigs was found to be between the 150 r and 200 r levels for single doses of whole body radiation. This established the guinea pig as a very radiation sensitive species.

Similar studies carried out on six week chickens showed a very large mortality during the first 24 hours. The median lethal dose was not calculated due to many interfering factors.

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L. Therapy for and Prophylaxis against X-ray Injuries, by Simmons, Fresser, Sacher, Herman and Jacobson.

In a search for some substances to use in therapy for and prophylaxis against acute x-ray injuries, studies were carried out on a number of substances which might be effective. Whole body x-radiation in doses of 600 r was given to animals (killing dosage). Various compounds were injected both prophylactically and therapeutically to determine what if any effect was obtained as evidenced by changes in the circulating leucocyte level of the blood.

The following compounds were tested: pentnucleotide, ascorbic acid, ribo-nucleic acid, crude liver extract, yellow bone marrow extract, phenol compounds (acetyl-methyl-amine and 2 chloro-5 hydroxy-toluene), BAL (British Anti-Lewisite) and ordinary normal saline. These compounds were all injected into the animals.

The lymphocyte and heterophyl counts were followed as indices as to the extent of the injury, and the value of any beneficial effect of the injected substance. None of the treatments prevented a decrease in these blood counts after irradiation. Normal saline injected in adequate quantities gave the most beneficial effect with a longer survival time, and less depression of the circulating white cell count.

M. The Effect of Age on the Radiosensitivity of Mice, by Zirkle, Anderson, Riley, and Curtis.

Mice of different ages ranging from 1.5 to 12 months have been exposed to gamma rays in an effort to determine the change in radiosensitivity with age. The dose necessary to cause 50 per cent killing was determined for each age group and sex. No change in sensitivity with age was observed with either sex although the experimental error in the case of the males was quite large. The females were found to be more resistant than the males. F. [redacted]

N. Effects of Total Body X-radiation on a Pre-existing Induced Anemia in Rabbits, by Jacobson, Marks and Simons.

1. The Response of Animals with a Phenyl Hydrazine Induced Anemia; The hematological effects of 800 r total body x-radiation on anemias produced by phenyl hydrazine administration were compared to the effects on normal untreated animals, on animals given phenyl hydrazine and allowed to recover spontaneously and on a group of animals which were given 800 r alone.

Animals receiving x-radiation alone developed an anemia maximum at 14 days with recovery at 23 days. Phenyl hydrazine produced anemias radiated at or near the point of maximal anemia developed no further anemia, and rapidly returned to normal. The phenyl hydrazine alone returned to normal somewhat more rapidly than either of the above groups.

Reticulocyte values after a high elevation returned to normal in two days in phenyl hydrazine treated animals. After x-ray and phenyl hydrazine they fell maximally in two days and returned to normal in nine days, and in controls with 800 r they fell maximally in two days and recovered in 23 days.

6. The Response of Animals with Bleeding Induced Anemia. A regenerative anemia was produced by the withdrawal of 30 cc. of blood by cardiac puncture daily for three days. A similar study to the above was carried out in these animals.

The findings in this study are similar to the above and indicate that x-ray superimposed on an anemic state does not produced further anemia with some differences due to the induced iron deficiency. The reticulocyte counts remained elevated for a period longer than with the phenyl hydrazine induced anemias.

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R.S.2 T. The Effects of Periodic Ga[redacted]tion in Mice, Guinea Pigs, and Rabbits, by Lorenz, Eschenbrenner, Heston, Berringer, Jacobson, Bacher, Bloom, Rhoades, and Murray.

Mice, guinea pigs and rabbits were given daily exposures over an eight-hour period to gamma rays from radium needles. The dosages received were 8.8 r, 4.4 r, 2.2 r, 1.1 r, and 0.11 r. Another group received 5.5 r given in a single hourly dosage to animals on a chronic daily exposure to 0.11 r. Additional animals received chronic exposures over the entire 24-hour day in doses of 8.8 r, 4.4 r, 2.2 r, 1.1 r, 0.55 r, and 0.11 r per day.

Conclusions which can be drawn at the present stage of completion of the experiment indicate that rabbits are comparatively sensitive to radiation. Guinea pigs on the other hand are extremely radio-sensitive rapidly developing a terminal state of aplastic anemia. No tumors or leukemias were observed in guinea pigs.

Mice are radio-insensitive as far as the blood picture is concerned but are radio-sensitive with respect to the development of lymphomas, ovarian tumors, and perhaps other forms of tumors. The development of lymphomas is directly related to the total dose received by the animal. The development of ovarian tumors at a relatively old age depends on the rate of administration of the radiation as well as the total amount received. The ovaries of mice are more radio-sensitive than those of guinea pigs, but the opposite is true for the testis.

All three species, the rabbit, guinea pig, and mouse, show widely different reactions to irradiation.

[redacted] -65-

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1. Breeding Experiments.

Using strain C3H mice (low tumor strain) tests for sterility, development of dominant mutations (particularly lethals) and development of genetic changes in progeny were carried out in the above study.

Males at 8.8 r were sterile after 1760 r had been received, but regained their fertility in approximately 2½ months. At 1100 r and 880 r a definite reduction in the size of the first litters was noted. No effect was noted at 660 r. When the 4.4 r and 2.2 r per day dosages were studies at the 1100 r period, no effect was noted as described in the 8.8 r per day experiment.

Females receiving 8.8 r per day showed definite sterility effects after receiving 770 r. These were more pronounced at the 880 r level. Reduction in litter size was noted at 550 r (8.8 r per day). Animals receiving 4.4 r per day also showed reduced litter size at the 550 r total dose. The 2.2 r and lower doses were normal at the 550 r total dose period.

The effect of the 8-hour exposure was slightly more severe than that of the 24-hour exposure. Females at 5.5 r per one hour showed sterility effects at the 770 r total dose. A single experiment using 300 r total body x-radiation in a single dose produced one litter of reduced size followed by complete sterility.

In the above studies, the female animals did not recover their fertility while the males regained approximately normal function.

No age effects were noted in these experiments.

At the time of report, no evidences of any genetic effects or changes had been found.

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[redacted]

Hematological studies carried out on these animals showed that in the guinea pig the platelet counts were the most sensitive index of irradiation. There was no change in other circulating elements in the guinea pig until immediately before death. In rats and mice no characteristic changes were found in the blood picture.

U. The Hemolytic Effect of Radiation, by Schwartz, Tinsley, and Fitz. The anemias following both acute and chronic irradiation are generally said to be due to the inhibition of erythropoesis. This is only a partial truth. A hemolytic component is often the major cause of the anemia. This is evidenced by the following.

Bile-renal fistula dogs show a marked increase in bilirubin excretion following total body irradiation. Unoperated dogs show increase fecal urobilinogen and urine bilirubin excretion following irradiation. Several humans given P^{32} showed an actual reticulocytosis during the period of rapid hemoglobin and red blood cell decrease. The hemoglobin fall is too rapid to be accounted for only by inhibition of red cell formation. When the assumption is made that the average red blood cell has a life of 100 days, a complete cessation of erythropoesis should result in a drop of approximately 1 per cent of the hemoglobin daily. Actually a two to seven per cent fall is noted during the most rapid period.

V. The Effect of Radiation on Coproporphyrin Excretion by Watson, Zagaria, and Schwartz.

The effect of external irradiation given in either single (300-2000 r) or multiple doses, and of internal irradiation has been studied in several dozen dogs. A consistent pattern of total porphyrin excretion has been found as follows.

[redacted]

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A marked depression of urine copro-excretion occurs rapidly. For several days before death, however, values rise and generally are several hundred per cent above control values by the day of death.

Adequate isomer studies are not yet available to permit a statement regarding the possible mechanism of the early decline. The late increase in copro excretion is undoubtedly associated with liver dysfunction.

W. The Effects of Radiation on Liver Function, by Schwartz, Porter Tinsley, and Wallace.

Liver function has been studied in humans by means of the urine urobilinogen excretion and the serum cephalin cholesterol floculation, colloidal gold, and thymol turbidity tests. In animals only the urine urobilinogen test has been found to be applicable.

There is a marked increase in the urine urobilinogen excretion during the last several days before death of animals given total body x-ray. Plutonium produces definite liver dysfunction as indicated by this test. The various metals especially beryllium and lead produce liver dysfunction of varying degree.

X. The Effects of Radiation on White Blood Cell Metabolism, by Schwartz, Cheney, Cohen, DeGrazia, Flox, Krizek, and Wattenberg.

These studies were planned especially for the investigation of the nucleic acids and their derivatives and associated compounds. White cells were isolated by the saponin method. They were broken down into various parts, analyses being made on the total sample, the trichloracetic acid soluble fraction, the alcohol soluble fraction and the residual sample. These analyses were for phosphorus and its combinations,

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ribose, desoxyribose and the ultraviolet absorption spectrum (Beckman).

Data has been collected in human, dog, and rabbits subjects. Certain changes have been observed to date.

A patient receiving total body x-ray and who died a few months later of a metastasizing parotid tumor showed a prolonged increase of several hundred per cent in the "adenosine triphosphate" phosphorus. This change preceded the drop in white blood cell count.

At approximately the time that the white blood count starts to fall a consistent increase in the ultraviolet absorption in the alcohol soluble fraction has been noted. This is greater than that of the phosphorus, ribose or desoxyribose increases. The identity of the substance or substances responsible for this relative increase is unknown.

Y. <u>Radiation-Induced Changes in Urine Ultraviolet Absorption Spectra</u> by Hatterberg and Schwartz.

Two general changes are seen in the urine ultraviolet absorption spectrum following subacute or chronic irradiation of dogs. These are: (1) increased absorption in the regions of 235 and 310 mu, and is due to hynurenic acid, a tryptophane derivative; and (2) increased absorption at 200 mu. due to uric acid.

A method is described for the quantitation of hynurenic acid by means of ultraviolet spectrophotometry. The quantitative increase in hynruenic acid excretion is thus measured.

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The possible significance of this phenomenon is discussed, for example, a correlation with urorosein, a tryptophane derivative obtained from human urine.

Z. The Effect of Irradiation on the Excretion of Allantoin and Uric Acid by Dogs and Rabbits, by Krizek, Singer, Silberbach, and Schwartz.

Preliminary results indicate that there is an increase in the uricolyte index (the ratio of uric acid to allantoin) indicating a relative inability of the animal to destroy uric acid. The possible inhibition of uricase after radiation is being investigated.

AA. Tumor Induction and Other Late Effects of Single Doses of X-rays on Rats and Mice, by Brues and Lisco.

Animals have been collected from other parts of the x-radiation program and are being held in order to study the possible development of tumors from varying amounts of 200 KVP x-rays. These animals are being studied primarily for tumor incidence.

The experiment is still in progress and results will not be available until most of the animals have either developed tumors or have died off.

BB. The Effects of Total Body X-ray on the Weights of Organs in the Rat, by Sacher and Brues.

Weights and water content of organs were investigated in a group of growing male rats which had received 1498 r at a 13.82 r per day average. The weights of the majority of the visceral organs were affected less than body weight as a whole, weights of muscle and femur were affetted in the same ratio as body weights, and spleen, thymus, gonads and gonadal fat were reduced out of proportion to body weight. Adrenal weights were normal. Water content per gram of tissue in muscle and bone of irradiated animals was

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4.3 per cent greater than in the controls. Thus the retardation in growth is similar to that seen in inanition except for the relatively greater effect on organs known to be radiosensitive.

Organ weights and total body weight of rats which had received single doses of x-ray up to 100 r several months previously showed no deviation from the normal.

CC. <u>The Effects of X-rays on Enzymes and Tissue Metabolism by</u> Barron, et al. To cause any effect on enzyme systems it requires a very large amount of x-radiation. This is in excess of 2000 r. Enzyme systems are not affected as are tissues because of the lack of many sulfhydryl groups (-SH) in their structure. Radiation produces oxidations which in turn act upon the SH groups. When an enzyme containing SH groups is treated with 100 r of x-ray (2000 VF), the enzyme is inhibited. Addition of glutathione, however, containing excess SH groups reactivates the enzyme. An effect (in vitro) using irradiation with doses as small as 1 r can be noted.

X-radiation likewise inhibits the SH groups in protein. If the dosage of irradiation is low, the above inhibition-reactivation phenomenon is noted. If the dosage is large, a denaturation of the protein of irreversible nature is noted.

Tissue studies show that doses as small as 25 r will cause changes in the respiration of liver, kidney, and intestine. Such tissues show no pathological change. This effect is noted in the intestine in as little as three hours after exposure. The tissue enzymes containing SH groups were most affected both in vivo and in vitro. Animals irradiated and killed at varying intervals after the exposure continued to show rather marked effects in those tissues rich in enzymes with SH groups.

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EE. Hyperheparinemia- A New Hemorrhagic Disease Caused by Overexposure to Ionizing Radiations, by Allen, Sanderson, and Jacobson.

A characteristic hemorrhagic disorder accompanies heavy exposure of the entire body of dogs to x-rays. This is a hemorrhagic disease whose nature has not been previously described. It is characterized by a prolonged clotting time, and has been observed in the guinea pig, the rabbit, the rat, the goat and the dog. It was also reported in some of the survivors of the Hiroshima and Nagaski bombings.

This hemorrhagic disease is due to an increase in the amount of circulating heparin or a heparin-like substance. While the chemical composition of heparin is not known, the active principle responsible for the clotting abnormality behaves like heparin both biologically, and chemically.

This abnormal bleeding can be controlled or prevented by the administration of toluidine blue.

The plasma prothrombin and fibrinogen are not significantly altered. The serum calcium may fall but this change does not produce the hemorrhagic state.

The profound thrombocytopenia associated with this disease appears to play little if any role in the bleeding tendency.

FF. Effect of Radiation on Lung Tumor Incidence in CF 1 Mice, by F. S. Henshaw, E. F. Riley, C. E. Stapleton, and L. D. Cupp.

By utilizing certain over-aged stock animals, some specific controls that were no longer needed, and some survivors of certain LD 50 studies, a fair amount of preliminary information has become available on the carcinogenic action of cadium gamma rays on lung

tissue in CF 1 mice. The lungs of 433 controls and 309 irradiated animals have been examined.

The doses of radiation were 525, 675, and 850 r applied as single exposures when the animals were three and one-half months of age.

All factors considered (age, sex, dose, etc.) essentially no difference has been found in tumor incidence, the number of tumors per animal, and the size or behavior of the tumors when the irradiated and non-irradiated animals are compared. For the conditions used, no significant evidence has been obtained indicating that the treatments given were carcinogenic for lung tissues.

The following statements apply for both irradiated and non-irradiated animals: (1) lung tumors begin to appear in CF 1 mice at three to four months of age, (2) the number of tumors per animal did not increase after six months of age, (3) while the number of tumors in a particular animal was as high as eleven, the average number per animal was between one and two, (4) the average number per animal of those that bore tumors was between two and three, and (5) the average incidence of animals with lung tumors, irrespective of sex or treatment was approximately 60 per cent.

GG. <u>An attempt to detect small amounts of irradiation injury</u>, by Cupp, and Henshaw.

A study of both normal and heavily irradiated blood serum failed to demonstrate any evidence of a decomposition product which caused the destruction of either normal or abnormal leucocytes in normal or leukemic animals.

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CATEGORY 3: COMPARATIVE EFFECTS OF [redacted] AND X OR GAMMA RAYS

C* A. Additivity of Fission Neutrons and Gamma Rays in Their Acute Lethal Action on Lice. Zirkle, Raper, Riley and Stapleton.

B. Comparative Action of Cyclotron Neutrons and X-rays.
1. Lethal Action on Lice and Rabbits. Hagen.
2. Hematological Effects on Rabbits. Jacobson, Zirkle and Hagen.
3. Statistical Analysis of Blood Data. Pearlman.

C. Acute Comparative Histopathology in Lice Due to Fission Neutrons and Gamma Rays. Snider.

D. Acute Comparative Histopathology in Rabbits Due to X-rays and Fission Neutrons. Snider.

C* E. Comparative Lethal Action of X-rays and Cyclotron Neutrons on Drosophila Eggs. Zirkle and Harrish.

C* F. Comparative Effectiveness and Additivity of Fission Neutrons and of Gamma Rays. Henshaw, Snider, Riley, Stapleton and Zirkle.

C* G. Comparative Late Effects of Periodic Doses of Fission Neutrons and of Gamma Rays. Henshaw, Snider, Riley, Stapleton, and Zirkle.

C* H. Comparative Delayed Effects of Single Doses of Fission Neutrons and of Gamma Rays. Henshaw, Snider, Riley and Stapleton.

C* I. Effects of Fast Neutrons on the Ability of Mice to Take Forced Exercise. Curtis and Stapleton.

C* Indicated that the work was carried out at the Clinton Laboratories and can be found in that list of reports.

[redacted]

1151874

CATEGORY 4: EFFE[redacted]EUTRONS

A. Acute Lethal Action of Slow Neutrons on Mice. Simmons, Riley, Stapleton.

C* B. Radioactivities Induced in Mammalian Tissues by Slow Neutrons. Curtis and Teresi.

C* C. Absorption and Reflection of Slow Neutrons by Tissues. Curtis and Cheka.

D. Acute Histopathological Effects of Single Doses of Slow Neutrons on Mice. Snider.

C* E. Delayed Effects of Single Doses of Slow Neutrons on Mice. Henshaw, Snider, Riley and Stapleton.

C* F. Effects of Periodic Doses of Slow Neutrons on Mice. Henshaw, Snider, Riley and Stapleton.

C* Indicated that the work was carried out at the Clinton Laboratories and can be found in that list of reports.

[redacted]

1151875

.11 to 17. Chronic studies with fast neutrons, slow neutrons and gamma rays. By: Henshaw.

MA 11-17 contains information indexed as Category F,G,H, Category A, MA 1 is listed under title Vitality.

Using penetrating radiations given in a single dose or in repeated chronic doses, experiments were designed with the following objectives in mind; (1) to determine the nature of the late effects (shortening of life, cataracts, tumor induction, hematological and histological changes etc.); (2) the threshold levels of these late effects from chronic exposure; and the (3) relative effectiveness of slow neutrons, fast neutrons and gamma rays in producing these late effects.

On chronic exposure to all types, an appearance of early aging of the animals was noted. An increase in leukemia and lymphomas over those appearing in the control animals was noted. The period of appearance of these leukemias and lymphomas was approximately the same time as the controls. No hematological changes were observed except in acute or heavy daily exposure.

The ratio of r (roentgens) to n (neutrons) for the acute single exposure was shown to be 7 to 8 in favor of the neutrons. For chronic exposures the biological effectiveness of the neutrons was from two to four times this figure.

The level of chronic neutron irradiation was shown to be approximately 1.15 n per day with the production of shortening of life, slight less increase in tumor incidence. (1.6 r. per day of gamma radiation produces only borderline changes in these categories.)

Using a high lung tumor strain (strain A) of mice, a series of animals were irradiated until 20% of the control group showed the presence of lung tumors. Examination of the irradiated animals showed no increase in the

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number of lung tumors. Another [illegible] of [illegible]

[illegible] urethane with resultant high increase in tumor production.

When a high leukemic strain (C58) of mice was used, the treated animals all died of the radiation by the time that spontaneous leukemia had developed in the controls.

1151877

3 A. Additivity of Fast Neutrons and Gamma Rays in Their Acute Lethal Action on Mice. by: R. E. Zirkle, J. R. Haper, E. F. Riley and G. E. Stapleton.

By exposing various groups of mice to graded doses, the median lethal dose of each of the following radiations was determined: (a) fast neutrons from the pile; (b) radiation G consisting of gamma rays contaminated with fast neutrons; (c) radiation F, a mixture of fast neutrons and radiation G. The LD50 of radiation G. Theoretically, the sum of these two fractions should be unity if fast neutrons and radiation F are radiobiological additive. Since the experiment deviation from unity (7 percent) is within the error of the measurements, is concluded that complete additivity of these two radiations has been demonstrated. It is assumed that this conclusion concerning additivity of fast neutrons and radiation G may be extended to additivity of fast neutrons and pure gamma rays.

The LD50 of fast neutrons, directly determined, was 91 n. The LD50 of pure gamma rays, indirectly determined from the experimental data, was 812 r. In another experiment, performed later for a different purpose a corresponding value of 840 r was obtained.

I. BX12-20. Vitality By: Henshaw.

An experiment on a method to determine whether any evidence of the effect of penetrating radiation can be detected and measured in the terms of fatigue using an exercise wheel and stimulating apparatus to prevent rest.

Results of this study do not allow interpretation at this time.

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Category 5: Effects of External Beta Irradiation

A. Gross Effects of Beta Irradiation of Restricted Surface of Rabbits.
Raper, Wirth, Barnes

B. Histopathological Effects of Beta Irradiation of Restricted Surface of Rabbits. By: Snider and Raper

C. Comparative Acute Lethal Action of Total Surface Beta Irradiation.
Raper, Wirth and Barnes

D. Gross Effects produced by Total Surface Beta Irradiation.
Raper and Barnes

E. Rate of Recovery from Total Surface Beta Irradiation.
Raper and Barnes

F. Additivity of External Beta and Gamma Irradiation
Raper, Barnes

G. Influence of Total Surface Beta Irradiation on Gross Metabolic Pattern of Rats. By: Anderson, Barnes

H. Acute Histopathological Effects of Single Doses of Total Surface Beta Irradiation on Mice. By: Snider and Raper

I. Changes in Peripheral Blood after Single Doses of External Beta Radiation. By: Raper and Barnes

J. Reactions of Human Skin to Single Doses of Beta Rays.
Wirth and Raper

K. Delayed Effects of Single Exposures to Total Surface Beta Irradiation
Raper, Henshaw and Snider

L. Effects of Periodic Total Surface Beta Irradiation.
Raper, Henshaw and Snider

M. Effects of External Irradiation with Beta Rays on the Peripheral Blood of Rabbits. By: Raper and Barnes

1151879

A. BA12-3 Gross Effects of [redacted] Irradiation on Restricted Surface of Rabbits. By: Hirth, Reiser and Barnes

The exposure to beta rays in doses from 1000 to 30,000 rep to restricted areas of rabbit skin and followed for nine months gives the following information. The reaction pattern is similar in doses from 5000 to 30,000 r. Main effects in their order of appearance were (1) erythema, (2) Pigmentation, (3) epilation, (4) Loss of outer layer of skin through one of three processes: (a) simultaneous separation of the superficial layers over the treated area, (b) dry desquamation and (c) wet desquamation; (5) crust formation, (6) healing and (7) regrowth of hair.

Late ulceration following healing was observed in a few animals.

The severity of the skin response varies with the dose but not strictly proportional with the dose. The time of appearance varied with the function of dose, appearing earlier at high dose levels.

The damage to the skin produced by massive doses of beta rays resembled thermal burns except for (1) various stages of beta burns required four to seven times as long to develop; (2) direct action of beta rays was confined to superficial tissues only.

The effects in general resemble the effects of other ionizing radiation such as x-rays, gamma rays and grenz rays (particularly the latter). The effects on human skin are thought to differ only little from those observed in the rabbit.

C. BA12-1 Comparative Lethal Effects of External Beta Irradiation J. R. Reiser, R. E. Zirkle, and R. K. Barnes. Oct. 1944 to Feb. 1946.

The acute lethal effects of total surface beta irradiation have been investigated in a series of laboratory animals ranging in size from 7 to 2000 gms. The median lethal dose, the LD_{50}, of external beta rays varied from 2200 roentgen equivalents physical in the smallest animal of the series to

17,500 rep in the largest animal.

Gamma ray survival studies with the same strains of animals demonstrated LD50's ranging from 510 r. to 1270 r, the sensitivity showing no correlation with size.

A comparison of the total energy absorbed in rep grams as beta rays and gamma rays, to produce 50% killing gave total beta total gamma ratios for the several animals of the series as follows: baby rats (one day old), 2.01; mice, 1.75; rats, 0.64; guinea pigs, 0.24; and rabbits, 0.92.

The relation between absorbed energy required to kill 50% of the individuals of each species and animal size may be expressed in the following equation: $t^E = KW$ where t^E is the total energy absorbed in rep grams, W, the weight of the animal and K a constant.

The small variation in the observed values of K for the different animals indicates negligible sensitivity difference.

D. F.A. BAL-1 Gross Effects of Total Surface Beta-Irradiation
By: Roper and Barnes Same Dates

Total surface irradiation of mice, rats and rabbits with beta ray results inconspicuous and characteristic gross damage. Within wide limits of dosage the pattern of effects varies little, while the severity of damage varies roughly with dose. There are only minor differences in the gross damage observed in the different animals.

The outstanding features of the gross response to beta irradiation, in the sequence in which they appear are as follows: inflammation of the eyelids and erythema on the ears and feet; closure of the eyes; epilation and desquamation around the eyes, mouth and nostrils; hyperemia and swelling of ear tips except in rabbit; general epilation over body; desquamation over extensive areas; resulting in severe ulceration; healing and limited regrowth of hair. Two late effects are frequently observed; late ulceration, involving the breakdown of previously healed skin, and tumor formation.

High doses of external beta rays profoundly affect the weight of the several animals.

The survival time of mice is significantly shortened by doses of beta rays 3000 rep or more. Two mechanisms are indicated as responsible for lethal action at high and at low dose levels.

E. BX12-4 Rate of Recovery from Total Surface Beta Irradiation.
By: Raper, J.R. and Barnes, K.K. Oct. 1944 to Feb. 1946

The recovery of mice from the effects of total surface irradiation with beta rays has been investigated for a period of 16 weeks following a single exposure.

A split dose technique was employed. A conditioning dose was followed at various intervals by graded test doses to determine the recovery of the animals from the effects of the conditioning exposure.

The total dose required in two exposures to produce 50% mortality increased rapidly as the interval between exposures was lengthened. Recovery proceeded very rapidly at first, 1.5 to 2 days and 8 days being required for half and complete recovery respectively. During the period 8 days to 16 weeks the recovery values exceed 100% i.e. the conditioning dose imparted a slight immunity against additional exposure.

F. P.M. BX12-5 Additivity of Lethal Effects of External Beta and Gamma Irradiation. By: Raper, J.R. and Barnes K.K. Oct. 1944 to Feb. 1946

Analysis of the survival of mice following added doses of external beta and gamma rays demonstrate that the acute lethal effects of the two radiations are not completely additive. Four series of experiments were performed; the results of two series indicate partial additivity of effects while the other two series indicate complete independence of action of the two radiations.

BA12-9 Water Metabolism By: [redacted]

This experiment was an attempt to determine whether the increased administration of water or saline would have an effect on the LD/50 of rats exposed to lethal doses of radiation.

Results of these experiments are negative.

BA12-6 Force Feeding By: Anderson

Rats when exposed to a lethal dose of gamma rays (1300 to 1400 r) all died within one week. 33 1/3% dextrose was injected into the peritoneal cavity before exposure, one the day of exposure and one the day of exposure with supplementary doses thereafter to determine whether any effect on mortality could be observed. All rats died at the same rate as the controls.

BA 12-19 The Carbonate Metabolism of Bone By: Curtis.

The purpose of this experiment was to study the metabolism of bone so as to better understand the deposition of various elements in bone, and if possible to suggest methods of decontamination.

The program consisted of the administration of CO_4 in the form of the carbonate and follow the time distribution of this substance. Following the assimilation a histological and radioautographic study of the bone for carbonate exchange is made. If such asstudy is successful, a study of various agents and their effect on bone decontamination can be carried out.

Problems

Whether inhaled substances (radioactive) can cause an increase in the lung tumor incidence over an extended period of time.

Whether chronic low dosage can increase the lung tumor incidence over an extended period of time.

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G. Rat Metabolism. By: Mulrooney, Barnes and Raper.

When groups of rats were exposed to beta radiation from phosphorus plaques in the dosage of 5,000, 7500 and 10,000 rep and followed carefully for a 70 day period the following findings were noted.

No change was observed up to the fifth day at which time a general decrease in food and water intake commenced and continued to the 15th day. After this time there was an increase in food and water consumption so that by the twenty-fifth day 30 to 50% more water was being assimilated by the experimental animals over the control rats; the food level increases up to 25 to 30% more than the controls. From the 25th to the 60th day the water intake remains the same, and after this time drops to control levels.

The weight lags in all experimental animals. They lose weight steadily between the 10th and 25th day at which point they stabilize. The control weight has not been regained at the 200th day period.

In an effort to discover whether beta ray burns can be correlated with thermal burns, a series of measurements of hematocrit and specific gravity of blood plasma were taken on the rats which had received total body irradiation with 7500 rep of beta rays. Techniques were selected which gave accurate readings with small samples of blood. The copper sulfate method was used for measuring the specific gravity of the plasma.

No significant change in the hematocrit was recorded, but specific gravity readings were below the normal range for rats in the 12th, 14th and 27th days after exposure. These results suggest: (1) that the plasma lost due to beta ray burns is lost at such a low rate that the blood volume remains unchanged but (2) that the lost plasma proteins cannot be replaced quite fast enough to keep their concentration normal. Since death attributed to

thermal burns is primarily due to a fall in blood volume, it would appear that the physiological changes taking place are quite different for the two types of burns.

H. Histopathological Studies on Mice Following Single Doses External Beta Ray Treatment. By: Snider, Raper.

Studies on mice (CF1) following single external beta ray treatments of 2500 and 5000 r. Damage was restricted to those superficial tissues generally susceptible to damage by irradiation namely the epidermis, derma, parts of the spleen and testis, and the bone marrow. Damage was more severe after the larger dose. Damage to the cells in the basal layer was found almost immediately after irradiation with degenerative signs becoming more conspicuous throughout the 8 and 24 hour intervals. At 15 to 21 days, the basal layer broke down completely in many regions of the body and many ulcerations were not healed at 3 months. In animals surviving the 3 month period, the epidermis contained thickened areas and down growths thought by some to have malignant tendencies. The derma also showed effects with edema, invasion by leucocytes, and occlusion of blood vessels. In general the effects on tissues were comparable with those of x-rays.

K. BA12-18 Delayed Effects of Single Exposures to Beta Rays.
By: Raper, Renshaw and Snider

Rats and guinea pigs were given graded doses of beta rays varying from 1500 to 15,000 rep. The early effects (see report on single dose radiation) were noted over a period of about three months. All doses except the 1500 rep caused increased mortality long after the repair of the acute process had been completed.

The reactions observed after this period here (1) alopecia - doses in excess of the 1500 for 2 species result in extensive epilation within two months. Such baldness may be permanent; (2) telangiectasis - occurs within several months. It is more common in the restricted surface experiments; (3) atrophy - the skin becomes dry, inelastic and cracks quite easily. This covers the entire surface in mice and isolated areas in rats, guinea pigs and rabbits; (4) keratosis - an abnormal growth of skin is seen in rats and mice of the 3000 to 5000 rep groups; (5) late ulceration - seen in all animals except guinea pigs. It appears in regions originally most severely damaged and may become infected; (6) opacity of eyes - mice which survive doses of 3000 rep develop opacities within 6 months after exposure; an occasional animal with the 1500 rep exposure is observed. Occasional rats develop opacities at 5000 rep. Rabbits and guinea pigs never develop this condition. (7) Induction of tumors is illustrated in the accompanying table on mice.

Tumor Frequency in Survivors in Mice

Dose	6 Month Period		8 Month Period		10 Month Period	
	% Survivors	% Tumors	% Survivors	% Tumors	% Survivors	% Tumors
0*	84	0	52.5	0	12.0	10
1500	90	0	84	0	30.0	6
2500*	58	0	57	6	4.6	0
3000	70	0	58	31	26.0	31
3000	60	0	40	44	2.7	100
4500	64	12	12	44	0.0	-
5000	12.5	0	7.5	0	0.0	-

* means sacrifice groups

The locations of the tumors can be divided into those on the ear and those elsewhere in the body. The former may disappear after once developed. Elsewhere on the skin of the body they are superficial, red, wart-like or subcutaneous in the mammary lines and may or may not be necrotic in nature. The superficial tumors are far more numerous than the subcutaneous tumors.

5 L DX12-2 The Effect of Ler[redacted] Surface Beta Radiation.
Keller, Hensin and Shaker.

The purposes of this study were (1) to establish a minimal periodic (chronic) dose of beta radiation which when delivered over a long period of time would produce no detectable effects on the organism; (2) to determine the minimal lethal dose (LD/50) for chronic beta radiation; (3) to compare the effect of beta rays and penetrating (gamma and neutron) irradiation; and (4) determine the tumor incidence under prolonged exposure to beta rays.

Rats (the subspecies) were exposed daily to beta radiation in doses from 0.5 to 625 rep per day until death had occurred. Suitable controls were handled in the identical fashion except for the exposure to irradiation.

The results of this experiment show that at the highest level (625 rep) the effects resemble those resulting from a single acute dose of beta rays. At the lower levels there was little effect of radiation. With the 625 rep dose the rats dies relatively soon with marked epilation. With 50 rep animals was lower than the controls. No detectable change was noted in the 0.5 rep animals. Periodic beta radiation also showed the development of a high proportion of subcutaneous tumors as contrasted with the predominance of epithelial tumors in the single acute dose. These consisted roughly as mammary carcinomas and sarcomas on preliminary examination.

The following conclusions were drawn from this study: (1) periodic beta ray exposure requires less rad doses to produce lethal effect than single acute exposures; (2) single high doses of beta radiation produce more marked immediate effects while with the periodic doses epilation was more extensive per unit dose and the effects on the feet and genitalia were much worse; (3) the acute dose produced a high proportion of epithelial tumors, while the periodic caused a predominance of subcutaneous tumors; (4) comparative effects of beta and gamma radiation indicate that while greater damage to the skin is

produced by beta rays, comparable doses of gamma rays also produce definite internal damage; low chronic doses of beta rays produce only superficial damage while penetrating radiation also gives definite internal damage; animals are able to withstand higher doses of beta radiation than gamma radiation.

5 L. Fmld-1 <u>The Effects of External Irradiation with Beta Rays on the Peripheral Blood of Rabbits</u>. By: <u>Raper</u> and <u>Barnes</u> <u>Same Dates</u>

Blood studies on rabbits which received total surface irradiation with beta rays, in doses ranging from 1500 rep to 59,000 rep, revealed no direct effect on the cellular constituents of peripheral blood. Sporadic increases of neutrophils beginning three weeks after irradiation with intermediate doses, 10,000 rep and 15,000 rep, coincided with infection of ulcerated skin.

No evidence of indirect effects on white blood cells was found nor was there any evidence to substantiate claims of lymphocyte stimulation by superficial irradiation.

The lack of effect is considered consistent with the physical properties of the radiation and the anatomical features of the rabbit.

1151888

BX 12-10 Biological Monitoring at Clinton Laboratories.
Curtis, Del, Andrews and Harris

In order to assess accurately the special hazards which were thought to exist at Clinton Laboratories, animals were placed around the grounds, and in buildings where these hazards might exist. Three groups of 12 rabbits each lived outdoors one group of 6 rabbits lived against the pile she, one group of 30 rabbits lived in an atmosphere composed of the undiluted exhaust gas from the pile, one group of 16 rats lived in a laboratory room where large quantities of plutonium were handled, and appropriate control animals were kept in the animal farm. These animals were examined periodically, including bloodcounts, and from time to time were sacrificed and their tissues examined histologically for damage and radio-chemically for the presence of radioactive material. Their fertility was tested by mating them from time to time. The experiment was terminated after about 1½ years by sacrificing and examining each animal.

It was concluded that none of these animals suffered any demonstrable damage as a result of their possible exposure. It was not possible to find any radioactive material in any of the tissues of these animals except the rats living in the plutonium laboratory, and here there was a small amount of plutonium on the skin and a little in the gastrointestinal tract but none in the internal organs including the lungs.

244-A1 B The Metabolism of Short-Lived Air-Borne Fission Products.
H.C. Lanz and J.D. Teres[redacted]

A Fission recoil apparatus has been constructed in which 1 sq. meter of bare uranium foil is subjected to neutron bombardment in the pile. Gas passing over the foil picks up the fission recoil atoms and carries them to animal exposure chambers and measuring equipment located outside the pile shield. The time taken for an atom to go from the foil to the exposure chamber (pick-up time) varied from 1.2 to 30 sec. A special ionization chamber was developed to measure the activity, which varied from 17 to 0.0 ec. cc. It has been shown that this gas contains not only the noble gas fission products but also all fission products which normally exist in the solid state.

Rats and mice exposed to this gas in an exposure chamber suffer damage and die, but no more so than would be expected from the amount of external gamma radiation which they received while in the exposure chamber.

A special apparatus was constructed which would allow rats to breathe the the gas without receiving external radiation. Rats receiving exposures of 8 hours per day for 5 consecutive days received burns of the mouth and upper respiratory tract. Some of these animals lived but most of them died, apparently from starvation because of failure to eat.

Metabolism experiments have shown that of the material that is inhaled, about 25% goes to the skeleton and is excreted very slowly; about 8% stays in the lungs and is excreted slowly; about 6% goes to the liver and is slowly excreted; and the remainder is excreted moderately rapidly. The highest specific activity is found in the thyroid.

A histological study of tissues of animals sacrificed at various times following exposure has shown that the most severe treatment possible with this apparatus has failed to produce any detectable tissue damage which could be attributed to internal deposition of radioactive materials.

[redacted]

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Radioautographs showed that the activity was deposited uniformly over the bronchial and alveolar surfaces.

It was concluded that for short-lived air-borne fission products the hazard is very much greater from external radiations than from internally deposited radioactive materials.

[redacted]

CATEGORY III. COMPARATIVE EFFECTS OF FAST NEUTRONS AND X-RAYS.

A. Comparative Action of Cyclotron Neutrons and X-rays, by Hagen Kirkie, Jacobson, and Pearlman.

1. Lethal Action on Mice and Rabbits: The median lethal dose and mean survival time were compared in mice and rabbits exposed to 200 KVP x-rays and fast neutrons generated by deuteron reaction on a beryllium probe. The mice used were CF 1 females weighing 20 grams; the rabbits were 2 kg. weight.

In the mice, a median lethal dose (LD 50) was shown to be 515 r of x-radiation and 55 n (the n as used in all of these accounts is that amount of ionization measured with a standard 100 r Victoreen chamber). This was determined over a three to twenty-day period. Thus for the mice, the ratio of x-rays to neutrons (x/n) is approximately 10. No ratio is obtainable for the survival curve because of the greater spread in the mortality of the neutron-exposed animals.

In the rabbits, a median lethal dose (LD 50) was obtained as 825 r for the x-radiation and 145 n for the neutron exposure. As in the mice, most of the mortality occurred between the 3rs and 20th day. The x/n ratio here is approximately 5.5. For the mean survival time the x/n ratio was determined at 6.4.

2. Hematological Effects: The effects on the circulating blood elements were studied after doses of x-radiation ranging from 25 r to 800 r, and after doses of neutron irradiation ranging from 9 n to 178 n.

The general pattern of response of the blood elements is qualitatively similar for fast neutrons and for x-rays. This has been described elsewhere in this account.

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[redacted]

3. Statistical Analysis of Blood Data: An analysis of the hematological data obtained in the above experiment indicated that following exposure to x-radiation and to neutron radiation there were intervals in which the response (reduction) in the circulating heterophyls was directly proportional to the dose and gave a linear relationship. Analyses of these periods in which such similarity was obtained with both x-rays and neutrons gave an x/n ratio of 6.3 to 1 which compares very favorably with that obtained in the survival time study. A less critical analysis of the lymphocyte response in a similar way gives an x/n ratio of 6.4 to 1.

An alternate analytical method gave similar results.

D. Acute Comparative Histopathology in Mice Due to Fission Neutrons and Gamma Rays, by Snider.

Histological observations were made on the following structures of CF 1 male and female mice after exposure to 65 n, 96 n, and 117 n units of fast neutrons. The bone marrow, thymus, lymph node and spleen suffered severe damage, and the intestinal epithelium was damaged to a lesser extent. All of these organs had completely recovered by 30 days after the two lower doses. The testis which was also badly damaged, showed a few signs of repair after 65 and 96 n. All other organs observed were resistant to irradiation by fast neutrons at these doses. Damage was more severe after the larger doses, but no alterations were characteristic of one dose and not of another. In general, the changes were similar to those described for x-ray damage.

CHAPTER IV. D. Acute Histopathological Effects of Single Doses of Slow Neutrons on Mice, by Snider

Histological observations were made on the following structures of CF 1 female mice after exposure to 400 arbitrary units of slow neutrons. The spleen, lymph node, bone marrow and thymus were organs most severely damaged by the treatment. All of these returned to the appearance of control animals by 30 days post treatment time. No damage was noted in bone, cartilage, skeletal muscle, peripheral nerve, autonomic ganglia, liver, kidney, pancreas, heart, lung, adrenal, stomach, fallopian tube, or colon. Minor damage was noted in the ovary and duodenum. The tissue response following slow neutron irradiation is similar to that following x-ray treatment.

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SECRET

CHAPTER I. EFFECTS OF EXTERNAL BETA IRRADIATION.

H. Histopathological Studies on Mice Following Single Doses of External Beta Ray Treatment, by Snider and Raper.

Studies on mice (CF 1) following single external beta ray treatments of 2500 and 500 r indicate that damage was restricted to those tissues generally susceptible to damage by irradiation namely, the epidermis, derma, parts of the spleen and testis, and the bone marrow. Damage was more severe after the larger dose. Damage to the cells in the basal layer was found almost immediately after irradiation with degenerative signs becoming more conspicuous throughout the 8 and 24 hour intervals. At 15 and 21 days, the basal layer broke down completely in many regions of the body and many ulcerations were not healed at three months. In animals surviving the three-month period, the epidermis contained thickened areas and downgrowths thought by some to have malignant tendencies. The derma also showed effects with edema, invasion by leucocytes, and occlusion of blood vessels. In general, the effects on tissues were comparable with those of x-rays.

D. The Metabolism of Inhaled Iodine, by Bailey, Wender and Abrams.

Radioactive iodine131 was obtained from a variety of sources. With the addition of an oxidizing agent iodine gas was evolved which was in turn mixed with air and inhaled by rats in an experimental chamber. The tissues were examined for I131 content.

Results obtained indicated that about 20 percent of the inhaled iodine was retained by the rats. At the end of a 30 minute exposure only 5% of the absorbed amount was in the lung indicating immediate absorption. The thyroid contained 10% of the retained iodine at 48 hrs. and the remaining 90% was excreted in the urine. The rate of iodine absorbed from the lung is similar to that of ingested iodine.

E. Tracer Studies with Inhaled 1.0 Year Ruthenium, by L. Bailey, I. Wender and R. Abrams.

This paper deals primarily with the fate of tracer quantities of airborne ruthenium after inhalation by rats.

1. In the course of the work it was necessary to have a simple quantitative test for Ru, and a colorimetric test was developed which depends on the blue color formed with rubeanic acid. It was possible to detect 2μg of Ru with 10 percent accuracy.

2. In order to expose animals to RuO_4 vapor, a generator was needed which would supply RuO_4 at a continuous and more or less uniform rate. Of a number of oxidizing agents investigated for this purpose, potassium permanganate in sulfuric acid was best suited.

3. A rough determination was made of the chemical toxicity of Ru for mice. By intracardiac injection, doses in the range of 3 μg/g were acutely toxic.

4. Methods have been developed for assaying tissues for radioactive ruthenium. The hard beta of the 1.0 yr. Ru-30 secondary system is counted. To avoid volatility

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losses, small tissues are minced, dried at 110° and counted. Larger tissues are muffled and a correction made for losses due to volatility.

5. Three types of Ru exposure were carried out — to RuO_4 vapor, "dry $RuCl_3$" aerosol, and $RuCl_3$ solution. In all cases the Ru was rapidly absorbed from the lungs and rapidly excreted via the urine and feces. The kidney tends to retain a considerably higher concentration than other tissues. The rapid excretion, approximately 90 percent in 30 days, minimizes the hazard resulting from internally deposited Ru.

1. Metabolism of Inhaled Fission Product Aerosols, by Abrams, Seibert, Potts Lohr and Postel.

This paper deals with the metabolism in rats of air-borne fission products. Four different isotopes were used, selected for their long lives and as representatives of the various chemical species occurring in fission. These were Sr^{89} (55 days), Zr^{95} (65 days), Y^{91} (57 days) and Ce^{144} (275 days). Aerosols of these materials were produced by atomizing aqueous solutions and by burning dried residues in a carbon arc.

Strontium differs from the other elements investigated in the ease with which it is absorbed from the lungs. Over 50% left the lungs within a matter of minutes, and 95% was gone within one hour. Of the Sr^{89} originally in the lung, 60 percent was deposited in the skeleton in less than 4 hours, and after 12 hours the total radiation dose to the skeleton exceeded that to the lungs. These results indicated that inhalation was as effective a means of administration as intravenous injection, and that lung damage as a result of inhalation of Sr^{89} would be of less importance than bone and bone marrow damage.

The other three elements Y, Zr and Ce may be grouped together insofar as they differ from Sr. They were all eliminated from the lung much more slowly than was Sr.

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Thus the times required for 50 percent elimination were Y^{91}, 12 days; Zr^{95}, 25 days; and Ce^{144}, 45 days. In all cases the skeleton was the majorsite of deposition and eventually contained 15 and 30 percent of the initial lung deposit. In no case in periods ranging up to 200 days did the total radiation dose to the bone exceed that to the lung, as it did within 24 hrs. with Sr. With all three elements the probability of radiation damage to the lung is great.

Very little deposition occurred in the soft tissues. The kidneys contained about 0.5 percent of the dose with all the elements. Concentration in the liver was lower than in the kidney with all isotopes except Ce^{144}. This element showed a very high initial liver deposition, but this was gradually eliminated so that after 2 months the liver concentration fell below that in the kidney.

J. BXL-16 - <u>The Effect of Clay on the Intestinal Absorption of Strontium</u>, by <u>L.J. Curtis</u>.

Rats have been fed active strontium which was absorbed on clay, while their controls were fed the same amount of active strontium in aqueous solution. There was no significant difference in the amount deposited in the skeleton between the two groups, and it si concluded that if ions are absorbed on inert material, they will be eluted during their passage through the gastrointestinal tract.

K. 143 LLH 2640 - <u>Accumulation and Distribution of Radioactive Strontium, Barium-Lanthanum, Fission Mixture and Sodium in Goldfish</u>, by <u>C. L. Prosser</u>, <u>M. Pervinsek</u>, <u>J. Arnold</u>, <u>G. Svihla</u> and <u>F. C. Tompkins</u>.

Small non-feeding goldfish accumulated radioactive strontium, barium-lanthanum fission mixture elements and sodium from solutions in which they were immersed. Accumulation was rapid initially and continued at a rate greater than the loss of radioactivity in the medium by decay and absorption. Hence, the ratio of the concentration of active material in the fish to that in the solution increased

with time. After a few days this concentration ratio is usually between 10 and 100. The concentration ratio was independent of dose over the range investigated (0.01 mc to 1.0 mc/kg.

Calcareous tissues, such as skeleton and scales, accumulated about 75 percent of the radio-strontium and radiobarium in the whole fish. Lanthanum and a mixture of cerium, yttrium and zirconium-columbium appeared to be accumulated in visceral tissues, especially the intestine, and to be more actively absorbed on feces and glass than were the alkaline earths. The comparable distribution particularly of bone-seekers, on immersion and injection indicated that these elements were taken up through gill mucous membranes and were distributed by the blood. Radio-sodium was present in greater proportion in the gills at six hours than later. Fission mixtures shifted in abundance from soft parts to bony portions of the gills during the first few days. Injection experiments indicated that there was no fecal excretion of strontium but that there was a small amount of fecal excretion of Ba140-La140 and of fission mixture. Some fission mixture components appeared to be taken out of the medium, either from feces or water, and precipitated along the intestinal folds. The yolk of eggs in ripe ovaries accumulated some components of fission mixture but not strontium or barium.

Young growing or regenerating bone accumulated more radioactive material than did older bone; cartilage accumulated none of the bone-seekers. Large mature gold-fish accumulated radioactive material more slowly than did small fish. This may have been in part related to their calcium turnover. Uptake of strontium 89 and uptake of fission mixture were shown to be greater when the surrounding pond water was low in calcium (1.8 ppm) than when it was high in calcium (36ppm). Inactive strontium also retarded the uptake of radiostrontium.

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When the fish were transferred from active to inactive water they lost some Ba-La and fission mixture radioactivity from soft tissues during the first few hours but they did not lose the latter part of their radioactivity any faster than by decay alone. The rate of loss of radiostrontium appeared to be unaffected by the calcium content of the water.

Goldfish were unable to absorb any appreciable quantities of fission elements from suspended clay.

C. <u>The Shift of Strontium^{89} from the Mother to Fetus and Young in Finkel and Brues.</u>

Strontium^{89} when injected into the mother is transmitted to fetus by the placental circulation, and after birth is ingested by the suckling animal in the milk.

The placental transmission is greatest when injected into the mother in the last three days of pregnancy. The total amount passing across the placental membranes becomes progressively less as the interval between the injection of the strontium^{89} and parturition increases. With injection during the last three days of pregnancy, however, the young are born with a higher per gram weight activity than that noted in the mother. This amount never reaches a concentration more than two times the per gram amount in the mother.

Animals born more than seven days after injection receive larger amounts of the strontium^{89} through the ingestion of the "active" milk than through the placental route.

In the case of the placental transmission the activity of the fetus is directly proportional to the dose of strontium^{89} administered to the mother. In the case of excretion into the milk and ingestion by the young animal, the higher doses yield relatively lesser amounts to the offspring.

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U. The Shift of Plutonium from the [redacted] Fetus and Young, by Finkel and Brues.

When plutonium in the form of the citrate is injected intravenously into a pregnant animal in the last three days of pregnancy, the fetus absorbs up to [illegible] of the dose through the placenta.

Plutonium has been shown to be transferred to the offspring through the milk in spite of the fact that this substance is not absorbed in the gastro-intestinal canal of the adult.

The survival of mothers given either plutonium or strontium[89] during pregnancy is better than the survival of animals given the same or weight dose not during pregnancy. In the case of the strontium[89] animals this could be due to the removal of the isotope by the fetus. In the instance of plutonium this cannot be the explanation.

Autoradiographs of the fetus show that the strontium[89] is in the points of calcification.

Bone tumors have occurred in the offspring of mothers receiving strontium[89] at the normal period of 200 days. The threshold of dosage is related to the specific activity in the fetus at birth rather than in the adult stage.

The retention by the new born animal approaches 100% as contrasted with animals more than 15 days of age and older.

Special Problems

Plutonium oxide has been injected into animals (beneath skin) in order to follow the chronic effects of slow internal absorption and possible tumor development. Doses of 1.0 mg in a single dose were injected subcutaneously in rats and rabbits.

[redacted]

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CATEGORY G: [illegible] Fission Products in the Body

* A. Metabolism and Distribution of Various Fission Products. Hamilton et al.

* B. Metabolism of Ingested and Injected Tellurium. Hamilton.

* C. Metabolism of Ingested Iodine. Hamilton.

D. Metabolism of Inhaled Iodine. Abrams, Bailey and Wender.

E. Metabolism of Inhaled Ruthenium. Abrams, Bailey and Wender.

* F. Absorption and Fixation of Fission Products by Plants. Overstreet and Jacobson.

* G. Retention of Fission Products by Soils. Overstreet and Jacobson.

* H. Attempts to remove fission products localized in Bone. Hamilton and Cu[illegible].

I. Absorption of Aerosols from the Lung and Metabolism of Fission Product Aerosols. Abrams et al.

J. Metabolism of Sr^{89} absorbed on clay when ingested. Curtis.

K. Metabolism of fission products by the Goldfish. Prosser et al.

L. Some Special Features of the Metabolism of Injected Sc^{89}. Anthony and Lathrop.

M. Some Special Features of the Metabolism of Sr-Cl^{45} and of Fission Mixture. Anthony and Morris.

O. Shift of Sr^{89} from Mother to Fetus. Shift of Pu from Mother to Fetus. Bruce and Finkel.

P. Shift of Sr^{89}, Y^{91} and Radium in Calcium-Metabolizing tissues of laying pigeons. Bloom and Murray.

* Q. Metabolism and Distribution of Plutonium in Rats. Hamilton et al.

R. Metabolism of Intravenously Injected Plutonium. Snyder and Kisielesk[illegible].

S. Metabolism and Distribution of Inhaled Plutonium. Abrams.

* T. Methods of Removal of Pu in Body. Hamilton et al.

U. Distribution of Pu in Very Young Animals. Finkel.

Stars in index A,B,C,F,G,H,[illegible],T to go with legend - report included in California Series elsewhere in this volume.

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CATEGORY VI. DISTRIBUTION OF INTERNAL EMITTERS IN THE BODY.

F1. Tracer Studies with Inhaled 1.0 Year Ruthenium, by Bailey, Wender and Abrams.

This paper deals primarily with the fate of tracer quantities of airborne ruthenium after inhalation by rats.

1. In the course of the work it was necessary to have a simple quantitative test for Ru, and a colorimetric test was developed which depends on the blue color formed with rubeanic acid. It was possible to detect 2 mg. of Ru with 10 per cent accuracy.

2. In order to expose animals to RuO_4 vapor, a generator was needed which would supply RuO_4 at a continuous and more or less uniform rate. Of a number of oxidizing agents investigated for this purpose, potassium permanganate in sulfuric acid was best suited.

A. Acute Radiotoxicity of Injected Na^{24}.
Introduction and Methods. Finkle.
Lethal Action. Finkle and Snyder.
Effects on Weight. Finkle and Snyder.
Effects on Peripheral Blood. Jacobson.
Histopathological Effects. Murray and Block.

B. Acute Radiotoxicity of Injected P^{32}:
Lethal Action. Anthony and Snyder.
Histopathology. Heller and Murray.
Hematological Effects. Jacobson and Simmons.

C. Acute Radiotoxicity of Ce-La^{140} for Rats and Mice.
Introduction. Finkle, Snyder, Tompkins and Prosser.
Metabolism of the Emitters and Their Distribution Among Organs.
Finkle, Snyder and Kisieleski.
Radioautographic Distribution of the Emitters. Murray, and Svihla.
Lethal Action. Snyder and Finkle.
Effects on Weight and Food Intake. Sacher and Snyder.
Effects on Peripheral Blood. Jacobson.
Histopathological Effects. Murray, Rhoades and Heller.

D. Acute Radiotoxicity of Injected Sr^{89} for Rats, Mice and Rabbits.
Introduction Methods. Anthony, Tompkins, Lathrop and Finkle.
Metabolism and Organ Distribution.. Anthony and Lathrop.
Lethal Action. Anthony, Lathrop, Snyder and Finkle.
Effects on Weight. Sacher and Anthony.
Hematological Effects. Jacobson and Simmons.
Histopathology, Radioautographs. Heller, Rhoades and Bloom.

E. Acute Radiotoxicity of Injected Radioactive Rare Earth Isotopes.
One Series for Cerium and One for Yttrium.
Introduction, Methods. Finkle and Anthony.
Metabolism and Organ Distribution. Finkle, Anthony, Lathrop and M. Morris.
Lethal Action. Anthony, Lathrop and Finkle.
Effects on Weight. Sacher and Anthony.
Hematological Effects. Jacobson.
Histopathological Effects, Radioautographs. Murray.

F. Acute Radiotoxicity of Injected Zr-Cb^{93}.
Introduction, Methods. Finkle and Anthony.
Metabolism and Organ Distribution. Finkle, Anthony and M. Morris.
Lethal Action. Finkle, Anthony and M. Morris.
Effects on Body Weight. Anthony and Sacher.
Hematological Effects. Jacobson and Simmons.
Histopathological Effects, Radioautographs. Heller and Pierce.

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G. Effects of Insoluble Injected Y^{91} and Other Fission Products.
Introduction; Methods. Brues and Finkle.
Metabolism. Anthony and Grundhauser.
Measurement of Dosage. Moon, Svihla, Grundhauser and Anthony.
Acute Lethal Action. Finkle and Anthony.
Chronic Lethal Action. Brues and Grundhauser.
Effects on Weight. Anthony, Sacher and Grundhauser.
Histopathological Effects. Pierce.
Hematological Effects. Simmons and Jacobson.

H. Acute Histopathological Effects of Injected Y^{91} Including Radioautographs. Pierce, Heller and Rhoades.

I. Acute Radiotoxicity of Injected Radium.
Introduction, Methods. W. Norris, Tompkins and Finkle.
Metabolism. W. Norris, Evans and Finkle.
Lethal Action. W. Norris, Anthony and Evans.
Effects on Weight. Sacher and W. Norris
Hematological Effects. Jacobson, Simmons and Sacher.
Histopathological Effects. Murray, Bellows, and Heller.

J. Effects of Inhaled Xe^{133}. Abrams, Rhoades and Jacobson.

K. Radio and Chemical Toxicity of Various Fission Elements for Goldfish. Prosser, Hagen and Grundhauser.

L. Lethal Action of Inhaled Radio-cerium. Abrams et al.

M. Histopathological Effects of Inhaled Radio-cerium. Rhoades and Abrams.

N. Late Effects of Beta Emitters (Ce, Y) Localized in the Lung. Abrams, Brues, Rhoades, Bloom and Murray.

O. Histopathological Effects of Radium and Uranium Near the Site of Injection. Rhoades.

P. Acute Action of Injected Sr^{89} on Large Animals (Dogs, Goats). Prosser, Painter, Swift, Jacobson and Edwards.

Q. Comparative Action of Injected Sr^{89} on Splenectomized and Non-splenectomized Mice. Jacobson and Simmons.

R. Studies on Polycythemia Patients Administered P^{32}. Jacobson and Schwartz.

S. Hematological Effects of Mixed Fission Products. Jacobson and Simmons.

T. Late Effects of Injected Sr^{89} on Dogs. Painter, Brues, Lisco and Swift.

U. Late Effects of Single and Repeated Injections of Sr^{89}. Brues, France, Lisco and Finkel.

V. Late Histopathology Due to Injected Sr^{89}. Bloom, Murray and Heller.

W. Late Effects of Injected Radium. Brues, France, Lisco and Finkel.

X. Late Histopathology Due to Injected Radium. Murray, Holler and Rhoades.

Y. Local Effects of Insoluble Beta Emitters Tattooed into the Skin. Brues and Finkel.

Z. Acute Toxicity of Plutonium for Mice and Rats.
Introduction. Finkle and Snyder.
Metabolism, Organ Distribution. Snyder and Kisieleski.
Lethal Action. Snyder, Kisieleski and Finkle.
Effects on Body Weight. Sacher and Snyder.
Hematological Effects. Jacobson, Sacher and Simmons.
Histopathology, Radioautographs. Rhoades, Bloom and Murray.

AA. Lethal Action of Inhaled Plutonium. Abrams et al.

BB. Late Effects of Plutonium Deposited in the Lung.
Gross Effects. Abrams and Brues.
Histopathology, Rhoades, Bloom and Murray.
Hematology. Jacobson and Simmons.

CC. Physiological Effects of Injected Plutonium on Dogs.
Clinical Observations. Painter and Swift.
Excretion and Distribution. Russell and Prosser.
Hematology. Painter, Jacobson and Edwards.
Porphyrin and Hemoglobin Metabolism. Schwartz and Zagrie.
Effects on Liver Function. Schwartz, Tinsley and Wallace.
Effects on Serum Proteins. Barron.

DD. Late Effects of Injected Plutonium on Mice and Rats. Brues, France, Lisco and Finkle.

EE. Late Histopathology Due to Injected Plutonium. Heller, Rhoades and Bloom.

FF. Late Effects of Injected Plutonium on Dogs. Painter, Brues, Lisco and Swift.

GG. Studies on Fluorescence of Plutonium. Price et al.

HH. Spectrophotometric Studies on Urine of Animals Containing Plutonium. Wattenberg and Schwartz.

II. Shift of Plutonium in the Calcium-Metabolizing Tissues of Laying Pigeons. Bloom and Murray.

JJ. Histopathological Effects of Plutonium Near the Site of Injection. Rhoades.

KK. Biological Measurement of Plutonium Damage to the Liver. Brues.

LL. Metabolism and Effects of Ingested Plutonium. Finkle, Snyder, Kisieleski and Lisco.

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LL. Absorption from Bound Sites and Attempts at Removal. Rimmie, Teresi and Cole.

MM. Effects of Plutonium on Tissue Metabolism. Barron et al.

OO. Effects of Plutonium on Enzyme Systems. Barron et al.

7a. The Effects of Na^{24}

Approximately 30 microcuries of radioactive sodium ^{24} (14 hr) per gram of body weight given intraperitoneally, kill 50% mice within 20 days. Within the limits of the methods employed, it was estimated that beta particles from sodium ^{24} are about as effective as single doses of x-rays in killing mice.

The mice excreted 33% of the injected sodium in the first day and 50% in the first three days. Similarly injected rats excreted the sodium more slowly. Increasing the total sodium chloride injection from four to twenty-four gm. per mouse increased the excretion in the first 24 hours by about 40%.

Radioactive sodium administered intraperitoneally produces a leukopenia, neutropenia and lymphopenia in mice within 6 days after doses of 23 microcuries/gm or more were given. No significant leucopenia occurs after a dose of 12 microcuries per gm.

b. The Acute Radiotoxicity of Injected P^{32} by Anthony, Snyder, Heller, Murray, Jacobson and Simmons.

ABC mice of 20 gms. average weight were given intraperitoneally doses of P^{32} ranging from 0.55 uc/gm to 8.4 uc/gm. Observations on the excretion, retention, distribution, clinical signs, survival, hematological and pathological findings were made.

The pooled excretion of urine and feces showed that during the first day 22% of the total dose was excreted. At the end of the third day 30% of the total dose had been recovered and at the end of two weeks, 65% of the total dose. Appropriate allowances for the radioactive decay were made.

At the end of three days the skeleton contained 19% per gram of the injected dose; the liver, 7.7%/gm; the kidney 7.2%/gm; the spleen, 2.1%/gm; the heart 5.2%/gm; the lungs, 6.3%/gm; the stomach 5.1%/gm; the intestine 4.0%/gm; the caecum 4.0%/gm; the large intestine 6.9%/gm. The carcass (containing

organs and tissues not examined individually, [redacted] 35% of the dose. No total recovery calculations are given.

A brief description of the clinical findings is given.

Survival studies show that the median lethal dose (LD 50) for 30 days is between 2.1 and 8.4 uc/gm, and is probably slightly less than 4.0 uc/gm as animals at 4.2 uc/gm survived an average of 18.2 days.

Pathological studies of the effect of I^{32} in toxic doses are given.

C. <u>The Acute Radiotoxicity and Metabolic Distribution of Barium^{140} Lanthanum^{140} in Rats and Mice.</u>

Mice and rats were injected intraperitoneally with toxic doses of radio-barium. Absorption was relatively rapid and excretion considerable during the first day. Mice usually excreted 25% of the injected dose by 12 hours and rats excreted a comparable amount by 50 hours. This excretion rate was comparable to that reported by Hamilton who found in rats a total excretion in 4 days of about 46% of Ba^{133} and 25% of Ba^{140}. Excretion declined rapidly so that after six days mice excreted 0.4% and rats 0.5% of the injected dose per day. In rats a little more than twice as much Ba^{140} was excreted by way of feces as by urine. This also agreed roughly with results at tracer levels.

In mice about 45% of the injected dose of Ba^{140} was deposited in bone and in rats about 60% of the dose. In both species these values represent practically all of the retained Ba^{140}. Absorption and decay curves on soft tissues, however show that most of the injected La^{140} was in spleen, liver and kidney immediately after injection. Limited data indicated that practically none of the La^{140} formed in bone by Ba^{140} decay left the bone for soft tissues.

It can be concluded that the excretion and distribution of radiobarium and the distribution of radio-lanthanum at toxic levels are comparable to those at tracer levels.

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The dose of $Ba^{140}La^{140}$ (measured as Ba^{140}) required to kill fifty percent of injected mice in one month was about 3 to 5 microcuries per gm. The dose required to kill half of the injected rats in 90 days was also about 4 microcuries per gm.

The weight curves indicate that there was definite retardation of growth in mice at 2.25 microcuries per gm. and in rats at 1.96 microcuries per gm. The threshold for weight loss was, about half the minlethal dose.

Hematological data indicated that in mice the threshold for effects on hemoglobin and red blood cell count must be above 1.0 microcuries per gm., that at 119 microcuries per gm. the heterophils and lymphocytes counts diminished and that the dose of 0.2 microcuries per gm. appeared to be just below threshold. In rats a dose of 0.5 microcuries per gm. decreased the white cell count by about 30 percent 8 days after injection.

Histological results in mice are of two sorts, (1) the damage to soft tissues particularly spleen, mesenteric lymph nodes and lymphatic and epithelial tissues of gastrointestinal tract probably due primarily to irradiation from lanthanum and (2) damage to bone and bone marrow due to irradiation from deposited barium. Damage in soft tissues was seen four hours after injection and damage in marrow was apparent at 17 hours. Much recovery in the soft tissues has occurred by 20 days.

The spleen took over much blood formation as the bone marrow was damaged. At 5 microcuries/gm. the red pulp became hyperactive with erythropoesis and granulocytopoesis while at 1.5 microcuries per gm. the marrow was not severely depleted and the red pulp showed no overactivity. The decrease in number of circulating heterophiles and lymphocytes paralleled the histological damage although the decrease in lymphocytes at 1.5 microcuries/gm was proportionally greater.

Threshold for bone marrow damage [redacted] between 1.5 and 0.5 microcuries per gm. and for spleen between 0.5 and 0.2 microcuries per gm. The damage to bone marrow was predicted in the metabolism and histographs showed that irradiation here was some ten times that in the shifts. The damage in the shift in the mice which received 5 microcuries per gm. was comparable with that due to 500 r of single dose x-ray. This correspondence is similar to the correspondence of the fluorine dose at 4 microcuries per gm. compared with 500 r. of total body radiation.

The lowest doses at which histological damage was detected in mice were 1.5 microcuries per gm. for marrow and 0.5 microcuries per gm. for spleen. The lowest dose at which a decrease in the count of circulating white blood cells was detected in mice was 1.9 microcuries per gm. and in rats of 0.5 microcuries per gm. The threshold for lethal action (in 3 months) was about 0.3 microcuries per gm. in mice and for weight loss 0.2 microcuries per gm.

<u>The Effect of $Ba^{140}La^{140}$ on the Hematological Constituents of the Peripheral Blood of Mice and Rats</u>, by <u>Jacobson</u>.

0.2 microcuries/gm. of Ba^{140} La^{140} administered intraperitoneally to mice produced only a questionably significant decrease in the peripheral blood elements. A dose of 1.9 microcuries/gm produced in the leucocytes, lymphocytes and heterophils a significant and rapid reduction sustained for approximately 60 days. Rapid reduction and death within 15 days following administration of 17 microcuries per gram. No changes in the erythrocyte and hemoglobin values were noted.

In rats a temporary depression in the leucocytes and lymphocytes following the intraperitoneal administration of 0.5 microcuries per gram. No effect on erythrocyte or hemoglobin values was noted. The results produced by 0.5 microcuries/gm. in rats were intermediate between the effects of 0.2 and 1.9 microcuries/g in mice.

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b. Acute Radiotoxicity of Injected [redacted], Mice and Rabbits, by Anthony, Terpening, Lehrer, and Finkel.

Male mice of 20 gms, Sprague-Dawley rats of 200 gms and Swiss rabbits of 1800-2500 gms were given intraperitoneally in doses ranging from 0.25 to 20 microcuries per gm.

Excretion studies indicated that the mice showed an overall excretion of 54% of the dose, 10% in the first 6 hours; 26% at 24 hours and 45-50% at 7 days. No difference in the size of the dose and rate of excretion was noted. Rats showed an excretion of 38% of the dose in the first few weeks; 10% at 6 hours, 19% at 24 hours and 35% at 7 days. In a differential between the ratio of urinary to fecal excretion the ratio at first is quite variable but at the end of the first week approaches a 1/1 relationship. In rabbits the total excretion between 70 and 80% in the first three weeks. Fecal excretion decreases to low levels at the end of 10 days time. A comparative study of all three species indicates that the rabbit, mouse and rat excrete Sr^{89} in decreasing order of total amount with the rabbit excreting twice that of the rat. Excretion is almost complete in 10-15 days in all species. In the next 90 days only about 10% of the injected dose is excreted.

Retention studies indicate that after three weeks mice retain about 45% of the injected amount; rats, 5% and rabbits 21%.

Distribution studies indicate that the soft tissues lose the Sr^{89} very rapidly so that at the end of the first 3 days less than 0.1% of the injected dose/gm of tissue can be measured. Bone contains all of the remaining amount. The femur has the highest concentration per gram.

Toxicity studies show that doses above 10 microcuries/gm cause 100% mortality in all species in less than 2 weeks. LD/50 studies for the 30 day period for the mice - 7 to 8 microcuries/gm; for the rat less than 5 microcuries

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per gm. and for the rabbit, less than 5 microcuries per gram. At the 150 day period the mice and rat show a two fold decrease in the required dosage while the rabbit shows only a 5% decrease.

A brief clinical description of the symptoms of Sr^{89} toxicity for animals is given. Gross pathological examination shows a decrease in lymphatic elements and a marked evidence of hemorrhagic tendency.

Weight loss apparent in animals showing no other sign of toxicity. This was related in some extent to decrease in food consumption.

<u>The Hematological effects of internal and parenterally administered Strontium^{89} in Animals, by Simons and Jacobson.</u>

Hematological studies of the peripheral blood of rabbits, rats and mice after the intraperitoneal or stomach tube administration of Sr^{89} in doses of from 0.015 to 14.5 uc/g for varying periods up to 37 are reported above.

With the techniques employed in this experiment a reduction in the heterophil values was the most sensitive indicator of acute and subacute effects. Moderate but significant reduction in heterophil values followed the intraperitoneal injection of 0.068 uc/g in ABC male mice; no effect was apparent after the administration of 0.015 uc/g by the same route in 2 strains. Progressively more significant and sustained reduction in heterophil and lymphocyte values followed higher doses. No anemia in mice occurred with doses of 0.034 uc/g and below but moderate and sustained anemia were apparent with doses of approximately 2.0 uc/g.

The minimal dose exerting a mean detectable reduction in heterophil values in rats was between 0.22 and 0.25 uc/g; doses above this producing a progressively more severe heterophil depression and lymphopenia. Intraperitoneal administration of the isotope was done in rats for comparison with the IV route; and intraperitoneal

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dose of [illegible] uc/[illegible] was required to [redacted] production of hematological values of significance in the rabbit.

B. Acute radiotoxicity of injected radioactive rare earth isotopes, by Finnie, Anthony, Lathrop, W. Morris, Secher, Jacobson and Murray.

1. Acute radiotoxicity of Cerium^{144}

Cerium^{144} (270 day half life) was administered intravenously into Sprague Dawley rats in the following dosages: - 2, 3, 5, 7, and 10 uc/[illegible]. The total excretion, retention, distribution, clinical changes and survival times were studied at the various dosage levels.

Total excretion studies showed that in the dosage of 2 uc/[illegible] 4.6% of the injected dose was excreted at the 6 hour period, 13% at the 100 hr. period, and at six weeks [illegible]%. In the 10 uc dosage, 1.7% was excreted at the 6 hr. period, 9% at the 100 hr. period and at six weeks 53% was excreted. The urinary excretion is a straight line function with a downward trend. The fecal excretion rose to maximum amounts at 168 hours at which time it was approximately 15 times the urinary value.

Studies on retention indicate that at the 6-7 day period 90% of the dose remained in the animal; at 3 weeks, 60% remained; and at 90 days, 54%. The distribution of the element shows that the liver and carcass contained the major amounts. The liver at 7 days contained 30% of the dose; at 3 weeks, 22%, and at 90 days, 5%. The carcass contained 45% at 3 weeks rising to 45% in 90 days. The major portion of this amount was in bone.

A brief clinical description of the behavior of the animals to lethal doses of cerium^{144} is given.

Survival studies show that the median lethal dose (ld 50) for 30 days is between 3 and 5 uc/[illegible]. For 120 days, it is between 2 and 3uc/[illegible]. The survival time is directly proportional to the dosage received.

Weight studies are now in preparation.

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E. Acute Radiotoxicity of Yttrium[91]

Yttrium[91] (57 days) was administered intravenously to Sprague Dawley rats in the following dosages: - 1, 3, 5, 7, and 10 uc/gm. The total excretion, retention, distribution, clinical effects and survival times were studied at the various dosage levels.

Total excretion studies showed that in 4 days 25% of the injected dose was excreted, and at 24 days, 28 to 29% of the injected dose. The excretion was directly proportional to the dosage given. The urine contained 90% of the total excretion at the 4 day period. At the 9th day, the feces had risen to 50% of the total.

Retention studies (60-90 days) showed that 6% of the total was retained by the animals with 5% in the carcass, 1% in the viscera and 2.5% in the femur. Total recovery values were 97% of the injected dose.

Distribution studies showed that the liver contained 8% of the dose; kidneys 2.0%; gastro-intestinal tract 2.0%; femur, 2.5%; heart, spleen and lungs, less than 1% of the dose.

A brief description of the clinical effects of yttrium[91] is given.

Survival studies showed that the median lethal dose (LD 50) for 30 days was between 1 and 3 uc/gm. The animals receiving 1 uc/gm died from 280 to 241 days after the dose was injected.

Weight studies showed that the lowest dose (1 uc/gm) depressed growth in these rats.

F. The Acute Radiotoxicity of Injected Zirconium-Columbium[93], by Finnie, Anthony, A. Morris, Secher, Jacobson, Simmons, Hexner and Pierce.

The data on this experiment is too incomplete to permit analysis at this time. The proposal is similar to that used in the remainder of the studies on the effects of internal irradiation and parenterally introduced substances.

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G. & H. The Effects of Insolubl[redacted] ^{91} and Other Fission Products, by Brues, Finkle, Anthony, Grundhauser, Loch, Svihla, Sacher, Jacobson, Simons and Pierce.

Rats were fed yttrium^{91} in single doses ranging from 1 uc/g to approximately 30 uc/gm. The median lethal dose (30 days) for the ingested yttrium^{91} was found to be 25 uc/gm. Death was caused by intestinal bleeding. Examination of surviving animals showed rats which had received from 10 to 20 uc/gm in a single dose continued to demonstrate intestinal lesions at 200 days and more.

Rats were also given yttrium^{91} daily in a chronic feeding experiment. The dosages used were up to 2 uc/gm for a three month period and up to 1 uc/gm for a six month period. During the feeding period the highest levels in both the three and six month experiments showed a slight retardation in growth and a lymphopenia. At the end of the feeding of the yttrium^{91} the animals clinically recovered. Two hundred days after the initial feeding some of the animals at the highest levels died and showed intestinal lesions at autopsy. No tumors have been proven histologically. At the present time only fibrosis and varying grades of intestinal obstruction can be related to the treatment.

Calculations have been attempted to demonstrate the dosage of radiation received by the intestinal canal from both acute and chronic yttrium^{91} exposure. In chronic radiation the dose is approximately 50% of the acute because of the depression of intestinal motility by the acute exposure in high concentration. The highest chronic dose is 1/10th of the acute lethal dose. With the 50% reduction due to increased bowel motility the actual exposure to beta ray damage is approximately 1/20th of the acute dose. In a separate experiment it was determined that to depress intestinal motility to the same degree as noted after acute high concentrations of Y^{91} requires approximately 600 r of x-radiation (200 KVP).

The total amount of the yttrium^{91} absorbed from the gut was determined as 10-4 to 10-5% of the administered dose.

[redacted]

1151916

Radiautographs were made of animals receiving yttrium^{91} and demonstrate the localization of the substance and its effect.

The Effects of Insoluble Ingested Y^{91} and Other Fission Products

The administration of the insoluble beta emitter Y^{91} to rats in single acute doses and the daily administration by gavage over extended periods may produce detectable hematological changes in the peripheral blood.

A single dose of 20 uc/g produces an erratic anemia and a lymphopenia. A dose of 10 uc/g produces a temporary lymphopenia. Heterophil values after administration of both of these doses are extremely erratic. No hematological effects follow a single dose of less than 10 uc/g. No anemia is produced by daily gavage of Y^{91} in amounts from 0.5 to 2.0 uc/g for a period of 178 days. A sustained increase in heterophil values occurs during this period of administration and lymphocyte values fall slowly to a minimum at 60 to 90 days.

No morphological change of significance is observed in the nucleated cells or erythrocytes after single or daily Y^{91} gavage.

1. Acute Radiotoxicity of Injected Radium, by W. Norris, Tompkins, Finkle, Evans, Mahoney, Sacher, Jacobson, Simmons, Murray, Petrunn and Heller.

Rats, mice and rabbits were injected intraperitoneally with soluble salts of radium in order to determine the acute toxicity of the substance. Sprague-Dawley rats of 200 gms. body weight received doses from 0.02 uc/gm to 0.5 uc/gm. ABC strain mice (males and females) of 20 gms. average body weight received 0.06 to 2.0 uc/gm. CF 1 female mice of 20 gms. average weight received 0.06 to 2.0 uc/gm. American Blue male rabbits of 2 kg. average weight received 0.004 to 0.1 uc/gm.

Excretion Studies. The amount of radium excreted in rats varies directly with the dosage used. In the 1.0 uc/gm group a 55% retention of the radium was found at 20 days while in the next 200 days an additional 1-2% only was excreted.

1151917

The percentage of retention decreased [redacted] dose so that at the 0.02 uc/gm level, a retention of only 25% was found at death.

In mice at the 2.0 uc/gm dosage level [illegible]% retention was noted at death which occurred in two weeks after the injection. The percentage retention decreased with the dose until at the 0.06 uc/gm level, only 10 to 15% was found at death. A strain difference in retention was found. The CF 1 mice showed greater decrease in retention with the lower doses than did the AKC mice. No sex difference was noted.

Limited analyses on the rabbits (small series) indicated that there is a decreased retention with the lower doses.

The total excretion in all three species showed a decrease which was exponential with time out to 175 days.

<u>Distribution and Retention.</u> Distribution studies carried out on rats only showed that after a short preliminary period 98% of the total dose was found in the skeleton with only 1% elsewhere. In some animals where increased calcification had been caused in soft tissues (aorta, lymph nodes, fibrous tissue), this calcification was attended by a great increase in the amount of radium deposited. As much as 5 to 20% of the total dose was often found in such calcifications.

Survival studies showed that length of life appeared to decrease exponentially with the dosage received. The median lethal dose (LD 50) at 30 days in the rat was approximately 1 uc/gm body weight and at 100 days, approximately 0.5 uc/gm. The survival studies on mice were almost identical with those in the rats. No sex differences were noted. The dosage used in the rabbit study was too low to effect an LD 50.

Clinical observations on the behavior of the three species are given.

[redacted]

1151918

weight changes in rats showed that at the level of 0.125 uc/gm an effect (loss) was noted. This was positive at 0.50 uc/gm in a 120 day period. Similar changes were observed in mice.

Pathological changes noted were the development of calcification at the dosage of 0.1 uc/gm minimum to a maximum at 0.25 uc/gm. These were most common in the stomach, liver and occasionally testicles. Aortic calcification was most pronounced at high levels (0.5 to 1.0 uc/gm) and occurred approximately at the 20 day period. The remainder of the pathological findings are characteristic of bone marrow depression which follows radium deposition.

The hematological changes which were noted were typical of those already described for radium poisoning.

J. <u>The Effects of Inhaled Xenon^{133}, by Abrams, Rhodes and Jacobson.</u>

An apparatus was designed for the exposure of mice and guinea pigs to radioactive xenon^{133}. These animals were given exposures up to 10^{-5} curies/cc for periods up to three weeks. This involved a maximum dosage of 7000 rem (roentgen-equivalents-physiological).

Of six animals (rats) exposed, three died. No evidence as to the cause of death. The remaining three animals were sacrificed and no histological evidences of damage were found.

No hematological changes were noted.

The guinea pigs were exposed in the same way to slightly lower levels. The results of this experiment were likewise negative.

K. <u>Radio and Chemical Toxicity of Various Fission Elements for Goldfish, by Prosser, Hagen and Grumhauser.</u>

The survival times after goldfish were immersed in solutions containing known concentrations of radiobari m-40-lanthanum^{140}, and strontium^{89} were determined.

The data have not yet been analyzed sufficiently to report.

L. [illegible] Acute Toxicity of Inhaled 275 [illegible] Ce[144], by Seibert and Abrams.

In an attempt to estimate the acute toxicity of insoluble radioactive material in the lungs, rats were exposed to smokes containing 275 mg Ce^{144}. Groups of 200 gm. rats were exposed to the following doses: 3.2, 14, 39, and 200 microcuries per rat. Weight loss was significant only in the 200 microcurie rats. These had a median survival time of 39 days and were all dead within 120 days. The 39 microcurie rats had a median survival time of 282 days, while 50 percent of the 14 microcurie animals were still alive after 300 days.

As has been demonstrated in tracer experiments, absorption from the lungs was slow, and liver and skeleton were the primary sites of deposition of the absorbed material. It was not possible to show any definite effect of dose on distribution. Preliminary histological examination revealed some tendency for the bronchial epithelium to undergo a squamous proliferation.

M. Late Effects of Beta Emitters Localized in the Lung, by Drues, and Lisco.

Rats which have inhaled concentrations of radioactive yttrium[91] and cerium[144] are being maintained for the development of possible latent effects.

At the present time the animals are still surviving and no pertinent or conclusive information is available.

P. The Acute Action of Injected Strontium[89] on Large Animals, by Prosser, Painter, Swift, Jacobson and Edwards.

This study has not been sufficiently analyzed at the present time for any report to be given.

SECRET

1151920

g. The Comparative Action of Injected [redacted] Splenectomized and Non-Splenectomized Mice, by Jacobson and [redacted]

Previous experiments with strontium[illegible] showed marked depression of the leucocyte series and no effect on the erythrocyte series when this substance was injected. A possible explanation was the presence of erythropoietic activity in the spleen. Accordingly the experiment was repeated with splenectomized animals, and while the control animals retaining their spleens showed normal erythrocyte counts, the splenectomized group became progressively more anemic. This confirmed the presence of functioning erythroblastic activity as previously postulated.

h. Studies on Polycythemic Animals Administered Phosphorus^{32}, by Jacobson and Simmons.

A polycythemic state is induced in rabbits by the administration of cobalt. Such a state is treated with P^{32} and the possible effects noted.

The studies are in progress at the present time, and no preliminary report can be given.

5. The Hematological Effects of Mixed Fission Products, by Jacobson and Simmons.

It was desired to know as to whether mixtures of the slugs used in the pile process contained elements which might have a possible effect on the hematological constituents of the blood. Accordingly mixtures of such slugs were dissolved and given to animals by stomach tube.

The doses of this mixture employed ranged from 0.5 to 3.0 uc/gm body weight. Following ingestion of a single dose a depression was noted in the leucocytes and lymphocytes which reached its maximum in 10 to 20 days. The observed depression was directly proportional to the dose in uc/gm.

[redacted]

1151921

T. The Late Effects of Injected Strontium[89] in Mice, by Bruce and Lisco.

Strontium[89] was injected into mice in doses as high as 0.2 uc/gm. These animals are being observed for the development of latent effects, particularly bone tumors.

U. The Late Effects of Single and Repeated Injections of Strontium[89], by Bruce, France, Lisco and Finkle.

The late effects of single doses of strontium[89] correspond closely to the effects of doses of the same dose repeated at monthly intervals. Strontium[89] in single doses of 0.5 and 1.0 uc/gm and in repeated doses of 0.5 and 1.0 uc/gm results in a very high incidence of bone tumors. The minimum latent period for the development of these tumors is 200 days. In two strains of mice, the bone tumor incidence in the surviving animals are respectively 90% and 60%. This is much higher than the incidence in mice of the same strain which are given radium.

Occasionally bone tumors have occurred following doses as low as 0.25 uc/gm given in a single injection. The latent period appears to be about 350 days.

The incidence of malignant lymphoma in the two strains of mice is significantly but not greatly increased with higher doses of strontium.

V. Late Histopathology due to Injected Strontium[89], by Bruce, Burrows and Keller.

This study is in preparation but not yet ready to report.

W. The Late Effects of Injected Radium, by Bruce, France, Lisco and Finkle.

Osteogenic sarcomas have been found in rats and mice which were injected with radium and survived more than 400 days. The incidence of tumors at the various dosage levels is as follows: - at the 0.03 uc/gm level, 5% of 40 mice; at the 0.06 uc/gm level, 5% of 20 rats; at 0.1 uc/gm, 30% of rabbits at 400 to 450 days; at 0.02 uc/gm 12.5% of rabbits at 65 days. (10 rabbits and 8 rabbits used in the latter two series).

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Calcification of the aorta and arterial tree was observed in rats and mice receiving doses of radium which permitted life for three or more days. The maximal incidence of this findings was between the dosage ranges of 0.25 and 1.0 uc/g body weight. These calcified regions contained high concentrations of radium. As noted previously other calcifications were found in the liver, stomach and occasionally testicles.

The hematological findings showed that the animals died of an aplastic anemia. This was associated with extreme emaciation.

Y. Local Effects of Insoluble Bone Emitters Tattooed Into the Skin, by Bruce and Finkle.

The original experiments were quite unsatisfactory and gave unpredictable results.

A new experiment using the subcutaneous and intramuscular injection of yttrium^{91} phosphate which is quite insoluble have been started. Preliminary studies indicate that this compound is practically intact at the injection site six months after its introduction.

Preliminary results are awaiting analysis.

6. The Toxicity and Metabolism of Plutonium in Laboratory Animals, (Mice and Rats), by Finkle, Sanger, Kisielski, Jacobson, Sacher and Simons.

The differences in the distribution of plutonium in rats and mice whether given as plus 4 nitrate or plus 6 nitrate were not great.

Mice, rabbits and rats used. Injected intravenously. Rats (Sprague Dawley) males doses 0.031 to 2.0 ug/g of +4 Nitrate (0.0015 - 0.125 uc/g) and +6Nitrate and Citrate; mice ABC males and females received 0.015 to 10ug/g of 4 nitrate and 6 nitrate and citrate (.0012 - 0.63 uc); rabbits received 0.1 ug/g (0.0063 uc/g) of +4 nitrate and 0.1-0.02 for +6 nitrate (.0063 uc/gm).

At 4 days after injection 20% of the dose was found in the liver of rats.

1151923

50% in mice. The spleen and lungs usually retained plutonium in approximately the same concentrations per gram as did the liver. The removal of the plutonium from the soft tissues was variable but there was usually a decrease in concentration by a factor of 10 or 20 in 100 days, but often livers could be found that contained at 100 days as much as 50% of the average initial concentration. At other times the half time of liver excretion was 14 days. The livers almost invariably still retained 1 to 2% of the dose for as long as 200 days. The spleen and lungs usually decreased at the same rate as or more rapidly than did the liver though in some cases the spleen or lungs contained as much as 5 times the concentration in the liver at 200 days.

Removal from the soft tissues was usually responsible for the high levels found in the blood stream. In rabbits from 0.2 to 0.4% of the dose was still in circulation at 40 days while in rats 0.1% was in circulation at 19 days.

In rats the plutonium leaving the soft tissues was largely excreted since there were decreases in the skeleton from the early figures. However in mice at least a portion of the plutonium removed from the liver went to the skeleton. The failure of the fecal excretion to plateau within a few days after injection was probably due to excretion of the plutonium leaving the liver. Retention in the skeleton was from 20% to 50% in rats and from 30 to 50% in mice.

At 200 days deposition in the skeleton averaged 10% of the retained dose per g. of bone ash. Slightly higher concentrations were found in the lumbar and thoracic sections of the spine and in the tibias while lower concentrations were found in the radii, skull, jawbones and ribs.

Intramuscular injection of plutonium plus 6 nitrate in mice resulted in much slower absorption. 2% was absorbed in 4 days and 40% in 60 days. Of the absorbed portion 20% was found in the liver initially. This decreased to 4% at

1151924

64 days and probably represented a balance between accumulation and removal from the liver.

The distribution of plutonium given intraperitoneally to mice was similar to that given intravenously. The absorption was slower and was not complete until sometime after 4 days. The citrate was absorbed more rapidly than the nitrate and the subsequent removal from the soft tissues was more rapid. The liver initially collected about 30% of the dose.

As with the internal distribution, differences in excretion after intravenous or intracardiac injection due to variations in the salt or valence were not striking. Variations within given series were relatively large but independent of the dose or of strain, according to the data obtained. Rats and mice excreted about the same amount in 50 days regardless of the differences enroute. The limited data indicated that rabbits excrete less than rats or mice.

Rats excreted 17% in 14 days and 27% in 8 weeks. The feces to urine ratio was about 3 to 1. The fecal excretion leveled off after 40 days. The rate of urinary and fecal excretion at 40 to 50 days was about 0.06% per day.

Mice excreted 12% in the first 2 weeks and 24% in 10 weeks. The rate at 10 weeks was about 0.07% per day.

The excretion of plutonium administered intramuscularly and intraperitoneally to mice was not followed.

Excretion of the plus 6 nitrate by rabbits was 4% during the first 7 days and at a rate of from 0.07 to 0.15% per day a 5-6 weeks. The feces to urine ratio was about 2 to 1.

1151925

The Survival and Growth of Plutonium Injected Animals.

In rats the 30 day LD50 was about 0.94 uc/g; 60 day, only slightly less and at 90 days, 0.063 uc/g; at 220 days 0.031 uc/g. Some of the lower doses 0.016 and 0.0032 uc/g appeared to be lethal but death due to secondary infection. Weight losses definitely detectable at levels as low as 0.0078 uc/g. At toxic levels the plutonium caused gastrointestinal hemorrhage and hyperemia or hemorrhage of lymph nodes and gonads. At sublethal levels (100 days or longer) severe atrophy of the testes, thymus and spleen (also nodes) was noted. Gross liver damage was noted down to a dose level of 0.014 uc/g. Skeletal lesions noted at 0.031 and 0.016. Only one osteogenic and 3 soft tissue tumors noted in animals given 0.014 to 0.031 uc/g and dying after 150 days.

In mice the 30 day LD50 was about 0.011 uc/g; 60 day 0.075 and 90 day 0.069. Lower doses not 50% lethal up to 200 days. Weight effects noted at 0.031 but not at 0.016 after 60 to 100 days. Pathology was similar to that noted for rats. 0.016 uc/g caused extensive graying of hair in 80% mice at 150 to 200 days.

Doses of 0.0003 uc/g and below were not lethal in rabbits up to 500 days. The higher dose caused a slow steady weight loss after 120 days.

The Effects of Ingested Plutonium

Rats were given plutonium plus 4 nitrate at 0.0031 uc/g and plus 6 nitrate at 0.005 uc/g orally. A single mouse was given 17.2 uc/g and analyzed after 26 days.

Of the ingestion experiments about 0.3% of the plutonium was absorbed from the gastrointestinal canal in one experimental group and in a second group in which tissues were analyzed an amount of 0.003% was found.

In the high dose mouse experiment the amount absorbed was 0.2% of the dose. No animals were killed by the plutonium given. No differences between the

4 and 6 valence states was noted.

xx. Acute Toxicity of Inhaled Plutonium. By Abrams, Seibert, Forker, Greenberg and Lisco.

Plutonium in the tetravalent state and in the absence of complexing agents was administered to the lungs of rats by tracheal intubation of solutions. Five groups of animals received doses ranging from 7.5 micrograms to 504 micrograms per 100 gm rat. All of these doses were lethal the mean survival time ranging from 203 days for the lowest dose to 26 days for the highest. Inhalation is the most dangerous route of administration, the 150 day MLD being only 0.1 that of intravenous injection.

Once in the lung, Pu seemed to be tightly bound. Even after 210 days, 25 percent of the initial dose was still in the lung. This may have been due to the inflamed and congested state of the lung. Approximately 15 percent of the initial dose found its way to the skeleton.

Except for the lungs no obvious pathological changes were seen in these animals. The damage produced in the lungs was considerable in all animals. At was characterized by severe inflammation, necrosis and abscess formation. Animals surviving a month or longer showed widespread chronic inflammation. This was accompanied in many animals by a striking squamous metaplasia of the bronchial epithelium which often showed remarkable proliferative activity.

7BB. Late Effects of Plutonium Deposited in the Lung. By Abrams, Bruce, Lisco, Rhoades Blom and Furman.

When plutonium is given by the administration of the plus 6 citrate (by lung drip) directly into the lung, not only severe pulmonary changes are produced but also those changes characteristic of injected plutonium elsewhere in the body.

When insoluble compounds of plutonium (such as the oxide) are inhaled, the pathology is limited to the lung and consists of scattered consolidations throughout the pulmonary tree containing rather characteristic early changes of microscopic nature.

The results of the present experiments are preliminary and sufficient time has not yet elapsed for the development of further alterations in the lung by the deposited plutonium.

The Hematological Effect of Plutonium administered by Lung Drip to Rats. By Simons and Jacobson.

Plutonium was administered in the plus 4 state to Sprague Dawley rats by lung drip. Groups of animals were given 31, 13, 4.3, 1.5 and 0.5 uc/g per rat. Hematological studies of the peripheral blood of these animals were made before and after treatment. Death of the animals in the group which received 31 uc per rat precluded sampling beyond 28 days. Prior to death, however, an initial rise in hemoglobin and erythrocytes and heterophils. A dose of 13 uc/rat produced an anemia, lymphopenia and heteropenia sustained throughout the 84 days of observation. Hematological studies were begun on the groups which were given 4.3, 1.5 and 0.5 uc/rat approximately 50 days after the administration of the material. The blood studies on the group which received 0.5 uc/rat were within normal limits and remained so throughout more than 200 days of observation. A questionably significant reduction in heterophils

1151928

and a lymphopenia was observed between the 60th and 260th day in the group which received 1.5 uc/rat. During this same period an anemia heterophil reduction and lymphopenia occurred in the groups which were given 4.3 uc/rat.

CC Physiological Effects of Injected Plutonium on Dogs
By: Prosser, Painter, Russell, Jacobson, Schwartz, Barron

Dogs were injected with plutonium plus 6 citrate and nitrate intravenously and intramuscularly in doses from 0.075 ug/g to 0.756 ug/gm. Twelve animals were used in this series. Of the five animals receiving more than 0.356 ug/gm, three died within 15 to 16 days of the injection.

Excretion at the fifteen day period varied from 6.93% to 24.53% in the high dose animals (0.287 to 0.756 ug/gm) and from 6.0 to 14.9% of the low dose animals (0.075 ug/gm).

Distribution studies showed a concentration (in ug per gram of tissue) were highest in the spleen, liver, bone and lymph nodes. The amount in the liver was 34% of the dose at the 16 day period for the plus 4 nitrate and 25.2% for the plus 6 citrate. The liver value at 234 days in one animal was 10.9%. The concentration in the spleen in two animals exceeded the liver concentration. Lymph nodes were relatively high in plutonium content. The gastro-intestinal canal and kidneys were quite low. The femur contained as high as 8.27% of the dose at 15 days. The highest amounts in the tissues (excluding bone) were in those rich in reticulo-endothelial tissue. The amount of absorption from intramuscular injection showed only 1% at the site of injection in three animals.

A description of the clinical findings in plutonium poisoning is given.

Hematological studies show that in animals receiving high doses

1151929

with early death, the total leuco[redacted] to less than 500 cells before death. The heterophils at this time had almost disappeared and the lymphocytes constituted almost all of the remaining cells. In the surviving animals there is an initial depression of the leucocyte count followed by a leveling off to a subnormal count. A terminal drop occurs immediately before death. The lymphocytes remain at a reduced level during the entire experiment. It is suggested that the extramedullary myelopoesis observed in some animals may contribute toward the maintenance of the circulating leucocyte level. In the acute animals (death within 10 days) the reticulocytes almost disappear immediately and are followed by a slow reduction in the red blood count and hemoglobin levels. In the surviving animals at mid dosage, the observed phenomena resemble the 12.5 r. daily x-ray animals. At the low levels (0.075 $\mu c/\mu m$) no effect on the red blood count was noted.

In the acute deaths the blood volume increased after the plutonium was administered. The red cell volume decreased at the same time. A dilution (by an increase in the plasma volume) served to maintain the blood volume at the slightly increased or normal level. In animals surviving several months the total cell mass decreased throughout life with a parallel drop in the blood volume. At the low dosages, no change in the blood orplasma volumes was noted.

All animals lost weight steadily. The intermediate levels showed a phenomen of loss and regaining of a portion of the lost weight. The low level animals showed no change in weight.

Pathological findings showed that before death hemorrhagic phenomena extensive edema (including ascites), oral ulceration and like changes of radition damage of severe nature were noted. Emaciation occurred in some of the lower level animals with resultant trophic ulcerations.

1151930

The pathological examination at death [redacted] that the acute deaths resembled those of high dosage x-radiation except that the hemorrhage is less common. The mid-level animals show marked anemia, hemorrhage, liver disease, ascites and emaciation. The low level animals show no radiation effect.

7DD. The Late Effects of Injected Plutonium in Mice and Rats.
By: Brues, Painter, Lisco, Swift.

The late effects of the injection of plutonium in its various forms consist of the development of (1) bone tumors and (2) liver disease.

Bone tumors have occurred in incidence of up to 25% of the animals treated with the optimal dosage range of between 0.5 and 0.05 ug/gm. One tumor has been observed at a level of 0.02 ug/gm.

Liver disease shows its maximum incidence of 50% at the above optimal dosage range. Pathological examination shows that liver changes consist of atrophy and degenerative or regenerative changes in the parenchymal cells. This often results in the development of ascites, anasarca and jaundice.

Bone tumors following plutonium administration usually appear in the axial skeleton (vertebrae, pelvis, jaw, acapulae) in contrast to the typical long bone (epiphyseal) which develops following strontium[89] administration.

Aplastic anemia is a common terminal result of plutonium administration.

Following the subcutaneous injection of plutonium citrate several fibrosarcomas have been observed at the injection site after 400 days. These have appeared with doses as low as 1.0 ug/gm administered.

7 FF. The Late Effects of Injected Plutonium on Dogs.
Brues, Painter, Lisco, Swift.

1151931

Animals which have previously received injections of plutonium are being followed for the development of latent effects. Sufficient time has not elapsed for even preliminary analysis of data.

7 KK. The biological Measurement of Plutonium Damage to the Liver. By Brues

The morphological picture, radioautographs and plutonium concentration of the liver are being compared in a series of animals previously injected with doses of plutonium which produce liver injury. Other animals are being treated with beta and gamma emitters to determine the comparative injurious effect produced.

Results are still in the preliminary stages and have been briefly discussed under several titles.

No physiological or functional studies are contemplated.

7 NN and 7 OO The Effects of Plutonium on Enzyme Systems and Tissues Metabolism. By: Barron et al

The effect of the alpha rays (plus chemical) of plutonium compounds on enzyme systems is similar to that as previously described for x-radiation, namely on the sulfhydryl systems (SH groups). The relative effect has been determined as approximately 1/10th that observed with an equivalent amount of x-radiation. That this is due primarily to the alpha radiation is presumed inasmuch as polonium has approximately the same effect as does plutonium.

The effect on tissues is likewise not as marked. The changes in the tissue metabolism are not similar to heavy metal (uranium) but to that of x-radiation. All tissues except bone were affected. A general denaturation of protein was produced with heavy exposure.

1151932

A. The Laws of Survival
Sacher, Cole

B. Normal Ranges of Variation in Blood Counts of Various Laboratory Animals. Jacobson, Sacher, Simmons

C. Catalog of Fixed Tissues
Harris

D. The Toxic Agent Hypothesis of Radiation Damage
Prosser, Painter

E. Toxicity of Metals in a Cold Slug after Extraction of Uranium
Jacobson, Simmons

F. Biological Monitoring at Clinton Laboratories.
Curtis, Day, Anderson, Morris.

[redacted]

1151933

CATEGORY 9 STUDIES [redacted] TOXICITY AND SPECIAL PAPERS

A. The Toxicity of Uranium Compounds
Tannenbaum and Silverstone

B. The Distribution of Uranium^{232} in the Tissues of Mice Following Injection of Uranium^{232} (plus o citrate).
Tannenbaum and Silverstone

C. The Microfluorimetric Determination of Uranium
Price, Ferretti and Schwartz

D. Uranium Distribution in Tissues of Experimental Animals
Price, Feretti and Schwartz

E. The Effect of Uranium Exposure on Urine Catalase Excretion
Kutz, Holt and Schwartz

F. Uranium Excretion Studies in Human Subjects
Ferretti, Price and Schwartz

G. The Effect of Metal Exposure on the Excretion of Naturally Occurring Porphyrins. By: Schwartz, Zegaria and Watson

H. The Effect of Bone Marrow Stimulation on Coproporphyrin Excretion
Schwartz, Glickman, Hunter and Wallace

I. The Effect of Uranium on Enzyme Systems, Protein and Tissue Metabolism
Barron et al.

1151934

[redacted]

A. The Toxicology of Uranium Compounds By Tannenbaum and Silverstone.

This report deals with the toxicology of uranium compounds following feeding or injection into mice. The criteria of toxicity were; appearance of the mice, weight changes, gross and microscopic pathological changes and mortality. The uranium content of the organs and tissues was determined.

On subcutaneous injection, uranium compounds are very toxic. Depending on the particular strain of mouse utilized in the experiment, a single subcutaneous injection of 0.5 to 2 mg. of uranium as the nitrate, produced a mortality in some members of a group; 4 mg. was lethal to all mice. Strain is a modifying factor in toxicity while age and sex play less important roles.

Injections of the above-mentioned amounts of uranium produced an acute intoxication; the most constant and striking pathological changeswas an acute necrotizing nephorsis. The deaths occurred during the acute intoxication (first two weeks); otherwise the mice recovered more or less completely. The main sites of uranium accumulation are the kidneys and bone. Disappearance from these organs is a slow process. Uranium is excreted in the urine and feces.

In the ingestion experiments the uranium compound was incorporated into the diet. Daily dosages of 10 mg. of uranium as the nitrate produced relatively low toxicity and no mortality, while dosages of 80 mg. of uranium daily were toxic and lethal. It is clear that the relatively low toxicity of uranium compounds in ingestion (compared with injection) is due to the low rate of absorption of these compounds from the gastrointestinal tract. The nature and duration of the intoxication, the gross and microscopic pathological changes and the distribution of uranium in the tissues were all similar to that found in the injection studies. Uranium accumulates slowly in the kidneys and bone, and disappears slowly after the removal of uranium from the diet

Studies on comparative toxicity reveal that the following compounds may be grouped with regard to their toxicity on ingestion

UO_2, U_3O_8, UF_4 are non toxic

UO_3 is toxic in high doses

UCl_4 is non-toxic in moderate doses; toxic in high doses

$UO_2(NO_3)_2$, UO_4, $Na_2U_2O_7$ are toxic in moderate doses

UO_2F_2 is toxic even at relatively low dosages.

In considering the toxicology of uranium compounds it is necessary to clearly distinguish between the exposure to uranium, the presence of uranium in the tissues, and uranium toxicity. The lack of relationship of the amount of uranium in the tissues and the state of intoxication at any one time is discussed. It is suggested that the acute intoxication be considered apart from the subsequent course. The significance of the anions of the ingested compound is discussed. For example, it is probable that UO_2F_2 is more a problem of fluorine toxicity than uranium toxicity. The course of chronic uranium poisoning is still to be recognized and described.

B. <u>The Distribution of Uranium^{232} in the Tissues of Mice Following Injection of Uranium^{232} plus 6 nitrate</u>. By: <u>Tannenbaum</u> et al

The distribution of uranium^{232} in the tissues of mice following a single intramuscular injection of the compound was determined. Mice injected with 1.5 micrograms of the compound were sacrificed for analysis in two weeks; mice injected with 4.5 micrograms, in four weeks. Approximately 90% of the material was excreted. Bone was the principal site of accumulation containing approximately 70% of the retained amounts. Kidney was next in importance. The liver contained 1% and the other organs a lesser amount. The distribution of uranium^{232} is similar to that of uranium^{238}. Both accumulate in bone and kidneys, and both are excreted to a relatively high degree immediately after injection.

The distribution figures on the six mice are as follows: the percentage of the dose retained in the animals varied from 9.5 to 11.1% of the dose; bone contained 66 to 76.3%; kidney contained 3.4 to 15.2% liver contained 0.8 to 1.6%; the injection site in three animals varied from 10.7 to 16.6%; the rest of the organs contained 0.5 to 0.8% of dose.

Comparison with experiments on the feeding and injection of uranium^{238} is given. Animals receiving this compound for 76 weeks as 1% of the diet (nitrate) showed 71% deposited in bone, 28% inkidney and 0.5% in liver. Animals injected with 2 mg. of uranium nitrate showed at the 41 day period 66% in bone, 33% in kidney and 1% in liver.

C. <u>The Microfluorimetric Determination of Uranium</u>. By: <u>Price, Ferretti</u> and <u>Schwartz</u>

The method described for the quantitative determination of uranium is sensitive to 0.0001 micrograms. As such, it is the most sensitive method for urnaium analysis with which the authors are familiar. This sensitivity and accuracy have been made possible by the development of a special florophotometer. The advantage of this instrument is that small samples can be analyzed and it is rarely necessary to resort to preliminary purification.

D. <u>Uranium Distribution In Tissues of Experimental Animals</u>. by above <u>authors</u>.

Studies in progress. To be completed in about three weeks. The chief interest is in the central nervous system uranium in chronic studies.

E. <u>The Effect of Uranium Exposure on Urine Catalase Excretion</u>. <u>Katz, Holt and Schwartz</u>.

Through rabbits injected with kidney-damaging doses of uranium excrete increased amounts of catalase in the urine, no difference was found in urine catalase activity in exposed and non-exposed project personnel.

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F. Uranium Excretion Studies in [redacted]. By: Ferritti, Price and Schwartz.

Urinary uranium excretion has been studied in personnel from various projects. Uranium excretion agreed quite well with uranium exposure. Relatively heavy exposure gave an average of 75 micrograms/liter excretion; moderate exposure, 46 micrograms/liter; and light exposure, an average of 16 micrograms per liter. Incidental exposure gave less than 5 micrograms per liter.

G. The Effect of Metal exposure on the Excretion of Naturally Occurring Porphyrine. By; Schwartz, Zaparia and Watson.

a. Lead Thirteen rabbits were administered up to 45 mg. of lead acetate per kg body weight. In all the total coproporphyrin excretion rose from an average of 10-15 micrograms per day to about 200-600 micrograms per day. Values returned to normal only after several weeks to several months. The increased copro-porphyrin excretion was found to be specifically due to the copro III isomer.

Red cell protoporphyrin also rose from control values of about 70 to over 200 micrograms per 100 cc. of cells.

There was no appreciable effect on the weight curve during this period.

b. Uranium Several dozen rabbits and one dog have been injected with 0.05 to 5 mg. of uranium nitrate per kg body weight. Large doses cause a precipitous drop in total urine coproporphyrin excretion which parallels the kidney damage as reflected in the decline in urine specific gravity. Small doses cause a slow increase of total coproporphyrin to 203 times the control values. Preliminary studies indicated that this increase was due to the type III isomer, but further study is desirable.

c. Combined Lead and Uranium

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c. Combined Lead and Uranium If lead is administered first to cause a marked rise in urine copro excretion, i.e. to 300 micrograms per day, the administration of a large amount (2.5 mg/Kg) of uranium to a rabbit causes a precipitous drop to a few micrograms per day within 24-48 hours. This is undoubtedly related to kidney damage.

d. Thorium Thorium given alone acts similarly to uranium, i.e. large doses (40-80 mg/Kg) depress and small doses slowly increase the total urine copro excretion. If lead is given first and followed by thorium the thorium produces a second pronounced increase in the urine copro excretion. This is completely unlike uranium. It should be pointed out, however, that urine specific gravity is not diminished by thorium, so that the mechanism of its action is quite certainly different from that of uranium. The possibility is being investigated that thorium mobilizes the lead which in turn produces the produces the porphyrinuria.

e. Beryllium in doses of 2-4mg/Kg has little effect on urine copro excretion. In most rabbits, however, it has a profound affect on liver function, as indicated by a marked increase in urine urobilinogen excretion.

f. Lanthanum in single or multiple doses of 20 mg/Kg or single doses of 30 mg/Kg has little effect on the urine copro excretion.

g. Arsenic in doses of 1.5 - 2.0 mg/Kg produces a marked prompt rise in urine copro excretion to values of 90 micrograms per day or more. Values return to normal within a few days.

h. Effect of Bal BAL is quite toxic as indicated by the increased urine urobilinogen and urine coproporphyrin excretion following administration of therapeutic doses of 6-20 mg/Kg (in peanut oil). Administered before, simultaneous with or after the administration of metals, it seemed to have no effect on their porphyrinapethic action.

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h. The Effect of Bone Marrow Stimulation on Coproporphyrin Excretion.
By: Schwartz, Glickman, Hunter and Wallace.

The dogs developed a severe anemia following the administration of phenylhydrazine. Fecal urobilinogen and urine bilirubin rose markedly in the following several days. These were followed by increased reticulocytosis to over 50%. At the same time both urine and fecal coproporphyrin excretion increased 200 to 300 percent. This increase was shown to be due to the type I isomer. The type III isomer was not affected. This is in contradistinction to lead exposure where the excretion of the type III isomer is increased.

Two rabbits similarly treated with phenylhydrazine showed questionable effects upon porphyrin excretion. Studies are still in progress.

i. The Effect of Uranium on Enzyme Systems, Protein and Tissue Metabolism
Barron et al.

Uranium when absorbed into the body combines with protein in a reversible state. The combination has been demonstrated to be with the albumin rather than the globulin fraction (plutonium). Uranium combines also with sodium bicarbonate also in a reversible state. Its affinity for the bicarbonate fraction is greatest in the plus 6 state; the affinity for the protein fraction is greatest in the plus 4 state.

Uranium likewise inhibits enzyme systems in a reversible nature. Enzymes when inactivated by the addition of uranium are reactivated when the metal is removed by the addition of either citric acid or sodium bicarbonate.

Extensive studies on the diffusability, filtration etc. of uranium compounds have been carried out. The effects of uranium on specific enzyme systems have been determined.

UNIVERSITY OF CALIFORNIA

J.G. HAMILTON, DIRECTOR

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The problems of the research program, initiated originally under the auspices of the O. S. R. D., 15 October 1942, and later incorporated into the framework of the Manhattan District Project, was to first evaluate the possible hazards arising from radioactive materials produced by uranium fission and, second, to explore the possible methods of treatment of individuals who might become infected with dangerous amounts of these substances. The first, and major phase of the research effort was devoted to a fairly exhaustive survey of the metabolism in rats of the more abundant long-lived fission products, thorium, protoactinium, neptunium, and plutonium. Parallel to this comparable, though less exhaustive, experiments were done to investigate the metabolism of the majority of these substances in plants and their deposition in various types of soils. The second aspect of the general program was concerned with possible therapeutic methods for treating individuals poisoned by these agents and devoted itself in considerable detail to the exploration of different procedures that possibly might prove of therapeutic value for the therapy of individuals infected with those of the long-lived fission products and plutonium, which are selectively accumulated in the skeleton.

The work under the general metabolic program included the study of the assimilation, distribution, retention, and elimination of the carrier-free radioactive isotopes of strontium, yttrium, zirconium, columbium, ruthenium, tellurium, iodine, xenon, cesium, barium, lanthanum, cerium, praseodymium, thorium, protoactinium, neptunium, plutonium, and americium, using rats as the experimental animals. With the exception of thorium and americium, these materials were administered by the three chief portals of entry into the body, namely, oral parenteral, and intrapulmonary. These tracer studies, in most instances, extended over periods of time up to and including sixty-four days. In the case of plutonium, the studies were for intervals ranging up to almost one year. In addition to the direct determination of the deposition of these various radio-elements in the twelve to fifteen most important organs, a very detailed series of radioautographic studies was completed with the two organs where deposition and retention was of greatest importance, namely skeleton and lung. In addition to the animal experiments, a very extensive study with plutonium 238 was

undertaken employing a relatively normal human subject from whom several highly important tissue samples were secured including bone. In this particular instance, a complete excretory record was obtained for almost one year. The results of all of these tracer metabolic studies may be summarized in the following manner for those radio-elements listed in the footnote.* The alkaline earths, rare earths, zirconium, columbium, thorium, protoactinium, neptunium, plutonium, and americium, when introduced into the body were deposited primarily in the skeleton and the average degree of retention of the absorbed radio-element, regardless of the route of administration, ranged from 25% to 75% of the amount initially absorbed. The fraction of these radio-elements retained by the skeleton is eliminated at rates which are less than their rates of radio-active decay with the exception of the 340 day cerium, possibly the 30 year strontium 90, Pu^{239}, and Am^{241}. The remaining five long-lived fission products, ruthenium, tellurium, iodine, xenon, and cesium do not show any significant degree of localization in the skeleton. In addition, there is no very striking deposition in any of the other tissues with the exception of the accumulation of iodine in the thyroid. The rates of elimination for all of these five radio-elements are much greater than their rates of radioactive decay, with the exception of radio-iodine (I^{131}) accumulated in the thyroid. In this particular case, the release of accumulated iodine in the thyroid is many times slower than its rate of radioactive decay. The members of the long-lived fission products group which are absorbed by way of the digestive tract to a significant degree include strontium, barium, tellurium, iodine, and cesium. The other members, notably yttrium zirconium, columbium, ruthenium, lanthanum, cerium, and praseodymium are not absorbed by way of the digestive tract to any significant degree, likewise negligible oral absorption was noted for Th, Pa, Np, Pu, and Am. A high degree of retention by the lungs has been observed for yttrium, zirconium, columbium, ruthenium, lanthanum, cerium, and praseodymium which frequently approaches and in some circumstances, exceeds the radioactive rates of decay of these substances. The pulmonary retention observed with strontium, barium, tellurium, iodine, and cesium, is negligible under the conditions of the experiment. The value of the general data summarized above was that qualitative estimates were possible in the evaluation of the relative degrees of hazard to health for those individuals who might come in contact with the agents listed above. Pulmonary retention was also high with Pa, Np, and Pu.

A large share of the total effort expended by the group working on tracer and metabolic studies was devoted to an evaluation of the behavior of plutonium in animals and in man. The results of these studies, which were far more exhaustive than the attention devoted to any one of the fission products and the other heavy elements, revealed the following important points. The absorption of plutonium

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*Sr^{89}, Sr^{90}, Ba^{140}, Y91, La^{140}, Ce 140, Pr^{143}, Zr^{95}, Cb^{95}, Th^{234}, Pa^{233}, Np^{239}, Pu^{238}, Pu^{239}, and Am^{241}, Ru^{103}, Te^{121}, I^{131}, Xe^{133}, Cs 134.

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from the digestive tract in rats is of the order of .007% of the orally administered dose, which is negligible. The chief organ of accumulation of plutonium absorbed into the blood stream is the skeleton. The rate of release of plutonium accumulated by the skeleton in rats is extremely slow; the estimated half-time of the excretion has been evaluated to be greater than two years. In the human study, the elimination was far less, giving a lower limit for the half-time rate of excretion from the skeleton of 50 years. Moreover, the radioautographic studies revealed that plutonium is deposited primarily in the region of the endosteal layer of the cortical and cancellous bone. Presumably, this material is accumulated within the osteoid matrix. Relatively little plutonium apparently enters the mineral portion of the bone both in rats and in man. This interesting behavior explains, in our estimation, the very high toxicity of plutonium since a large proportion of the alpha rays can enter the radio-sensitive bone marrow. An extensive series of inhalation studies were done employing aerosols of PuO_2, produced by burning chlorides and nitrates of plutonium and the metal. These studies revealed that of the order of 10% of the inhaled material in rats is deposited within the alveoli where its rate of release was of the order of a fraction of 1% per day of the retained amount. Comparable results were obtained from studies of the inhalation of fine aerosols of soluble compounds of plutonium with the observation that a significant fraction was absorbed through the lungs and deposited in the skeleton, while in the case of the oxide smokes, negligible absorption through the lungs occurred.

A survey of the deposition of fission products and plutonium in plants and soils, revealed that all of these radio-elements tended to be immobilized by soil colloids to a very high degree. Plants grown in such infected soils demonstrated an extremely high selective accumulation of these materials in the roots. With the exception of strontium, only minute quantities appear in the stems and leaves. This phase of the work, of course, indicates that the release of fission products or plutonium into the agricultural areas will probably be attended by a very serious problem of considerable duration.

The second phase of the project at Berkeley encompassed the decontamination of skeletal deposits of fissionable materials. Up to the present, experiments have been confined to plutonium, and to the radioactive isotopes of strontium, yttrium, and cerium which are produced by nuclear fission.

The behavior of these elements was compared under conditions profoundly affecting bone metabolism. Growing rats fed a diet low in calcium absorbed over 25 times as much Sr* from the gut as did adults receiving adequate calcium. There was a four-fold difference in retention of injected Sr* between the two groups, but no appreciable effect on Y*, Ce*, or Pu*.

Severe phosphate deficiency was produced in rats with a synthetic diet containing washed blood fibrin, or by adding aluminum

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hydroxide to a normal diet to precipitate the dietary phosphate and prevent its absorption. The deficiency produced severe decalcification of bone, and reduced the retention of Sr* three or four fold, but had no appreciable effect on the retention of Y*, Ce*, or Pu*. In advanced stages, large areas of the bone consisted of organic matrix free from bone salts. Radioautographs of undecalcified bones from such animals showed that Sr* was deposited exclusively in the remaining mineral bone salts of the shaft, while Y*, Zr*, Ce*, and Pu* were deposited in the uncalcified organic matrix. These experiments indicate th t Sr* follows the path of calcium metabolism and is deposited in the mineral of bone, while the metabolism of the other elements appears to be unrelated to that of calcium, and they are deposited in the organic matrix of bone. Even in the forming bone of a healing fracture, Pu* and Y* were deposited in the callus several days before the onset of calcification and the deposition of Sr*.

The elimination of Sr*, Y*, Ce*, and Pu* by rats was followed for several months to determine the effect of prolonged treatment with parathormone, ammonium chloride, citrate, or other agents used in treating chronic lead and radium poisoning. No significant effect was observed, except for a small increase in the excretion of Sr*. This does not hold out much hope for decontamination by such methods.

A procedure was suggested for reducing the toxicity of the plutonium deposits in the skeleton by overlayering them with new non-radioactive bone. This would shield the sensitive cells of the bone marrow and bone from the short range alpha particles. Various means of decalcification followed by new bone formation were investigated, and radioautographs of the bones showed that overlayering actually did take place.

Experiments were also carried out on various phases of bone metabolism, including rickets and scurvy, and on the factors involved in the new bone formation associated with fracture healing.

The work planned for the coming fiscal year includes the following major items. First, we plan to perform more adequate inhalation studies with rats using the more important long-lived fission products. Many of the earlier inhalation studies with fission products were unsatisfactory due to the unavailability at that time of what we now consider suitable technical methods. The projected experiments include the inhalation of oxides as well as soluble compounds of radioelements such as yttrium, zirconium, and columbium, ruthenium, cerium, and praseodymium. These represent the members of the long-lived fission products group which show a high degree of retention in pulmonary tissues. In addition to the animal studies, which are in themselves relatively straight forward and brief, it is planned to undertake inhalation studies with these agents using normal human subjects. A very satisfactory technique which has already demonstrated its effectiveness has been developed and subjected to trial by one of the members

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of the Berkeley group. Here, of course, it will be necessary to use the shorter-lived isotopes and under such conditions, the experiments can be done without risk to the subjects. Secondly, tracer studies in rats are planned employing carrier-free radioactive isotopes of the following agents; notably, gallum, germanium, arsenic, selenium, rubidium, molybdenum, element 43, rhodium, paladium, silver, cadmium, indium, tin, antimony, neodymium, element 61, samarium, and europium. As many of these different elements will be studied as time permits. Thirdly, tracer studies will be initiated employing radium, actinium, uranium, americium, and curium in animals and humans using clinical material available from the Medical School. In addition, tracer studies employing the most important of the long-lived fission products in such human subjects will be attempted if time is sufficient. Fourth, tracer studies are planned with such elements which although not naturally radioactive, can be made so by exposure to irradiation by a chain reacting pile. These include possible materials of construction; notably, chromium, iron, nickel, etc. Fifth, tracer studies are planned using a radioactive isotope of beryllium, (Be^7), in order to study the metabolism of this substance, which is most certainly warranted in view of the ominous toxic characteristics of th element which is apparent in industries where this substance is encountered in large amounts.

Under the decontamination part of the program during the coming year, it is proposed to extend these investigations to include uranium, thorium, actinium, protoactinium, radium, and elements 95 and 96. Metabolic studies of the effect of age, dietary calcium, and phosphate deficiency will indicate whether or not the element in question follows the path of calcium metabolism. Radioautographs of bones from injected phosphate deficient rats will show whether the site of deposition is exclusively in the mineral bone salt, or in the organic matrix.

Agents which might increase the elimination of the elements under consideration will be investigated. These will include compounds forming soluble complexes with the metals, BAL and non-toxic metals (such as Zr and Hf) which might competitively reduce the deposition in bone.

The experiments on overlayering of plutonium deposits by new bone will be extended to other elements which emit alpha particles. Toxicity experiments will be carried out on small mammals to determine the effect of overlayering on mortality. The overlayering experiments are also to be carried out on puppies and cats, adding aluminum hydroxide to the normal diet to produce decalcification. Phosphate deficiency, particularly that produced by adding aluminum hydroxide to a normal diet, will be further investigated in animals, and possibly in selected clinical cases, to determine its value as a means of producing bone resorption. Experiments will be carried out to obtain information on the character and reactions of bone, including the nature of the organic matrix in which these elements are deposited. Three types of animals should be particularly useful for these experiments: growing animals which are actively forming new bone, phosphate deficient or

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rachitic animals in which bone salt is resorbed leaving large areas of uncalcified organic matrix; and animals with healing fractures in which there is a very active local formation of new bone in the callus. The effect of protein deficiency and plasmapheresis on the organic bone matrix will also be studied, to determine the influence on the deposition of these elements.

Another phase of the project at Berkeley was the use of radio-elements in agricultural research. The absorption of mineral nutrients by plant roots must be accorded a rank with photosynthesis as one of the truly vital processes of plant growth. The process constitutes a major field of study for the plant physiologist, and in its diverse aspects, is the control theme of all investigations dealing with soil fertility. Hitherto, ion-absorption by roots has been studied almost exclusively in terms of net gains and losses of ions by the root system or by the culture medium. Virtually no attempts have been made to study the process from the point of view of plant anatomy. Little information is available as to which cells or regions of the root system are involved in the processes of mineral absorption, ion-exchange, etc. Almost nothing is known concerning the physiological role of ions such as Na^{+}, K^{+}, Ca^{++}, and the so-called "trace elements." In the proposed work, these vital and essentially untouched fields will be investigated with the use of radioactive isotopes.

It is anticipated that many of the above mentioned physiological problems in agricultural research may be solved by the development of methods of preparing radioautographs of plant tissues. By these means, it should be possible to study the mechanism of absorption of many inorganic ions by roots. Also, it might well be possible to determine the role of particular elements in the development of particular tissues in the growing plant structure.

Inseparable from the general problem of mineral nutrition of plants are the innumerable problems presented by the complex colloid chemistry of the soil itself. There again our knowledge of the surface chemistry of soil is far from adequate. Existing information concerning ion-absorption reactions in soils is confined chiefly to more divalent ions. With the use of radio-elements it is expected that the behavior in soil of most of the elements of the periodic system can be determined with great convenience. It is hoped that this brief outline will provide some conception of the magnitude of the problems to be attacked. A satisfactory investigation of the field will take many years even with the best of techniques. Nonetheless, it is felt that such a plan of research will amply establish the fact that information gained in the development of the atomic bomb also will contribute immeasurably to one of the most basic endeavors of man-growing of plants in soil.

The foregoing summary may serve to present the general pattern of work to be pursued in the College of Agriculture. Certain aspects of the work are of concern to the Manhattan District and should be

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incorporated in the work planned for the biological program during the coming year. These aspects deal with the behavior in soil and plants of those radio-elements created in the production and applications of nuclear energy. Studies of a preliminary nature in this laboratory strongly support the conclusion that the accidental or intentional release of fission products to soil or irrigation water may constitute a grave agricultural hazard. It is proposed that these studies be continued in the ensuing year to embrace problems of toxic limits and of soil decontamination for the more important long-lived radioelements involved in nuclear fission.

In addition to this program, work under a separate section has been started on the study of the effect of fission recoils on tissue. This is of importance from the health aspect and also from the fundamental point of view. The broad purpose of this work is to determine the specific biological effects of fission recoils produced by slow-neutron bombardment of fissionable materials administered to animals. The work was started by preparing colloidal uranous oxide, and studying its toxicity when administered intravenously to laboratory mice. It was found that one milligram in suspension may be given intravenously without lethal effect, as long as there are no soluble uranium compounds mixed with the UO_2. Almost all of the colloidal suspension is taken up by the liver and the spleen, which will hold these compounds until they are changed to soluble uranium compounds. A large fraction of the soluble part of the colloidal U_3O_8 is taken up by the kidneys. Colloidal U_3O_8 is, therefore, much more toxic than colloidal UO_2 (50% of animals die after 0.9 milligrams). Using UO_2, the density of uranium in the liver and spleen is around one milligram per cubic centimeter, high enough to observe the effects of fission without interference from other biological effects of thermal neutrons, especially if uranium enriched with the isotope 235 is used. In fact, calculations and measurements show that a certain amount of gamma ray background is also tolerated. Thus, the first experiments will be carried out with a paraffin moderator and the 60" Cyclotron. We intend to observe the effect of fission recoils in the spleen. Similar studies using P^{32} have shown that, given adequate dosage, the spleen will shrink to less than half of its size. In dogs, the spleen can be made to disappear by this method of selective irradiation. Secondly, histological sections will be prepared. Thirdly, the nucleic acid metabolism of the liver will be studied under the influence of fission, by means of tracer amounts of P^{32}. Finally, the distribution of fission products in the animal will be measured - i.e. radioiodine, radio-strontium, etc. If the results show that fission recoils are very effective in producing local changes or biological effects, other uranium compounds will be prepared (possibly with the help of Dr. Calvin's group). Already, work has been started on tying uranium to Type II pneumococcus antibody. Reports in the literature indicate that it is possible to attach uranium to antibodies in such a way that, after intravenous injection, the antibodies will cary the uranium with them, uniting with the antigen. There they react with the antigen, depositing the uranium in the tissue. This mechanism will be studied with the hope of preparing suitable uranium-protein combinations which could be deposited in any given part of the body [redacted]ecially in regions

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of tumors. There are possibilities of attaching uranium also to azo dyes, some of which may be taken up selectively by tumors.

Parallel with these investigations on uranium, work has been started on boron and lithium combinations suitable for studying the (n,d) reaction. The line of work will be the same as in the case of uranium; we will start using colloidal suspensions of insoluble salts, and later synthesize other combinations. Decrease in nucleic acid metabolism and possibly reduction in size of the spleen, when attained by means of chronic radio-phosphate, boron, lithium, and uranium after fission, etc., may give us important information on the biological effect of specific ionization, or better, specific energy loss. Zirkle's data indicates that alpha particles are seven times as effective for the same energy loss in the cell as gamma rays or x-rays. Fission recoils ionize thirty times more powerfully than alpha rays; their specific effect may possibly be significantly higher. If the uranium fission work leads to significant results, we may attempt to study the effect of fission of plutonium, and possibly thorium. The case of plutonium is especially interesting, since this element is selectively taken up in the periosteum. Since the ranges of fission recoils are very short, there may be a possibility of damage to the periosteum alone, without influencing any of the surrounding tissues. The study of the effects on the growth of bone and on the development of bone cancer may be of importance. This whole study has bearing on the health problems of pile workers and on the mechanism of action of ionizing irradiations, and indirectly on the cancer problem.

We feel that the experimental program now in progress and outlined for the coming year carries out an important duality of interest. From the point of view of fundamental research, it is of considerable importance to explore the behavior of elements in animals and man that heretofore have been exposed to little or no investigation. Not only is the information of interest for its own factual worth but already it has been shown that detailed investigation of the behavior of the long-lived fission products and plutonium in the skeleton have brought out some significant points of interest relating to the overall field of bone metabolism. It is certain that further investigation will increase our knowledge of this most important physiological field. Moreover, it is quite apparent that a number of the less abundant fission products embrace elements of considerable medical and toxicological interest entirely apart from their concern in the field of atomic energy. On the other side of the picture, it is apparent, of course, that a large share of this work is of immediate and practical concern to the many problems arising in the field of health protection incident to the development of nuclear energy. In particular, the tracer studies with fissionable materials and the further study of therapeutic methods for the treatment of individuals who may in the future become poisoned with either of these substances or the long-lived fission products is of considerable importance in our estimation.

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COLUMBIA UNIVERSITY

PROGRAM

C. FAILLA, DIRECTOR

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COLUMBIA UNIVERSITY PROJECT [redacted]

A. The Measurement of Fast Neutrons for Biological Dosage, by G. Failla.

B. Effects of Small Daily Doses of Fast Neutrons on Mice, by T. C. Evans.

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The Measurement of Fast Neutrons for Biological Dosage

by G. Failla

The purpose of this experiment is to measure the tissue dose delivered by a beam of fast neutrons in terms of the ionizing energy liberated in the tissue. This information is needed in order to determine the true biological effectiveness of fast neutrons and to evaluate the results of biological experiments conducted in different branches of the Manhattan Project.

Method. The extrapolation ionization chamber method, previously devised, has been further developed as regards both the constrution of the ionization chamber and the detecting instrument. The electrodes of the ionization chamber are made of material similar to animal tissue in its atomic constituents. Two independent determinations will be made: (1) With air as the ionized medium (2) With a gas mixture having the same atomic constituents as the electrodes of the chamber. The final result should be the same in both cases and therefore this provides a check of the accuracy of the method.

Extent of Progress. The work up to the present has involved the construction and testing of apparatus and the making of a great many measurements under different conditions. Numerous difficulties have been overcome and the experimental procedure is now quite satisfactory. Work is still in progress and it is preferable not to give the results obtained thus far, since they are subject to modification.

In order to determine the biological effectiveness of fast neutrons in the case of biological experiments carried out in other parts of the Project, similar measurements will have to be made with the neutron beams used and under the physical conditions obtaining at the time the animals were irradiated.

It is expected that final results will be obtained by July 1, 1946, in the case of the measurements in which air is used as the ionized gas.

Effects of Small Daily Doses of Fast Neutrons on Mice

by T. C. Evans

For the purpose of determining the ratio of biological effects of multiple small doses of 200 KV x-rays and neutrons, it was necessary to carry out a series of pilot experiments with a single treatment. The percentage of survival, the median lethal time and hematological effects were used as indices of comparison. The experiments indicated that the dosage of 8 r gave almost identical findings as those with a dosage of 1 N.

A second comparison pilot experiment using multiple dosages of 80 r and 10 N per day for 25 days showed similar effects on the median

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lethal time and hemogram.

In the selection of daily doses of neutrons which were to be used in the daily experiments covering a period of one and a half years, the following choices were made: 1.4, 0.14, 0.07, and 0.014 N equivalent to, respectively, 11, 1.1, 0.55, and 0.11 r, according to the above mentioned ratio. These dosages were given (using the cyclotron as a source) five times weekly. The animals used were Swiss and CF1 mice. Twenty-five males and twenty-five females of each type were used at each of the above dosage levels. Each strain and each sex of animals had a suitable control of twenty-five. Five hundred mice were used in this experiment.

In the largest chronic dose (1.4N) after five months of irradiation the animals started to die. All animals had started to lose weight with both leucopenia and lymphopenia being produced at this time. The groups were down to a 50% survival level a few weeks later. The CF1 mice did not survive as long as the Swiss strain. The approximate mean lethal time was 49% of the control time. Cataracts began to appear in the exposed animals after approximately 80% of the total group had died.

In the next dosage level, 0.14 N, the mean lethal time was 86% of the control time. The effects on the blood were not as definite as observed at the higher dosage level, a slight reduction in leucocytes being observed as compared with the controls. A light reduction in fertility was noted in the males and a light but definite reduction in the frequency of the estrous cycle in the female. There was a slight increase in the incidence of cataract when the number of survivors had been reduced to a few animals.

In the two low dosage levels (0.07 and 0.014 N) no noticeable effects have been observed as regards the survival time, weight loss, blood counts, pathology, changes in oestrus, or breeding. By the time the latter studies (Oestrus and breeding) could be done the animals had aged considerably but a comparison of the control and experimental groups gave very similar observations.

In all groups of older animals various forms of neoplasma have developed. The comparative frequency in control and experimental groups has not yet been determined completely.

Pathological study of the tissues from animals dying in the experiments described above gives the following information. With a maximum single dose of neutrons, there is an aplasia of bone marrow, marked reduction in lymphoid tissue, degenerative changes leading to aplasia of the testis (germinal elements only), destruction of intestinal mucose, and general atrophic changes. In the 80 r/day - 10 N/day experiment, the effect were more or less sharply localized to the blood forming organs and gonads. With the 1.4 n/day dose the most marked effect was the development of testicular atrophy. Some slight depression of bone marrow activity was noted. Splenic atrophy was produced with an increase in fibrosis. Many changes compatible with early aging were noted.

The highest dosage level (1.4 N) was repeated for the specific purpose of following the effect on estrus change, sperm production and cataract formation. Swiss animals were used. In two experiments cataracts appeared in animals surviving after 80% of the original experimental group had died. In the male group sterility was produced about the fifth week of exposure. In the females the estrous was completely inhibited after about 12 weeks of exposure.

A group of female animals of hybrid strain with black coats and pigmented eyes were also studied at the high level (1.4 N). Cataracts were formed in approximately the same fashion and degree as observed in the albino pure strains. An interesting additional observation is that the hair turned gray after about 6 weeks of exposure and later became pure white in those animals surviving the median lethal time.

Attempts were made to correlate the testicular weight with exposure level and damage. In those animals receiving 1.4 N a definite reduction was noted; at 0.14 N change was doubtful; and at 0.07 and 0.014 N no change was noted. Splenic weight showed definite reduction in the highest dose level. Occasional enlarged spleens were noted in the 0.07 and 0.014 N and control groups.

An additional experiment was carried out using 11 r and 1.4 n per day to determine whether x-radiation also produced cataracts. At the time when 1.4 N showed weight reduction and mortality increase, the 11 r of x-radiation animals showed no change. With the continuation of the experiment the effect of the 1.4 N was shown to be definitely more severe than the 11 4; and the approximate biological effect ratio was tentatively placed as 12 r = 1 N. Cataracts were produced by x-rays but not to the same extent.

Several other phases of this work will be reported later.

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UNIVERSITY OF CALIFORNIA

J.G. HAMILTON, DIRECTOR

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Fish Program (OSRD E134, E135, E145)

Site elected - University of Washington Department of Fisheries Research under direction of Dr. Lauren Donaldson.

Purpose - Hazards to fishes by both external radiation and radiation from fission products deposited within them.

Hazards related to chemical dispersion in river water from effluent of pile at H.E.W.

Hazards due to temperature elevation.

The major interest in this study is the attempted protection of the salmon runs in the Columbia River, and correction of any processing method which might be deleterious to the safety and future of these runs.

It is also necessary to remove the government from any claim as to the injury to the large and lucrative salmon industry in the Columbia River.

Dates of Work - August 15, 1943 to present.

Protocol of the Experiments Conducted (as of present date).

External Radiation (by controlled x-ray dosage)

1. Chinook Salmon adults treated with 25, 50, 100r.; male and female; spawned and any effect in size, growth, etc.

2. Hatching of above eggs (1) to young and observation for effect. The 100 r fry released to return from the sea in 1946, 1947, 1948.

3. Sockeye Salmon adults treated with 25, 50, 100 r; male and female; spawned and possible effects observed.

4. Hatching of above (3, and 25, 50 r fry released to return 1946, 1947, 1948.

5. Steelhead Trout Adults treated with 250, 500, 1000 r. Observation as to effects on growth, mortality and histological change.

6. Chinook eyed eggs 25, 50, 100 r. Hatching rate and observation of fry.

7. Chinook Eyed Eggs 250, 500, 1000, 2500, 5000, 10,000 r. Hatching and observation for mortality, growth, histological effect.

8. Silver Salmon adults 25, 50, 100, 250, 500, 1000 r. effects on skin and external organs.

9. Rainbow Trout adult male, female, 500, 1000, 2500 r. Death of fish, effect on spawning to hatching of eggs.

10. Chinook Fingerlings male and female 100, 250, 500, 750, 1000, 1250, 2500, 5000 r. Observation of mortality and sectioning of tissues for histological effect.

11. Rainbow Trout yearlings male and female; 25, 100, 500, 1000, 2500 r. Observations on the above to sexual maturity in spring of 1945.

12. Chinook adults male and female 25, 50, 100 r to spawning.

13. Hatching of eggs (12) to fry stage and observation for effect. Release 50 r. and controls to return in 1948.

14. Steelhead male and female 25, 50, 100 r. Treated and spawned.

15. Eggs from above (14) Hatching and young observed. Release 100 r and controls to return in 1947, 1948.

16. Goldfish male and female treated with 25, 50, 100, 250, 500, 750, 1000, 1250 and 2500 r. Observations as to effect on mortality, histological changes and growth.

Chemical and Temperature Changes

A series of special ponds was constructed at the H.E.[illegible] and so arranged that the water supply be connected with the effluent of the plant in a variety of ways so that the effect of various concentrations of plant effluent at various temperatures could be tested on salmon and trout adults, fingerlings and eggs.

Concentrations adopted using river water as controls were effluent pure containing all the salts and being cooled before the tank; effluent refrigerated, and effluent mixed with river water in the following concentrations: - 1 to 3; 1 to 10; 1 to 50; 1 to 250; 1 to 500 and 1 to 1000. Steelhead trout and chinook salmon were tested in the same tanks divided by partitions. The fertilized salmon eggs were placed in identical concentrations later in the experiment.

Various tests of special materials used by the plant (oils and the like) were also tested in the normally used dilutions in such tanks and the effect on the fishes and eggs observed.

Absorbed Radioactivity

Adult fish, eggs and fry were exposed to concentrations of effluent water containing both long and short lived fission products. Following a standard exposure these were killed, ashed and examined for residual radio-

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activity which remained. In addition fish of various species were collected from the Columbia River and examined in a similar fashion. This was to determine as to whether enough comparable radioactivity had been absorbed to cause damage.

Current Observations

Radiation - Adult Fish - no observable change in any species made after exposures to 25, 50, 100 r. Definite increase in mortality in trout exposed to 1000 r or more. Changes at 500 r show very little distinct evidence of damage in growth, weight changes. Some cytological changes of transient nature observed in the gut and anterior kidney (base of the hematopoetic systems. In small goldfish the changes occur at about the same level (1000 r) with total mortality at 2500 r.

Eggs. In the eyed stage (in which the eye pigmentation first begins to show there is a definite retardation in growth at 1000 r although histological change is first apparent at a lower level 250 r. As in the above the most sensitive tissues are the gut and anterior kidney. A 50 percent mortality figure occurs around the 1000 r level as observed over a period of time and at a level in excess of this but less probably than the observed 2500 r. a 100 percent mortality is reached.

In fingerlings a retardation in growth occurs at 500 r with histological changes appearing at this level in the spleen and anterior kidney which in this form are sites of blood formation. At 1250 r an approximate 25 percent mortality is reached for the several species. At some point in excess of 2500 r. a 100 percent mortality occurs. Unfortunately no level of an intermediate stage between 2500 and 5000 was used.

In the radiation of parent fish under 500 r. no changes have been observed of significant nature as to size of hatch, fertility, abnormality of developing eggs and the like. Significant changes do not appear up to the 1000 r. level as noted above.

Statistical analysis of all data concerning the above problems is in progress, but no reports have been submitted for survey.

Data will appear throughout 1948 on the results of the fish that have gone out to sea and returned to spawn.

Chemical and Temperature Changes

Both fishes and eggs exposed to the effluent unrefrigerated, refrigerated and in a 1 to 3 concentration show increases in mortality of significant degree.

Certain of the chemicals also increase mortality due to actual chemical injury to gill structures and possible respiration of the egg.

Increase in temperature does not influence the severity of the development but only in the rapidity of onset.

Absorbed Radioactivity

After living in effluent (pile) water for as long as two months under controlled conditions, the absorbed and deposited radioactivity was measured. In no instance was it great enough to cause damage. The major portion of the deposition was in the form of the short lived products. Only traces of long lived radioactive materials were found. As correlated with the effect on the effect of external radiation, the dosage received was almost negligible compared to that required to produce damage as such.

Application of Results

Knowledge of the total amount of external radiation necessary to cause damage to fishes of various sizes and ages is applied directly to the analysis of total radioactivity liberated by the piles into the Columbia River. The assumption that this concentration 10^{-10} as diluted is totally absorbed and all is granted. Further application of the Clinton Laboratory studies on the absorption of the various products and their effects after known exposures is also included. In this fashion concentrations can be kept below the hazard level for salmon. Also this is true with respect to the chemical and temperature hazards as well.

Future Program

The program through the next year is already authorized and consists of (1) follow up of the present experiments extending through 1948.

(2) Continuation of the exposures to chemical materials and also observations of possible effect in the Columbia River.

(3) Completion of the histological studies already in progress on fishes damaged by the variety of X-ray dosages.

(4) No program which is not completely related to district operation is necessary.

Remuneration: No additional funds necessary to complete present study.