1967
BIOLOGY
BNWL-714

4-

**PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1967
TO THE
USAEC DIVISION OF BIOLOGY AND MEDICINE
VOLUME I BIOLOGICAL SCIENCES
MAY 1968**

**AEC RESEARCH &
DEVELOPMENT REPORT**

RE WOODLEY 31002 326 JUN 18 1968
A. McCann 30719 329 OCT 23 1968
D. W. Dole 30776 325 FEB 4 1969
H. R. Palmer 30644 329 FEB 10 1969
K. R. Larson 32288 122 R 2014 JUL 30 1969

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

PACIFIC NORTHWEST LABORATORY

RICHLAND, WASHINGTON

operated by

BATTELLE MEMORIAL INSTITUTE

for the

UNITED STATES ATOMIC ENERGY COMMISSION UNDER CONTRACT AT(45-1)-1830

PRINTED BY/ FOR THE U. S. ATOMIC ENERGY COMMISSION

BNWL- 714
UC-48, Biology and Medicine

3 3679 00067 7825

PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1967
TO THE
USAEC DIVISION OF BIOLOGY AND MEDICINE

VOLUME I BIOLOGICAL SCIENCES

by
The Staff of the
Biology Department

Edited by
R. C. Thompson, Paulette Teal,
and Evelyn G. Swezea

May 1968

FIRST UNRESTRICTED
DISTRIBUTION MADE

MAY 14 '68

PACIFIC NORTHWEST LABORATORY
RICHLAND, WASHINGTON

BNWL-714

This is the seventeenth Annual Report of the Biology Department. Previous volumes are: HW-25021 (1951), HW-28636 (1952), HW-30437 (1953), HW-35917 (1954), HW-41500 (1955), HW-47500 (1956), HW-53500 (1957), HW-59500 (1958), HW-65500 (1959), HW-69500 (1960), HW-72500 (1961), HW-76000 (1962), HW-80500 (1963), BNWL-122 (1964), BNWL-280 (1965), and BNWL-480 (1966).

Volume II of this report to the USAEC Division of Biology and Medicine covers work in the physical sciences and is issued in four parts: (1) Atmospheric Sciences, BNWL-715, Part 1; (2) Radiological Sciences, BNWL-715, Part 2; (3) Earth Sciences, BNWL-715, Part 3; (4) Instrumentation, BNWL-715, Part 4.

Printed in the United States of America
Available from
Clearinghouse for Federal Scientific and Technical Information
National Bureau of Standards, U.S. Department of Commerce
Springfield, Virginia 22151
Price: Printed Copy \$3.00; Microfiche \$0.65

FOREWORD

This seventeenth in a continuing series of Biology Department Annual Reports follows the general format of the past several years with certain minor changes of an evolutionary nature. Because of the large number of reports relating to our chronic ^{90}Sr feeding study in miniature swine, we have set up a separate section for them. The other major sections correspond approximately to those of last year's Annual Report, except that we have been a little less conscious of AEC budget categories and have titled the sections and distributed the reports among them, in what seemed to us the most logical manner.

Each major section of this document is introduced by a few paragraphs which review the contents of that section and highlight what might be considered the major accomplishments in that area. Following each of these summarizing introductions is a list of the past year's publications in that area of research. As in Annual Reports of past years, a complete list of Biology Department publications appears together with a listing of staff at the end of the document.

A highlight of the year was the Symposium on Diagnosis and Treatment of Deposited Radionuclides, held in Richland, May 15-17, and jointly sponsored by the AEC's Division of Biology and Medicine, the Hanford Environmental Health Foundation, and Battelle-Northwest. Scheduled for May 19-23, 1968, is a Symposium on Myeloproliferative Disorders in Animals and Man, which will be jointly sponsored by the Division of Biology and Medicine and Battelle-Northwest.

On September 18 and 19, 1967, our Biomedical Program was reviewed for the Division of Biology and Medicine by a distinguished group of scientists that included Drs. C. L. Comar, R. D. DeMoss, R. D. Evans, E. E. Osgood, and J. N. Stannard. Their comments, while generally complimentary, have provided us with many valuable suggestions that we will endeavor to implement during the coming year. As a first step in that direction, an Advisory Committee to the Biomedical Program of the Biology Department was being formed at year end.

The only major organizational change during 1967 was the splitting of the Technical and Administrative Support Section into two Sections-- a Biology Administration Section, managed by P. M. Potter, and a Technical Services Section, managed by L. J. Kirby.

Throughout 1967, we were engaged in the planning of our new Biology Laboratory. At year end, architects were putting the final touches on the plans which should be ready for construction bids in the spring of 1968. Although optimism concerning construction funds seems particularly hazardous at this point in time, we have every assurance that we should be installed in our new quarters by late 1969 or early 1970.

H. A. Kornberg, Manager
Biology Department

TABLE OF CONTENTS

FOREWORD.	iii
RADIATION EFFECTS STUDIES	1.1
Publications.	1.2
Factors Affecting Fluid and Electrolyte Transport in the Irradiated Rat Intestine - M. F. Sullivan	1.3
The Influence of Pancreatic Secretions in the Intestinal Radiation Syndrome - B. W. Wachholz	1.7
Effect of Abdominal X-Irradiation on the Intestinal Lymph Protein - B. W. Wachholz	1.9
Late Effects of Skin Irradiation - M. T. Karagianes, E. B. Howard, and J. L. Palotay	1.10
Late Effects of Intradermally Administered Plutonium in Swine - Beatrice J. McClanahan, E. B. Howard, H. A. Ragan, and J. L. Beamer	1.12
Survival of Transfused Autologous Erythrocytes in the Hanford Miniature Swine - H. A. Ragan, M. F. Gillis, M. T. Karagianes, and J. M. Thomas	1.14
Acute Gamma Irradiation Effects in Juvenile Chinook Salmon - D. G. Watson and G. B. Pauley	1.16
The Radiosensitivity of Alligators - W. J. Bair, J. F. Park, and W. J. Clarke	1.18
STRONTIUM-90 STUDIES INMINIATURESWINE	2.1
Publications.	2.2
Histopathology of ⁹⁰ Sr-Induced Leukemic Processes in Swine - E. B. Howard, W. J. Clarke, and M. T. Karagianes	2.3
Induction of Bone Tumors in Miniature Swine Following a Single Injection of ⁹⁰ Sr - E. B. Howard, W. J. Clarke, and M. T. Karagianes	2.8
Hematology and Clinical Chemistry of ⁹⁰ Sr-Fed Swine - Patricia L. Hackett and Glenda S. Vogt	2.10
Serum Protein Alterations in ⁹⁰ Sr-Fed Swine - E. B. Howard and C. C. Jannke	2.14
Marrow Hemopoiesis in Normal Pitman-Moore Swine - P. H. Cooper	2.16
Phytohemagglutinin Response of Leukocytes from ⁹⁰ Sr-Fed Swine - E. B. Howard and C. C. Jannke	2.18
Possible Viral Association in ⁹⁰ Sr-Induced Porcine Leukemia - E. B. Howard, W. J. Clarke, R. N. Ushijima, and M. E. Frazier	2.19
Familial Patterns in the Development of Hematopoietic Disorders in ⁹⁰ Sr-Fed Swine - Beatrice J. McClanahan, R. F. Palmer, and Patricia L. Hackett	2.21
Radiostrontium Retention on Swine Teeth - C. R. Watson	2.23
INHALATIONSTUDIES	3.1
Publications.	3.2
Chronic Effects of Inhaled ²³⁹ PuO ₂ in Beagles - J. F. Park, W. J. Clarke, and W. J. Bair	3.3

Dynamic Simulation of Retention and Translocation of Inhaled Plutonium Oxide in Beagle Dogs - B. O. Stuart and P. J. Dionne	3.5
Use of In Vivo Counting to Determine Retention of Inhaled ^{239}Pu in Dogs - K. L. Swinth and W. J. Bair	3.8
The Determination of Plutonium to Americium Ratios in Biological Specimens - Y. M. Jones and P. O. Jackson	3.11
Plutonium-Americium Ratios in Dogs After Inhalation of $^{239}\text{PuO}_2$ - W. J. Bair and R. W. Perkins	3.12
Radionuclide Scanning Facility for Dogs - C. R. Watson	3.15
Heterophile Antibody Formation in Rats and Beagles After $^{239}\text{PuO}_2$ Inhalation - J. V. Dilley	3.17
Biliary Excretion of Plutonium After Pulmonary Deposition in Rats - N. Matsusaka and W. J. Bair	3.20
Acute Toxicity of Inhaled Plutonium-239 Nitrate in Beagles - J. F. Park, W. J. Bair, and E. B. Howard	3.22
Long-Term Studies of Inhaled ^{144}Ce - $^{144}\text{PrO}_2$ in Beagles - B. O. Stuart	3.27
Inhalation Studies with Promethium Oxide in Beagle Dogs - B. O. Stuart	3.29
In Vivo Separation of Uranium and Thorium After Inhalation of Uranium Ore by Beagles - B. O. Stuart and Y. M. Jones	3.32
Effects of X-Radiation on Lipid Synthesis by Lung Tissue - E. G. Tombropoulos	3.34
Incorporation of Palmitate into Lipids by Subcellular Fractions of Lung Tissue - E. G. Tombropoulos	3.35
The Hemogram of Rats Anesthetized with Methoxyflurane During Blood Sampling - L. R. Richardson, J. F. Park, and R. J. Olson	3.37
SPACE NUCLEAR SYSTEMS STUDIES	4.1
Publications	4.2
Pulmonary Deposition and Retention of $^{238}\text{PuO}_2$ Microspheres in Beagles - D. H. Willard and B. O. Stuart	4.3
A Constant-Feed, Uniform Dispersion Device for Large Particle Inhalation Studies - B. O. Stuart and M. D. Snyder	4.7
Acute Toxicity of Inhaled Crushed $^{238}\text{PuO}_2$ Microspheres in Beagles - D. H. Willard	4.9
Comparative Toxicity of Inhaled $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ in Rats - B. O. Stuart and W. J. Bair	4.11
Gastrointestinal Passage Time and Absorption of $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ Microspheres in Miniature Swine - V. H. Smith, M. T. Karagianes, and C. R. Watson	4.12
In Vivo Thermoluminescence Dosimetry of Ingested ^{60}Co in Swine - C. R. Watson, M. F. Gillis, M. T. Karagianes, and V. H. Smith	4.16
The Solubility of $^{238}\text{PuO}_2$ Microspheres in Simulated Gastric Juice - V. H. Smith	4.18
Effects of ^{238}Pu Microspheres Lodged on Leaf Surfaces - J. J. C. Hsieh	4.20
Electron Microscopic Determination of Specific Leaf Surface Area - J. J. C. Hsieh and J. M. Thomas	4.21

Temperature of Surfaces in Contact with $^{238}\text{PuO}_2$ Microspheres - J. J. C. Hsieh, F. P. Hungate, and W. C. Roesch	4.23
RADIOELEMENT TOXICITY STUDIES	5.1
Publications.	5.2
Effects of Radioiodine in Sheep and Swine - M. T. Karagianes, J. L. Beamer, M. F. Gillis, and J. L. Palotay	5.3
Physicochemical Form as a Determinant of the Toxicity of ^{238}Pu in the Rat - D. D. Mahlum and M. R. Sikov	5.5
Effect of Age and Physicochemical Form on Plutonium Toxicity and Metabolism in the Rat - M. R. Sikov and D. D. Mahlum	5.8
Age Dependence of Radioiodine Metabolism and Effect in Rats - M. R. Sikov and D. D. Mahlum	5.15
Age-Related Differences in the Metabolism of ^{137}Cs by the Rat - D. D. Mahlum and M. R. Sikov	5.18
Metabolism of ^{143}Pm in a Miniature Swine - V. H. Smith, R. L. Amster, and C. R. Watson	5.20
Production and Dosimetry of Experimental Quantities of ^{237}Pu - I. C. Nelson, A. J. Haverfield, and W. W. Schultz	5.24
RADIOELEMENT REMOVAL STUDIES	6.1
Publications.	6.2
Plutonium-Contaminated Wound Studies - Beatrice J. McClanahan.	6.3
Treatment of Promethium Deposition in the Rat - V. H. Smith	6.7
Removal of Inhaled $^{239}\text{PuO}_2$ in Rats - J. V. Dilley and K. E. McDonald	6.9
Removal of Inhaled $^{239}\text{PuF}_4$ in Beagles - J. V. Dilley and K. E. McDonald	6.11
In Vivo and In Vitro Chelation of Plutonium by α -Lipoic Acid and DTPA - J. V. Dilley	6.12
Phagocytosis and Translocation of Intraperitoneally Injected $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ Particles - C. L. Sanders	6.13
Effect of DTPA and Calcium on Intraperitoneally Injected $^{239}\text{PuO}_2$ Particles - C. L. Sanders and W. J. Bair	6.17
The Ultrastructure of Peritoneal Mononuclear Phagocytes Exposed In Vivo to $^{239}\text{PuO}_2$ Particles - C. L. Sanders and R. R. Adee	6.20
MOLECULAR AND CELLULAR LEVEL STUDIES	7.1
Publications,	7.2
Tryptophan Transport in Neurospora: Effect of Intracellular Pools - W. R. Wiley	7.3
Tryptophan Transport in Neurospora: Tryptophan Binding Protein Released by Osmotic Shock - W. R. Wiley	7.6
Regulation of Enzyme Synthesis in <u>Neurospora Crassa</u> - J. R. Turner	7.10
Acid Mucopolysaccharide Localization in Intestinal Absorptive Cells After Irradiation - J. D. Berlin	7.14
Intranuclear Inclusions in Leydig Cells of Human Testes - J. D. Berlin	7.18

Metabolism of Acetate by Trout Tissues at High Temperatures - J. M. Dean	7.21
Effect of Acclimation Temperature on Mitochondrial Enzymes in Fish - R. S. Caldwell	7.23
TERRESTRIAL ECOLOGY STUDIES	8.1
Publications.	8.2
Eskimo Food Chain Studies - W. C. Hanson and L. L. Eberhardt .	8.3
Computer Simulation of ^{137}Cs in an Arctic Food Chain - L. L. Eberhardt and W. C. Hanson	8.5
Effects of Acute Radiation in Native Rodents - T. P. O'Farrell and G. E. Cosgrove	8.7
Radiation Effects in Free-Ranging Pocket Mice - T. P. O'Farrell	8.10
The Retention of ^{137}Cs by Darkling Beetles - W. H. Rickard and T. E. Hakonson	8.13
The Influence of Wildfire on Ground Dwelling Beetles - W. H. Rickard	8.14
Phychrometric Determination of Water Potential of Desert Plants - J. J. C. Hsieh	8.16
The Relationship of Water Potential and Water Content in a Local Soil - J. J. C. Hsieh	8.19
Absorption of $^3\text{H}_2\text{O}$ and ^{22}Na from Saline Soil Layers by Greasewood - O. Van der Borght and F. P. Hungate	8.20
Effect of Sagebrush Distillate on the Germination of Cheatgrass Seed - J. D. Davis	8.23
Uptake of ^{241}Am and ^{239}Pu by Plants - J. F. Cline	8.24
AQUATIC ECOLOGY	9.1
Publications.	9.2
-Effect of Elevated Temperatures on Mortality and Growth of Young Chinook Salmon - P. A. Olson and R. E. Nakatani . .	9.3
Lethal Temperature Relations of Juvenile Columbia River Chinook Salmon - J. M. Dean and C. C. Coutant	9.5
Behavior of Adult Salmon and Steelhead Trout Migrating Past Hanford Thermal Discharges - C. C. Coutant	9.10
Effect of Temperature on the Development Rate of Bottom Organisms - C. C. Coutant	9.13
Chinook Salmon Spawning Near Hanford - 1967 - D. G. Watson .	9.14
Columnaris Exposure and Antibody Production in Seaward and Upstream Migrant Sockeye Salmon - M. P. Fujihara	9.16
Retention of Radionuclides in Columbia River Bottom Organisms - C. C. Coutant	9.21
Uptake and Retention of ^{65}Zn by Periphyton - C. E. Cushing .	9.23
Behavior of Certain Radionuclides in a Marine Benthic Amphipod - F. A. Cross	9.25
Some Sites for Radionuclide Binding in Fish - D. R. Buhler .	9.26
Modeling of Aquatic Systems - C. C. Coutant and C. R. Cole .	9.30
Tumor Incidence Among Freshwater Mussel Populations - G. B. Pauley	9.33

The Pathology of "Spongy Disease" in Freshwater Mussels -	
G. B. Pauley	9.34
BIOLOGY DEPARTMENT STAFF	10.1
PUBLICATIONS AND PRESENTATIONS	11.1
Documents	11.1
Open Literature Publications	11.1
Presentations at Society Meetings and Symposia	11.3
INDEX OF INVESTIGATORS	12.1

RADIATION EFFECTS STUDIES

Our continuing program of gastrointestinal studies is represented in this section by three reports. Continuing the tradition of several past years, one of these tries to unravel the interrelated effects of radiation and bile on the transport of fluid and electrolytes in the intestine. The other two are of an exploratory nature, looking to the possible involvement of pancreatic secretions and mesenteric lymph in the intestinal radiation syndrome. A fourth report on ultrastructural effects of intestinal irradiation is included in the section on Molecular and Cellular Level Studies.

Two reports are concerned with late effects of skin irradiation. One of these describes a carcinoma in a pig exposed 12 years ago to ^{106}Ru - ^{106}Rh betas. The other describes effects observed 5 years after intradermal injection of plutonium in swine and is of particular interest for the systemic effects noted, including a neoplastic hepatic lymph node. Plans were formulated during the year for an ambitious program of skin irradiation studies directed toward comparing the effects of point- versus extended-source exposures.

The remaining three reports in this section are not related to major programs. One describes observations on the transfusion of erythrocytes in our miniature swine; the other two describe radiation effects in species not widely studied. To our knowledge, no one has previously irradiated alligators, and there has been only very limited previous investigation of radiation effects in fish. In both instances, the effects observed show similarities to effects widely studied in other species, but are notably different in respect to the time relationships involved.

RADIATION EFFECTS STUDIES

PUBLICATIONS

Bair, W. J., J. F. Park, and
W. J. Clarke. "The Radiosensitivity
of Alligators," Radiation Research,
vol. 31, p. 532. 1967. (Abstr.)

McKenney, J. R. "Chloride Transport
by Isolated Rat Intestine,"
Federation Proc., vol. 26, p. 260.
1967. (Abstr.)

Sullivan, J. F. "The Effect of Bile
on Intestinal Function of Irradiated
Rats," Radiation Research, vol. 31,
pp. 592-593. 1967. (Abstr.)

Sullivan, M. F. "Intestinal and
Gonadal Injury After Exposure to
Fission Neutrons," Proceedings of
the First International Symposium on
the Biological Interpretations of
Use from Accelerator Produced
Radiation, Berkeley, March 13-16,
1967. USAEC Report TID-4500,
pp. 180-191. 1967.

FACTORS AFFECTING FLUID AND ELECTROLYTE TRANSPORT IN THE IRRADIATED
RAT INTESTINE

Transport of ^{22}Na , ^{42}K , and ^3H from the lumen of the irradiated intestine was decreased 3 days after 1250 R. The accumulation of intravenously injected water and electrolytes in the gut lumen was due largely to decreased movement out of the lumen rather than to increased movement in the opposite direction. The quantity of ^{22}Na accumulating in the lumen was directly related to the volume of fluid in the lumen at the time of the test and not to its content of electrolytes or bile salt.

Investigator:
M. F. Sullivan
Technical Assistance:
Alma L. Crosby

The presence of bile in the irradiated intestine is known to influence the symptomatology and mortality resulting from radiation exposures in the intestinal radiation syndrome dose range (above 1000 R). Functional measurements made by an in situ perfusion method indicated that diversion of the bile away from the lumen of the intestine by an external fistula resulted in decreased transport of sodium and water from the lumen to the blood, in irradiated or unirradiated animals (Annual Report, 1966). This decrease was not noted when labeled electrolytes and fluid were injected into a ligated loop of the small in-

testine, suggesting that either the method or the perfusion medium was responsible for the altered response of the bile-free intestine.

This report describes experiments made to measure the movement of labeled potassium, sodium, and water either from a ligated segment of the lumen of the small intestine to blood (insorption), or from blood into a ligated segment of the intestine (exsorption). Results are also presented which suggest a mechanism by which radiation may cause excessive sodium loss and by which bile diversion can modify the extent of that loss.

Observations

The common bile duct of rats was cannulated with polyethylene tubing (choledochostomy) prior to exposure of the abdominal region to 250 kVp X rays. Fluid and electrolytes lost externally with the bile were replaced through a duodenal cannula. Functional measurements were made at 3 days after X-irradiation or at an equivalent time in control animals. These functional measurements involved injection of the tracer radioisotopes into the ligated small intestine (for insorption measurements)

or tail vein (for exsorption measurements) of lightly anesthetized animals. After 30 min, the rats were killed and the ligated small intestine removed for assay of the intraluminal contents. Measurements of ^{42}K and ^{22}Na were made in separate experiments and also in the same study by allowing the short-lived potassium isotope to decay before recounting.

Results of these measurements are shown in Figure 1. They indicate that transport out of the intestine is not altered by choledochostomy, but that both sodium and potassium movement

FIGURE 1. Sodium, Potassium, and Water Transport in the Rat Small Intestine (Each bar represents averaged data from three or more rats.)

out of the intestine is markedly depressed by 1250 R. The severity of this impairment of sodium movement (but not potassium) was lessened in the absence of bile.

Movement of ^{22}Na , ^{42}K , and ^3H from blood to lumen appeared to be increased by radiation, suggesting a change in the permeability of the small bowel. This effect was largely eliminated by choledochostomy. Other studies, however, showed that movement of ^{22}Na and ^3H from blood to lumen was almost instantaneous. The maximum quantity of these radionuclides usually appeared within the lumen of the intestine of unirradiated animals within 1 min after their intravenous injection, as shown in Figure 2. Only in the irradiated rats was there a gradual accumulation

within the lumen over the 30 min period following injection. This would seem to clearly indicate that defective insorption rather than effects on exsorption was primarily responsible for the changes noted in the exsorption portion of Figure 1.

The lumen of the intestine of irradiated rats contained more fluid and sodium than either normal animals or animals with choledochostomy. Experiments were performed to determine if differences in the quantity of either water or electrolytes present could be responsible for the accumulation of sodium in the intestinal lumen of irradiated animals.

The small intestine was ligated as previously described and injected with 10 ml of either phosphate buffer, water, or 0.9% saline containing

FIGURE 2. Time Course of ^{22}Na Accumulation in the Rat Small Intestine After Intravenous Injection (Each point represents data from a single animal unless otherwise noted beside point.)

0.02M sodium taurocholate. Results showing the accumulation of ^{22}Na in the gut lumen 30 min after intravenous injection, and also the lumen content of stable sodium, are shown in Figure 3. These results indicate that the quantity of sodium accumulated in the intestine from the blood-stream bears a close relationship to the fluid volume in the lumen, irrespective of what may be dissolved in that fluid, or past history of irradiation or choledochostomy.

Conclusions

The movement of labeled sodium, potassium, and water from the lumen of the small intestine to the blood was decreased 3 days after exposure of the abdominal region of rats to 1250 R. Diversion of the bile prior to exposure decreased the effect of irradiation on sodium insorption but had no influence on either ^{42}K or ^3H transport. Blood to lumen accumulation of ^{22}Na , ^{42}K , and ^3H increased

FIGURE 3. Sodium Accumulation in the Rat Small Intestine After Intravenous Injection of ^{22}Na (Each bar represents averaged data from three or more rats.)

RADIATION EFFECTS STUDIES

after radiation, but time studies indicated that this accumulation was due primarily to decreased movement out of the small intestine rather than to an increased rate of movement into the intestine.

Bile diversion prevented the radiation-induced accumulation of intravenously injected electrolytes and fluid in the gut. This may be due primarily to an effect of bile on sodium insorption, but is related to a more general effect on fluid volume in the intestine. The accumulation of

sodium in the intestinal lumen was most markedly influenced by fluid volume, and in the presence of excess fluid was relatively insensitive to the quantity of sodium initially present in the luminal contents, or to the presence of the bile salt, sodium taurocholate, at the time of the functional test. The influence of bile on the intestinal radiation syndrome must, therefore, occur over a time span much longer than the 30 min test period used in these studies.

THE INFLUENCE OF PANCREATIC SECRETIONS IN THE INTESTINAL RADIATION SYNDROME

The absence or presence of pancreatic fluid in the intestine does not appear to significantly alter the morphological response of the intestinal mucosa to abdominal X-irradiation.

Investigator:
B. W. Wachholz
Technical Assistance:
S. A. Hughes

We have previously shown that bile is a contributing factor in the development of the "intestinal radiation syndrome" following abdominal X-irradiation in rats; that diversion of bile prior to exposure prevents the diarrhea associated with the syndrome, causes a relative increase in the numbers of mucus-filled goblet cells in the epithelial lining of the mucosa, and results in certain functional changes. It was considered desirable to investigate whether

pancreatic fluid might also influence the radiation damage observed.

Observations

A duodenal cannula and an internal biliary fistula were established in each of the rats employed in these studies. The internal biliary fistula extended from just distal to the last bifurcation of the common bile duct to just proximal to the ampulla of Vater in the duodenum. This fistula was

brought outside the body where it could be examined to ensure that bile was flowing into the intestine. This fistula made it possible to cannulate the common bile duct and drain the pancreatic fluid externally without interfering with the normal flow of bile to the intestine. Half the animals were equipped with such an external draining pancreatic fistula. Animals from each group were exposed or sham-exposed to 1250 R abdominal X-irradiation (250 kVp, 30 mA, 0.25 mm Cu plus 1.0 mm Al filtration) and sacrificed at either 3 or 4 days post-exposure. Tissue sections were obtained from ten areas of the intestine, extending from the proximal duodenum to the descending colon.

In another group of rats only a duodenal cannula and an internal biliary fistula were established prior to exposure or sham-exposure, following which the animals were infused through the duodenal cannula with pancreatic fluid at the rate of 0.02 cc/min, thereby increasing the volume of pancreatic fluid in the intestine to at least twice the normal volume. The pancreatic fluid was obtained

from donor rats with an external pancreatic fistula. Tissue sections were also obtained from these infused animals at 3 days and at 4 days postexposure.

In none of these animals were there observable clinical symptoms different from those usually associated with gastrointestinal radiation death. Examination of the intestinal mucosa revealed no morphological changes different from those commonly observed after exposure of the intestine to 1250 R.

Conclusions

Judging from gross clinical symptoms and from histological examination of the mucosa of the gastrointestinal tract 3 and 4 days following 1250 R abdominal X-irradiation, there was no effect from either diversion of pancreatic fluid from the intestine or infusion of an excess of pancreatic fluid. Effects previously observed to occur following bile duct cannulation must, therefore, be due to the absence of bile rather than to any effect of pancreatic fluid.

EFFECT OF ABDOMINAL X-IRRADIATION ON THE INTESTINAL LYMPH PROTEIN

Neither the relative quantity of albumin in the blood serum and intestinal lymph nor the plasma albumin-to-lymph albumin ratio was consistently affected by 1250 R abdominal X-irradiation.

Investigator:
B. W. Wachholz
Technical Assistance:
S. A. Hughes

Despite the extensive work in this laboratory and elsewhere on the response of the gastrointestinal tract to X-irradiation, there has to our knowledge been no investigation of the possible role of intestinal lymph. Although the transport of absorbed substances from the lumen of the intestine to the lymph has been much studied, the effect of radiation on this function, or on the constituents of lymph and their relationship to blood serum, has not been previously investigated.

Observations

A heparinized polyethylene cannula was inserted into the intestinal lymph duct of rats, passed between the vena cava and the aorta and brought outside the animal close to the vertebral column. Lymph was collected at 24-hr intervals in dried heparinized collecting tubes and blood samples were obtained from the tail vein just prior to surgery and at daily intervals thereafter. Three days following cannulation of the intestinal lymph duct, the abdomen of half the animals was exposed to 1250 R X-irradiation.

Lymph and blood serum samples were frozen until an electrophoretic protein analysis was performed. The re-

sults of these analyses are shown in Figure 1. There is clearly no significant change in the relative proportion of albumin in the total proteins of serum or lymph following abdominal X-irradiation. With few exceptions, the concentration of protein in the serum was greater than that found in the lymph by a factor of 1.2-3.0. Though quite variable, there was no consistent change in this factor following irradiation.

Conclusions

There appears to be no significant change in the relative or absolute concentrations of albumin and globulin in blood serum or intestinal lymph following 1250 R abdominal X-irradiation.

These are very preliminary observations, however, and before final conclusions can be drawn such factors must be evaluated as the possible effect of the surgical procedure and prolonged sampling which might result in dehydration; and the possible effects of compensating, increased protein synthesis. From earlier studies it would seem quite clear that protein, and particularly albumin, is lost into the irradiated intestine.

FIGURE 1. Percentage of Albumin in the Blood Serum and in the Intestinal Lymph Prior to and Following 1250 R Abdominal X-Irradiation of Intestinal Lymph Duct Cannulated Rats (Each value is a mean from at least three rats.)

LATE EFFECTS OF SKIN IRRADIATION

Skin lesions produced by the topical application of ^{106}Ru particles 12 years ago in a 6 month old sow had not changed in gross appearance in the past few years. A skin site exposed to 19,470 μCi hr showed histopathologic evidence of an early squamous cell carcinoma.

Investigators:
M. T. Karagianes
E. B. Howard
J. L. Palotay

A comparative study was initiated 12 years ago to determine acute and chronic effects of beta radiation from ^{90}Sr and ^{32}P plaques applied to pig, sheep, and rabbit skin (Annual Report, 1956). Some animals were also exposed at this time to ^{106}Ru particles. We have previously described a fibrosarcoma that developed 4 years postexposure in a rabbit exposed to 16,000 rad of ^{32}P

beta radiation (Annual Report, 1959), and a metastasizing fibrosarcoma and keratoacanthoma that developed 9 years postexposure in a sheep exposed to 16,000 rad of ^{32}P beta radiation (Annual Report, 1965).

Observations

A pig exposed to ^{106}Ru particles at five skin sites died of unknown

cause 12 years postexposure. The exposure sites had not changed in gross appearance since the last annual observation. One of the sites, which received an exposure of 19,470 $\mu\text{Ci hr}$, on histopathologic examination disclosed sloughing of the epithelium with underlying inflammatory reactions of polymorphonuclear and mononuclear cell types (Figure 1). One region of the epithelium had undergone a marked proliferative reaction which invaded the underlying subepithelial connective tissue. Squamous epithelial cells, producing keratin, and having mitotic figures, were observed in the nest of proliferating epithelial cells. This lesion was considered an early squamous cell carcinoma. The extent of proliferation and invasion was minimal at the time of death. Other sites, exposed at lower dose levels, showed varying degrees of pathology, which were not clearly dose-dependent nor neoplastic.

Conclusions

The period between exposure and recognition of neoplasia in this pig was 12.5 years. This diagnosis of neoplasia is consistent with the

FIGURE 1. Early Squamous Cell Carcinoma in Skin of Pig Exposed to ^{106}Ru Particles

earlier speculation (Annual Report, 1965) that the latent period for skin neoplasia in swine should exceed that observed for sheep (9 years) and rabbits (4 years). While in accord with reasonable expectation such a conclusion can hardly be considered established, in view of the very limited number of observations and the differing types of exposure employed.

LATE EFFECTS OF INTRADERMALLY ADMINISTERED PLUTONIUM IN SWINE

Five years after multiple intradermal injections of plutonium nitrate, liver and skeletal burdens were 6.5 and 9.2%, respectively, of the administered dose. The skin sites retained 2-7% of the injected plutonium. The liver was diffusely cirrhotic with severe fatty degeneration and necrosis of the hepatic cord cells. Some of the lymph nodes displayed a significant hyperplasia in the germinal follicles, and the hepatic node was lymphomatous.

Investigators:

*Beatrice J. McClanahan
E. B. Howard
H. A. Ragan
J. L. Beamer*

Five years ago, at 9 months of age, ten Hanford Miniature Swine were injected intradermally with plutonium nitrate (Annual Report, 1962). One of these animals was killed in 1967 because of marked clinical degeneration and anorexia. This animal had received a total of 94 μ Ci of plutonium at dose levels ranging from 0.0016 to 5 μ Ci per injection site.

Histopathologic examination of liver sections revealed a wide-spread destructive process with very severe fatty degeneration of hepatic cells. There was extensive necrosis and a regenerative hyperplasia involving

Observations

Table I lists the plutonium content of tissues measured 5 years after multiple intradermal injections. Most of the absorbed plutonium was present in liver and skeleton. These values are slightly lower than those observed in an animal killed 4 years after injection when liver and skeleton burdens were found to be 11 and 13%, respectively (Annual Report, 1966). Certain lymph nodes, notably the atlantal and prefemoral, contained substantial amounts of plutonium, confirming earlier observations. Plutonium retention at the skin sites ranged from 2-7%.

TABLE I. *Retention of Plutonium 5 Years After Intradermal Injection.*

<u>Tissue</u>	<u>Percent of Administered Dose</u>
Skeleton	9.2
Liver	6.6
Kidney	0.034
Spleen	0.047
Ovaries	0.0017
Adrenal	0.057
Lymph nodes(a)	
Superficial inguinal	0.016
Prefemoral	0.10
Prescapular	0.003
Hepatic	0.018
Atlantal	0.76

(a) *Values are expressed as percent dose per gram of tissue*

focal regions of the hepatic cord cells. Some bile duct proliferation and fibroplasia was seen. The superficial inguinal, gastric, and hepatic lymph nodes displayed a significant proliferative response of the germinal centers with the production of a spectrum of immature lymphoid elements such as is seen in cases of lymphoma. This was especially evident in the neoplastic hepatic node (Figure 1). Many of the cells were of large histiocytic appearance with large pale nuclei. Others were more mature lymphoid cells, some of which were undergoing degeneration with shrinking of the nuclei. Mitotic cells were apparent in the germinal centers of hyperplastic nodes and in sheets of neoplastic cells. The other lymph nodes appeared relatively normal except for some slight hyperplasia in the atlantal and prefemoral nodes.

Skin sections from sites injected with 0.008 or 0.0016 μCi plutonium exhibited only minimal epithelial changes. At higher injection levels the basal layer of the epithelium was most significantly altered, evidencing hypertrophy, atypical cells, and increased mitotic activity. Figure 2 shows a section from a 0.04 μCi site. It consisted of a mass of mature connective tissue in the dermis covered by epithelium which was undergoing hyperkeratosis in some regions. One portion of the epithelium was considerably more active with a small nodule above the surface consisting of thickened epithelium with some connective tissue in the center. There were two inclusion cysts containing keratin in this projection. The basal layer of epithelial cells in this region was considerably more active, with frequent mitotic cells. No neoplasia was apparent.

128X

FIGURE 1. Neoplastic Hepatic Lymph Node

128X

FIGURE 2. Skin Section from 0.04 μCi Injection Site

Conclusions

The large fraction of plutonium retained 5 years after intradermal injections, and the cellular changes observed, underscore the importance

of prompt excision of plutonium-contaminated wounds, and the need for procedures to reduce translocation and accelerate excretion of absorbed plutonium.

SURVIVAL OF TRANSFUSED AUTOLOGOUS ERYTHROCYTES IN THE HANFORD MINIATURE SWINE

The survival of transfused autologous erythrocytes was studied in eight female Hanford Miniature Swine, 8 to 16 months of age, using the ^{51}Cr -labeling technique. The mean half-life observed over a 42 day period was 24 days with standard deviation of 1.8 days.

Investigators:
H. A. Ragan
M. F. Gillis
M. T. Karagianes
J. M. Thomas*

The survival of transfused erythrocytes may be followed by labeling their hemoglobin with ^{51}Cr . Blood to be transfused is mixed with a sodium chromate solution of high ^{51}Cr specific activity. Hexavalent chromium penetrates the erythrocyte wall and attaches to the hemoglobin where it is retained in a reduced trivalent state. Excess hexavalent chromium is reduced to the trivalent state which will not penetrate the erythrocytes and which is rapidly excreted from the transfused animal.

Measurement of the ^{51}Cr gamma in standard erythrocyte samples withdrawn at intervals following transfusion produces a declining curve characterizing the survival of the transfused erythrocytes.

Observations

Eight female Hanford Miniature Swine, aged 8-16 months, were placed under light anesthesia with thiamylal sodium and the cranial vena cava catheterized. Twenty cubic centimeters of blood were withdrawn and labeled using approximately 200 μCi sodium chromate (^{51}Cr) solution. Thirty minutes after reinjection, a heparinized blood sample was withdrawn, and the catheter removed. Heparinized samples were taken at 10 more times during the following 42 days using no anesthetic. Packed cell volumes were recorded for each sample and counting data normalized to 1 cm^3 of erythrocytes. Samples were counted in a deep-well scintillation counter with fixed geometry

* *Mathematics Department*

and pulse height discrimination at the 0.32 MeV photopeak of ^{51}Cr .

Semilog plots (example, Figure 1) showed that in all cases, discounting data obtained during the first 48 hr where splanchnic pooling associated with anesthesia perturbed the erythrocyte distribution, the survival curves could be satisfactorily represented by an exponential expression. Excluding data from samples taken during the first 48 hr, a least squares analysis gave results for the biologic half-life as shown in Table I.

Conclusions

The exponential behavior of the survival curve is characteristic of this technique and probably reflects effects of trauma during tagging and reinjection of the cells rather than any basic physiologic mechanism. Similar results were obtained in unpublished work performed on the Pitman-Moore strain of miniature swine at

this laboratory in 1963. In that study a half-life of 31 days with a standard deviation of 4 days was indicated by results on five animals. The results of the present study indicate that strain differences in hematologic characteristics may exist, necessitating appropriate attention to control data during future radiotoxicity experiments on the Hanford Miniature strain.

TABLE I. Half-Lives for Transfused Autologous Erythrocytes in Female Hanford Miniature Swine

Animal Number	Age, months	Half-Life, days
1	8	27
2	8	22
3	9	23
4	9	25
5	9	21
6	11	25
7	12	23
8	16	25
Mean $\pm$ Standard Deviation		24.0 $\pm$ 1.8

FIGURE 1. Survival Curve for Transfused Autologous Erythrocytes in a Hanford Miniature Swine

ACUTE GAMMA IRRADIATION EFFECTS IN JUVENILE CHINOOK SALMON

Exposure to ^{60}Co -gamma doses of 3000 R and above produced a transient erythema. No other gross changes were noted until just prior to death. Mortality and histopathological changes were dose dependent over a range from 550 to 5470 R.

Investigators:

D. G. Watson

G. B. Pauley

Technical Assistance:

L. H. Heaton

R. M. Steele

In earlier unpublished irradiation studies, an ephemeral reddening of the snouts of irradiated chinook salmon was observed, which seemed analogous to the radiation erythema characteristically shown in mammals. The present investigation was designed to further define this effect and other acute radiation responses of juvenile chinook salmon.

Observations

Four treatment groups of about 35 juvenile chinook salmon (average initial weight, 10 g) were exposed to 550, 1080, 3080, and 5470 R ^{60}Co gammas at a dose rate of approximately 270 R/min. Exposures were made on freely swimming fish. A fifth group of controls was sham irradiated to duplicate the handling experienced by the experimental groups. All fish were kept in unmodified river water which increased in temperature from 16 °C at the start of the experiment to nearly 21 °C at the end. The terminal temperature is well above the optimum for salmon and probably contributed significantly to the mortality rates of all groups.

Erythema was evident at the two highest exposure levels and was exhibited by only about one-third of the fish. A pronounced reddening of the snout, the fins, particularly the base of the paired fins, and to a lesser degree, the skin appeared approximately 20 hr postexposure. Maximum intensity occurred at about 30 hr and the effect had disappeared by 96 hr. The redness appeared to be caused by an enlargement and engorgement of blood vessels near the body surface. No further gross differences, except for a slight nervousness and snapping of the lower jaw, were noted until about 24 hr before death when some of the irradiated fish showed a darkening of the skin and some loss of equilibrium.

Time to death was dose dependent (Figure 1). In the lower exposure groups and in the controls, mortality was undoubtedly influenced by stresses such as the increase in water temperature above optimum levels and the detention of the fish beyond their normal downstream migration period.

Blood cell numbers were reduced by irradiation in a dose-dependent fashion as shown in Figure 2. There

FIGURE 1. Mortality of Juvenile Chinook Salmon Exposed to Acute ^{60}Co Irradiation

was an indication of a partial recovery in the two lowest irradiation levels. Hematocrit values showed no correlation to radiation dose, with packed cell volumes ranging from 37 to 52%.

Histopathologic examination of serially sampled fish showed that all experimental groups exhibited:

- Edema in the connective tissue of the snout
- An increased number of red blood cells in the spleen and some small petechia on the surface of this organ
- An acute inflammation of the gill lamellae with edema and congestion.

This latter effect led to hyperplasia of the epithelium of the gill fila-

FIGURE 2. Effect of Acute ^{60}Co irradiation on White Blood Cell Count in Juvenile Chinook Salmon (Each point represents a mean value from three fish.)

ments between lamellae and hypertrophy of the epithelial cells of the lamellae. Severity of these symptoms was dose dependent.

In the 3080 R group, the hyperplastic gill epithelium had pycnotic nuclei and the hypertrophic epithelium possessed nuclei that showed karyolysis and karyorrhexis. The most significant change occurred in the kidney of this group with shrinking and necrosis of the renal capsules, hypertrophy and necrosis of the hematopoietic tissue, and pycnotic nuclei in the renal tubules and capsules.

Damage was most evident in the 5470 R group. Renal tubules were shrunk and starting to slough from their basement membrane. The hematopoietic tissue was highly necrotic and almost completely lacking basophilia. Red blood cells were lacking

in the liver and the kidney tubule area. There was a great increase in red blood cells in the spleen altering the normal architecture of this organ so that the splenic nodules were unrecognizable in most cases. When nodules were present, they showed an increased nuclear activity as indicated by the presence of large numbers of mitotic figures. In the gill filaments, hyperplasia and hypertrophy were especially noticeable with a confluent tissue formed between many of the lamellae. A phenomenon unique to this group was the apparent necrosis of the mucous cells of the midgut, as evidenced by their shrinking and sloughing.

Conclusions

The transient erythema seen in some fish exposed to gamma doses of 3000 R or greater appears to be similar in nature to that which occurs in mammalian species. The other damaging effects described are clearly dose dependent, and are, to some degree, evident at doses as low as 550 R. The effect; observed, particularly the mortality data, cannot be extrapolated in any absolute sense to conditions other than those pertaining in the present experiment. This reservation must be made because of the presence of other stressful factors, as clearly evidenced in control mortality.

THE RADIOSENSITIVITY OF ALLIGATORS

The 60 day acute lethal X-ray dose for alligators was 900-1000 R. Death was due to hematopoietic depression, small intestine damage, and bacterial infection.

Investigators:

*W. J. Bair
J. F. Park
W. J. Clarke*

Technical Assistance:

*D. L. Catt
J. D. Maulsby
Glenda S. Vogt*

The availability of a number of alligators ranging in weight from 600-2000 g presented an opportunity to study the sensitivity of these animals to whole-body X-radiation. The objectives of the study were to define the acute radiation syndrome and determine the acute lethal dose.

Observations

The study was comprised of two experiments, one started in November and another in July. In both experiments the alligators were housed outside in a closed pen containing a pond through which Columbia River water was

circulated at 70 °F. The pens were heated with heat lamps in November. A total of 55 alligators divided into groups of four or six each were given whole-body X-ray exposures with a 250 kV Maxitron. The X-ray beam was filtered with 0.5 mm Cu and 1 mm Al. The dose rate was 50 R/min. Ten alligators were held as controls. In the first experiment blood samples were obtained by heart puncture for hematology. Body weights were recovered in both experiments. The alligators were fed live trout weekly.

No deaths occurred the first month after exposure, even among those given 9000 R, with the exception of one given 800 R (Figure 1). This alligator apparently died from gastrointestinal problems not related to the X-ray exposure since large quantities of rocks and debris were found in its stomach. No histologi-

cal changes were noted which could be attributed to radiation. All alligators given 2500 R or more died between 31 and 55 days after exposure. Deaths among those given 900 to 1500 R occurred 37-63 days postexposure. There were 12 survivors when the experiment was terminated after 65 days. None of the controls died.

The relationship between dose and 60-day mortality is shown in Figure 2. One hundred percent mortality occurred in animals receiving 2500 R or more. Also, all of the animals receiving 1100 R died. The LD_{50} 60-day dose can reasonably be estimated as being between 900 and 1000 R. The fact that the two experiments were performed at different times of the year, summer and late fall, did not appear to be reflected in the mortality response.

The animals showed lethargy, partial paralysis, and ulceration around the mouth. However, not even the 9000 R animals showed changes

FIGURE 1. Survival Time for Ir-radiated Alligators (Results shown for two experiments)

FIGURE 2. Sixty-Day Mortality in Alligators After whole-Body X-Radiation

earlier than about 2 weeks after exposure. Weight loss was not a significant response to irradiation until about the week preceding death. The maximum weight loss was about 30% and was seen in several groups. However, at least one alligator in each group given up to 3500 R showed a net weight gain. Hemoglobin levels, packed cell volumes, and red cell counts remained unchanged at all dose levels at which hematology was studied, from 1500 to 9000 R (PCV = 25%, Hgb = 8%, RBC = $5 \times 10^5/\text{mm}^3$). The only significant change occurred in the leukocyte counts. The normal leukocyte counts ranged between 12 and 30,000 cells/mm³. The leukocyte levels decreased steadily until death. The maximum decrease occurred in alligators given the highest radiation doses. A week before death, the levels were 10-20% of control values.

Gross pathology consisted of slight hemorrhage in the gastrointestinal tract, and excess fluid in abdominal and thoracic cavities. In

general, the gastrointestinal tract showed little change. A number of the alligators had caseous abscesses in the lung, liver, and stomach. Many of those which were bled by heart puncture showed caseous lesions around the heart. Cultures of these lesions and abscesses generally showed coliform bacteria.

Histologic findings included:

- Loss of spermatogonia
- Degeneration and necrosis of the small intestine mucosa, liver, and spleen
- Alveolar and septal edema in lung
- Decreased bone marrow activity.

Conclusions

It was concluded that death was due to a combination of hematopoietic depression and small intestine damage plus bacterial infection. The most unusual feature of the findings was the long delayed response at dose levels as high as 9000 R.

STRONTIUM-90 STUDIES IN MINIATURE SWINE

Investigation of the biological effects of daily ingestion of ^{90}Sr in miniature swine was initiated at this laboratory in 1958. During the course of this continuing study, 718 swine, spanning three generations, have been exposed to ^{90}Sr feeding levels ranging from 1-3100 $\mu\text{Ci/day}$. An additional 270 untreated littermates have served as controls. Currently, 128 experimental swine, with 7-10 years of exposure and 63 controls, are being maintained for lifetime observations and study.

The originally anticipated primary "end point" of these studies was the production of osteosarcomas. However, the rather early appearance of a disproportionate number of hemopoietic and lymphoreticular disorders necessitated certain changes in our thinking and planning, and opened new areas of research presently being actively pursued, as evidenced by the reports included in this section of our Annual Report.

A total of 58 swine have thus far developed myelolymphoproliferative disorders after cumulative skeletal radiation doses of 100-19,000 rad. Six of these animals also exhibited bone tumors incidental to their primary hemopoietic disorders. In many respects, the leukemogenic processes observed in these swine mimic the spontaneous leukemias occurring in animals and man. Therefore, these animals provide an unusual opportunity for fundamental investigations of cellular kinetics, chromosomal alterations, "activated" virus-tumor cell interactions, etc. in these disease states.

It is notable that the parent generation of ^{90}Sr swine exposed from age 9 months have evidenced primarily myeloproliferative disorders while their offspring have, in addition, shown a markedly increased incidence of lymphoproliferative disorders. This suggests that exposure of the fetus and/or young animal may be a critical factor in the development of lymphoproliferative disorders, and a new series of exposures are being initiated to specifically investigate this facet of the leukemogenic problem.

In terms of original objectives, we have demonstrated the effect of chronic ^{90}Sr ingestion over a range of dose levels. The importance of marrow damage as opposed to osteosarcoma development is quite clear, though much remains to be done in delineating the more subtle effects that may afford mechanistic explanations for the more gross observations. Quantitation of radiation dose at the anatomic levels of interest also remains a challenging problem. It is also evident that a feeding level lower than $1\text{ }\mu\text{Ci/day}$ should be established, since effects are being observed at this presently lowest level of feeding.

PUBLICATIONS

McClellan, R. O., J. L. Beamer, and P. L. Sheldon. "Some Aspects of Strontium-90 Metabolism in Miniature Swine Ingesting Strontium-90 Daily," pp. 213-222. In: J. M. A. Lenihan, J. F. Loutit, and J. H. Martin (eds.) Strontium Metabolism, Academic Press, Inc. 1967.

HISTOPATHOLOGY OF ^{90}Sr -INDUCED LEUKEMIC PROCESSES IN SWINE

Histopathologic and hematologic evaluation of the lesions occurring in miniature swine chronically ingesting ^{90}Sr has resulted in the classification of 58 cases of hemopoietic neoplasms. The characteristics of these conditions are similar to the spontaneously occurring leukemias. An attempt has been made to evaluate the pathogenesis of the hemopoietic disorders and to discuss possible etiologic factors.

Investigators:

E. B. Howard

W. J. Clarke

M. T. Karagianes

Previous progress reports have described the experimental design of the chronic oral ^{90}Sr toxicity study in miniature swine and the occurrence of hemopoietic neoplasia in animals on various levels of feeding (Annual Reports, 1959 et seq.). Additional cases have been developing with increasing frequency, resulting in a total of 58 cases of hemopoietic neoplasia at this writing. Interesting variants which developed during the past year prompted intensive histologic and hematologic study in an effort to properly classify these types of proliferative disease or leukemia.

For the purpose of categorizing the types of hemopoietic proliferations observed, the nomenclature advanced by Dameshek and Gunz⁽¹⁾ has

been found useful and will be used as described below.

Nonneoplastic Hemopoietic Disorders

Extramedullary Myelopoiesis (myeloid metaplasia)

Proliferation of a normal spectrum of myeloid elements in sites other than bone marrow, such as liver, spleen, and lymph nodes. These tissues are normally considered potential sites for myelopoietic activity on demand.

Lymphoid Hyperplasia

Controlled proliferation of germinal follicles in normal lymphatic

tissue with increased production of lymphocytes, with no involvement of other tissues or blood.

Neoplastic Hemopoietic Disorders

Myeloproliferative Disorders

Excess proliferation of myeloid elements in bone marrow, liver, spleen, lymph nodes and sites other than these where extensive proliferation of myeloid elements would not normally be expected in response to demand. These include kidney, myocardium, lung, gonads, and brain. The cell population can vary from a normal myeloid spectra to a predominance of one cell type, or consist of completely undifferentiated cells.

- Leukemic - showing manifestations of tissue proliferation in peripheral blood.
 - a. Granulocytic leukemia
 - b. Erythrocytic leukemia
- Nonleukemic - not showing manifestations in peripheral blood.

Lymphoproliferative Disorders

Excessive proliferation of lymphatic elements in sites other than those considered normal lymphatic tissues.

- Leukemic - showing manifestations of neoplastic lymphoproliferation in the peripheral blood.
- Nonleukemic - no peripheral blood manifestations of lymphoid neoplasia.

Reticuloproliferative Disorders

Generalized proliferation of undifferentiated "stem" cells involving the hemolymphatic system and other tissues.

Leukemic - occurrence of undifferentiated blast cells in the peripheral blood.

- Nonleukemic - no peripheral blood manifestations of neoplastic process.

Plasmoproliferative Disorders

Neoplastic proliferation of lymphoid elements in the lymphatic system and other tissues with resulting production of plasma cells as a prominent characteristic.

Hodgkin's-like Disorder

Atypical neoplastic process involving the lymphoreticular system with a heterogeneous cell population consisting of lymphoid elements, plasma cells, eosinophils, reticulum cells and abnormal binucleated cells.

Observations

Utilizing the above histopathologic criteria, the 58 cases of ^{90}Sr -induced porcine hemopoietic neoplasias have been categorized as shown in Table I.

The bone marrow of animals on higher levels of ^{90}Sr feeding (625 and 3100 $\mu\text{Ci/day}$) was extensively damaged, with a paucity of myelogenous tissue, and with replacement of medullary spaces by fibrous connective

TABLE I. *Experimental Design, Indicating the Distribution of Cases of Hemopoietic Neoplasia in ^{90}Sr -Fed Miniature Swine*

History					Pathologic Diagnosis ^(a)				
^{90}Sr , $\mu\text{Ci/day}$	Generation	Animals on Experiment	Age at Death, months	Average krad Dose	M	L	R	P	H
3100	P	5	12-13	5-7	2				
625	P	6	17-19	3-8	2				
	F ₁	35	3.0-3.5	5	14				
		2 ^(b)	3.5-28			2			
125	P	12	8	19	3	1			
	F ₁	72	24-47	10-20	12	6	2	1	1
25	P	18	52	2.5	1				
	F ₁ , F ₂	146	50-62	3-4	2	1		1	
5	P	8	47	0.3		1			
	F ₁ , F ₂	98	--	--		--			
1	P	24	--	--		1			
	F ₁ , F ₂	23	71-81	0.3		3			
		1 ^(b)	60	0.01		1			
0	P	49	--	--					
	F ₁ , F ₂	221	--	--					
Totals					36	17	2	2	1

(a) *Myeloproliferative (M), lymphoproliferative (L), reticuloproliferative (R), plasmoproliferative (P), or Hodgkin's-Like disease (H)*

(b) *Exposed in utero + 6 weeks after weaning*

tissue. Simultaneously there was extensive extramedullary myelopoiesis (myeloid metaplasia) in the liver, spleen, and lymph nodes. The histopathologic characteristics of the tissues in these animals were comparable to the spontaneous myelosclerosis (myeloid metaplasia syndrome) which is thought to be a precursor phase to myelogenous leukemia of man. Those animals that had only extramedullary myelopoiesis at death or serial necropsy examination are not included in Table I.

At lower feeding levels the histologic appearance of the marrow varied from normal to a shift toward a higher percentage of blast cells. In animals developing frank hemopoietic neoplasia, the marrow often consisted primarily of blast elements, while in others the marrow appeared normal or only defective in megakaryocyte population.

In the hemopoietic neoplasias, gross lesions were observed at necropsy as whitish foci on the surface of the liver, spleen, kidneys, and

lymph nodes. Lesions were also observed grossly or histologically in the gonads, myocardium, lung, and brain. The spleen and lymph nodes were usually markedly enlarged, and the liver was often friable. Visceral and subcutaneous hemorrhages were often found due to the marked thrombocytopenia that was usually present.

In general, the more extensive and disseminated the neoplastic process, the more anaplastic the tumor cell population. In destructive, infiltrative lesions, the tumor cells were usually seen as homogeneous sheets of undifferentiated blast cells with many mitotic cells. Late in the disease these tumor cells were found to invade and fill the vascular spaces in the spleen and lymph nodes, often resulting in a clinical leukemic state.

As would be expected from the extensive damage to bone marrow by deposited ^{90}Sr , the most common proliferative syndrome involved myeloid cell types (Table I). These varied from cases with a spectra of proliferating myeloid elements differentiating along the lines of granulocytes, erythrocytes, and thrombocytes, to other cases in which there appeared to be selective proliferation of myeloblasts, or in rare instances, erythroblasts and some myeloblasts. This latter condition has been observed with a leukemic hemogram, and appears to fit the description of leukoerythroleukemia, or "polyphasic myeloproliferative disease," as described in man. In studying the development of this

condition by hematologic evaluation, it appears that the erythroblastic proliferation is the most significant feature, with the myeloblastic elements appearing in the blood late in the disease.

The significant number of lymphoproliferative disorders that have appeared in ^{90}Sr -fed animals (Table I) requires special consideration, since these would not necessarily be expected to result from bone marrow damage. It is possible that damage to the stem cell could result in selective proliferation of lymphoid elements. In some instances, the marrow was populated with neoplastic lymphoid cells, while in other cases the marrow appeared normal. The majority of lymphoproliferative disorders occurred in the F_1 and F_2 generation offspring of sows being fed ^{90}Sr . Three animals that developed lymphocytic neoplasia had received exposure only in utero, while nursing, and for 6 weeks after weaning. The in utero exposure to the thymus of the fetus must be considered as a possibly significant etiologic factor. One pig showed a thymic mass in the anterior mediastinum, histologically diagnosed as a thymoma. This was an adult pig in which the thymus should have been completely involuted. With these observations in mind, the in utero exposure of the fetus must be considered a potentially significant factor.

To pursue the possible role of in utero exposure in leukemogenesis, a new experiment was initiated in which sows will be bred after reaching ^{90}Sr

equilibrium, and the piglets will be transferred to normal sows for nursing. After weaning, they will be kept for observation without further radioisotope feeding.

Conclusions

It must be concluded from the accumulating evidence of this study that the radiation insult from chronically ingested ⁹⁰Sr results in a pluripotent neoplastic disease. These hemopoietic neoplastic processes mimic the spectra of naturally occurring leukemias. Granulocytic, erythrocytic, lymphocytic, reticulum cell, and plasmocytic proliferations have been observed. This seems to indicate that there has been stem cell damage with subsequent selective proliferation and differentiation of one main cell type.

It seems reasonable to speculate that the myeloid neoplasia developed subsequent to marrow damage and myeloid metaplasia of the reticulo-endothelial system. The myeloid metaplasia developed in response to a demand for blood elements, and per-

haps progressed to a state where control mechanism(s) regulating proliferation became defective and leukemia resulted.

In instances of lymphoreticular neoplasia at lower feeding levels, in utero exposure to the thymus and lymphatic tissue may play a significant role. Immunologic factors also may be involved, as a marked increase in serum immunoglobulin content is often noticed in these animals. This facet is separately discussed elsewhere in this Annual Report.

Finally, the role of radiation-activated virus as an etiologic factor must be considered, as this has been found to be significant in the induction of certain murine leukemias. Similar virus-like particles have been found in our leukemic miniature swine, as is discussed elsewhere in this Annual Report.

Reference

1. Dameshek, W. and F. Gunz. *Leukemia, 2nd Edition.* Grune and Stratton, Inc., New York. 1964.

INDUCTION OF BONE TUMORS IN MINIATURE SWINE FOLLOWING A SINGLE INJECTION OF ^{90}Sr

Three cases of bone neoplasia were observed in two pigs following a single intravenous injection of ^{90}Sr . These tumors were histologically and anatomically comparable to those previously reported in pigs exposed by chronic ingestion of ^{90}Sr , and were classified as osteosarcomas and giant cell tumors involving the skull and mandible.

Investigators:

E. B. Howard
W. J. Clarke
M. T. Karagianes

Technical Assistance:

V. T. Faubert

Skeletal deposition of parenterally administered ^{90}Sr in rats and dogs has resulted in the induction of bone tumors as one of the most significant lesions associated with chronic toxicity of this radionuclide. By contrast, chronic oral administration of ^{90}Sr to miniature swine has resulted in a high incidence of hemopoietic neoplasia during the 8 years of this study, with the occurrence of bone tumors in six pigs reported for the first time last year (Annual Report, 1966). No additional cases of bone neoplasia were observed during the past year.

Observations

In a group of 17 pigs given single intravenous injections of $64 \mu\text{Ci/kg}$ ^{90}Sr , three cases of bone neoplasia were recently observed in two pigs (Table I). A giant cell tumor (Figure 1), an osteosarcoma of the mandible, and an osteosarcoma of the skull (Figure 2) were the types seen. The sites of origin of these bone tumors

TABLE I. Bone Tumors in Swine Following a Single Intravenous Injection of $64 \mu\text{Ci}$ $^{90}\text{Sr/kg}$

Age at Exposure, months	Age at Death, months	Tumor Type(a)	Tumor Site	Est'd Dose, krad
6	46	G.C.T.	Mandible	9
36	118	O.S.	Skull	12
		O.S.	Mandible	12

(a) O.S. = osteosarcoma

G.C.T. = Giant cell tumor

were similar to those previously reported from the chronic feeding study, with the proliferative lesion arising in or near the periosteal surface of the bones. This is substantiated by the marked proliferation of multinucleated tumor cells (osteoclasts), which are believed to develop from the periosteum. Also, in nonneoplastic bone lesions consisting of reactive bone growth and exostosis, the periosteum is the tissue most extensively affected.

50X

FIGURE 2. Section from Giant Cell Tumor of the Maxilla Showing Numerous Proliferating Multinucleated Giant Cells Eroding Areas of Both Reactive and Normal Bone (H&E Stain)

50X

FIGURE 2. Section from Osteosarcoma of the Skull Showing Marked Extraperiosteal Proliferation of Neoplastic Spindle-Shaped Cells and Deposits of Newly Formed Osteoid Tissue (H&E Stain)

Conclusions

These tumors, induced by a single injection of ^{90}Sr , were similar to those induced by chronic ^{90}Sr feeding in site of occurrence, cell type, and cumulative dose to appearance. The

marked difference in latent period in the two animals may reflect the enhanced sensitivity of the younger animal with its greater volume of proliferating cells in the growing bone.

HEMATOLOGY AND CLINICAL CHEMISTRY OF ^{90}Sr -FED SWINE

Cellular profiles and levels of serum LDH, SGOT, SGPT, and alkaline phosphatase during the development of granulocytic leukemia are compared with those accompanying non-neoplastic extramedullary hemopoiesis.

Investigators:

*Patricia L. Hackett
Glenda S. Vogt*

Technical Assistance:

*Eugenia T. Edmerson
Darlene G. Hunter
Margaret L. Lawson
Martha C. Perkins
Starlene M. White*

During the past year, the major effort in clinical laboratory studies was placed on a detailed evaluation of individual animals throughout the development of terminal hematologic disorders. Additional cytochemical techniques were refined to aid in the classification of these disorders. Serum enzyme patterns were determined as possible indicators of early cellular damage.

Observations

Hematologic and serum enzyme results from animals developing hemopoietic disorders during 1967 are summarized in Table I. The types of hematologic changes observed are well illustrated by a comparison of two litter mates, Animals 1847 and 1857. Distinguishing traits in the development of myeloproliferative or granulocytic leukemia (neoplastic) are demonstrated by the cellular profile in Animal 1857, while values for 1847 typify the blood picture in non-neoplastic extramedullary myelopoiesis (Figure 1). Since these animals were

progeny of a sow which received 125 $\mu\text{Ci/day}$ of ^{90}Sr , their cellular levels were lower than those of control animals throughout most of their lives. The terminal hematologic profile of 1857 was characterized by a marked elevation in platelets and mature granulocytes, as well as in immature erythrocytes and immature granulocytes. In contrast, corresponding values for 1847 showed an early attempt to maintain cellular levels by the release of immature forms into the peripheral blood, with an ultimate deterioration in all cell series.

The appearance of appreciable numbers of immature cells, particularly granulocytes, was an overt signal for the development of terminal events. Accumulated average bone dose in these animals was calculated to be 11,000-12,000 rad.

Since the neoplasms and sites of extramedullary hemopoiesis are commonly found in the liver, assays were made for serum levels of alkaline phosphatase, lactic acid dehydrogenase (LDH) and the transaminases,

TABLE I. Hemopoietic Disorders Observed in ^{90}Sr -Fed Swine During 1967

Hemopoietic Disorder	Animal Number	^{90}Sr Feeding Level, $\mu\text{Ci/day}$	Generation	Age at Death, months	Terminal Blood Values					
					PCV, %	WBC, $10^3/\text{mm}^3$	Serum Enzymes ^(a)			
							Alk. Phos.	LDH	SGOT	SGPT
Myelo-proliferative	997	125	Parent	67	23	4.1	1.7	550	32	35
	982	125	Parent	67	16	5.5	0.6	580	23	24
	1257	125	F ₁	56	31	21	3.0	680	93	24
	1857	125	F ₁	34	28	160	3.6	1660	50	23
Lympho-proliferative	170	1	Parent	95	40	15				
	612	1	F ₂	81	33	24				
	384	25	F ₁	92	13	42				
Plasmo-proliferative	1845	125	F ₁	28	44	4.3	0.3	550	33	37
Extramedullary myelopoiesis	355	1	Parent	91	28	30				
	980	125	Parent	70	22	7.1	1.0	690	30	28
	1847	125	F ₁	33	17	5.5	1.8	1620	51	15
None		0	Parent		41	12	1.9	540	28	34

(a) Enzyme units: alkaline phosphatase, $\mu\text{M/ml/hr}$; lactic acid dehydrogenase, Wroblewski and LaDue units; glutamic-oxalacetic transaminase, Karmen units; glutamic-pyruvic transaminase, Wroblewski and LaDue units.

glutamic-oxalacetic transaminase (SGOT), and glutamic-pyruvic transaminase (SGPT), all of which are good indicators for hepato-cellular damage. Serum LDH and SGOT levels showed the greatest change (Figure 2), and their increased values appeared to coincide with the appearance of immature granulocytes. Previous work has shown that increased LDH activity may also reflect erythrocyte breakdown or organ damage resulting from virus infections.(1)

Results from alkaline phosphatase and SGPT assays were less enlightening. No specific clinical changes coincided with the elevation of alkaline phosphatase in Animal 1847; SGPT levels in both animals very

gradually decreased with no striking differences.

A sequential study of serum enzyme levels was also performed on a group of animals receiving 625 μCi of ^{90}Sr /day to observe changes in marrow turnover resulting from ^{90}Sr deposition. Although the ^{90}Sr feeding produced a 50% decrease in leukocyte levels, no significant alterations were detected in serum enzymes. Experimental manipulation of these animals, involving a series of bone marrow biopsies, however, resulted in a marked increase in SGOT levels for a short time. LDH values also were increased and remained elevated for a longer period.

FIGURE 1. Comparison of Hematologic Constituents in Myeloproliferation (Animal 1857) and Extramedullary Myelopoiesis (Animal 1847)

FIGURE 2. Comparison of Serum Enzyme Values in Myeloproliferation (Animal 1857) and Extramedullary Myelopoiesis (Animal 1847)

Conclusions

The appearance of immature cells and their definitive classification in peripheral blood smears is still the best indicator of the onset of a hemopoietic disorder. Preliminary studies on serum enzyme levels indicate that although significant changes do occur, they may not be directly related to the development of the blood dyscrasia. Further studies

will be necessary to determine if serum enzyme differences are apparent in the other types of hemopoietic disorders such as reticuloproliferative, erythro-leukemia, and lymphoid hyperplasia.

Reference

1. Ebert, P. S., M. A. Chirigos, L. A. Fields, and P. A. Ellsworth. *Life Sciences*, vol. 6, pp. 1963-1971. 1967.

SERUM PROTEIN ALTERATIONS IN ^{90}Sr -FED SWINE

Electrophoretic resolution of serum proteins from pigs developing hemopoietic neoplasia following chronic ingestion of ^{90}Sr has indicated a sharp alteration in the albumin and γ -globulin components. Serial disc electrophoresis of sera from pigs started on 625 $\mu\text{Ci/day}$ of ^{90}Sr has indicated the early appearance of a protein component not detectable in the sera of control pigs or experimental pigs before feeding.

Investigators:

E. B. Howard

C. C. Jannke

Technical Assistance:

Marilyn J. Bottorff

Fractionation and quantitation of serum proteins are sensitive procedures for the detection of pathologic processes, including inflammatory, neoplastic, immunologic, and toxicologic diseases. Observed deviations in serum proteins have proved to be of diagnostic and prognostic value in some instances.

This report describes alterations observed in the sera of miniature swine developing myeloproliferative

and lymphoproliferative syndromes following chronic ingestion of ^{90}Sr , as detected by routine microzone cellulose acetate electrophoresis. A more definitive study of early serum protein changes, evaluated by disc acrylamide gel electrophoresis (Canalco), is in progress in a group of pigs started on high level (625 μCi) daily feeding of ^{90}Sr , and early results from this study are also reported.

Observations

Serum analyses were performed periodically on ^{90}Sr -fed pigs through the preleukemic to the leukemic state, which occurred 2-4 years after initiation of ^{90}Sr feeding. There was a marked decrease in the albumin/globulin ratio prior to the onset of the leukemic state, which was due partly to an absolute decrease in albumin content and partly to an increase in the globulins, especially the γ -globulin (Figure 1).

Disc electrophoresis, at bi-weekly intervals, of serum from five pigs being fed 625 $\mu\text{Ci/day}$ of ^{90}Sr and from three control pigs has resulted in excellent resolution of the major protein components, and has also revealed some interesting changes. A new protein component, or a marked increase in a component that is not normally detected, has appeared in

each of the five experimental pigs. In each case, this protein has identical electrophoretic mobility on the discontinuous acrylamide gel system. This component, shown in tubes B of Figure 2, developed about 3-4 months after beginning ^{90}Sr feeding, and was not present in pre-experimental serum samples nor in samples from control pigs.

Conclusions

The significance of these protein changes has not been determined. Immunoelectrophoresis is being employed to identify the newly appearing component and in vitro tissue culture systems will be employed to evaluate possible biological activity. The component may be a seromucoid resulting from nonspecific tissue

FIGURE 1. Serum Protein, Indicating Alterations with Time After ^{90}Sr Ingestion (Note marked increase in γ -globulin and drop in albumin content as leukemic crisis developed. Total protein content increases with rise in γ -globulin.)

FIGURE 2. Disc Acrylamide Gel Electrophoresis of Pre-experiment Sera (A) and Serum at 6 Months After ^{90}Sr Feeding (B) from Three Miniature Swine [Note homogeneous protein component (arrow) in each sera, at 6 months, which was not seen in normal sera.]

damage associated with the ^{90}Sr feeding, or it may represent an "acute phase" protein. It may be a leukopoietic or erythropoietic stimulating

protein such as has been described as migrating in the α_2 -globulin region.

MARROW HEMOPOIESIS IN NORMAL PITMAN-MOORE SWINE

Analysis of labeled mitotic figure curves following tritiated thymidine injection indicates that swine erythrocytic and myelocytic precursor DNA synthesis time is 6-7 hr. The total generation time increases as the maturity of the cells increases. Erythrocytic G_2 time of 1-2 hr was shorter than myelocytic G_2 time of 4-6 hr.

Investigator:
P. H. Cooper

Earlier studies suggested that in swine granulocyte precursors spend more time than do erythrocyte precursors in the premitosis stage of the generation cycle (Annual Report, 1966). The present experiment was designed to study marrow cell generation cycles in the parent generation of ^{90}Sr swine. The study includes two control swine and three swine fed 625 μCi ^{90}Sr /day beginning at 2-1/2 years of age. Presented in this report are results from one of the control swine.

Observations

A Pitman-Moore sow weighing 80 kg was injected with 8 mCi tritiated thymidine. Autoradiograms were prepared of sternal marrow aspirated at various times after injection. Mitotic figures were examined for

the presence or absence of developed silver grains overlying the nucleus. Mitotic figures were classified on the basis of cytoplasmic staining characteristics and size, and were placed in one of five groups. Myeloblasts and rubriblasts were listed as unclassified immature cells. Pro-rubricytes, rubricytes, promyelocytes, and myelocytes made up the other four groups. Table I contains a summary of the generation cycle times derived for these five classes of cells.

The total generation time increased as maturity progressed, while DNA synthesis time remained relatively constant. If mitotic times in rubricytes and myelocytes were overestimated, as may be the case, then DNA synthesis time would have also increased with increasing maturity.

TABLE I. *Generation Cycle Times in Hours^(a)*

	Max <u>G₁</u>	Min <u>S</u>	Min <u>G₂</u>	<u>M</u>	<u>G_t</u>
Unclassified immature cells	3	6	1	1	11
Prorubricytes	3	6	1	1	11
Rubricytes	5	6	2	6	19
Promyelocytes	1	7	4	2	14
Myelocytes	2	7	6	4	19

(a) G_1 = Pre-DNA synthesis time

S = DNA synthesis time

G_2 = Post-DNA synthesis or premitosis time

M = Mitosis time

G_t = Total generation cycle

Longer G_2 times in the myelocytic series, as compared to the erythrocytic series, was the most striking result.

The DNA synthesis time found for swine hematopoietic cells agrees well with the DNA synthesis time reported for dog marrow cells, but is shorter than the 14-hr DNA synthesis time reported for human hematopoietic

cells. Human erythroid and myeloid precursors and dog erythroid precursors appear to spend the same amount of time in G_2 as swine erythroid precursors, but a shorter amount of time than swine myeloid precursors.

Conclusions

Since G_2 is more radiosensitive than other stages in the generation cycle, longer G_2 in myelocytic cells than in erythrocytic cells could have serious implications in radiation studies utilizing swine. The finding in swine ingesting 125 or 625 μCi ^{90}Sr /day of near normal red blood cell counts in the presence of greatly decreased blood neutrophil counts could be explained by longer exposure of myelocytic cells in the sensitive G_2 stage. Considered more generally, the observed differences in G_2 times emphasize the need for caution in comparing hematopoietic abnormalities between species until more specific information concerning generation cycle times is available for these species.

PHYTOHEMAGGLUTININ RESPONSE OF LEUKOCYTES FROM ^{90}Sr -FED SWINE

Chronic ingestion of ^{90}Sr by miniature swine resulted in an alteration of peripheral leukocytes such that they failed to respond, in vitro, to stimulation by phytohemagglutinin. This may indicate an immunologic incompetence which could play a role in the leukemogenic process.

Investigators:
E. B. Howard
C. C. Jannke

Chronic ingestion of ^{90}Sr by miniature swine has resulted in a high incidence of hemopoietic neoplasms, including clinical leukemias. The etiology and pathogenesis of these lesions have not been determined. Several experiments have been initiated to evaluate the immunologic status of these animals during ^{90}Sr feeding. Immunologic incompetency of the host may be a necessary prerequisite for the effectiveness of leukemogenic stimuli. Leukemogenic viruses may be suppressed by the normal antibody-producing system and may be able to express their pathogenicity only if this system is defective or suppressed by factors such as radiation or chemicals.

Cultured peripheral lymphocytes respond to phytohemagglutinin (PHA) stimulation by undergoing transformation to blast forms. This response has been considered to reflect the ability of these cells to respond to an antigenic stimulus, although the mechanism is not entirely clear. The experiment described in this report determined the responsiveness to PHA of cultured peripheral leukocytes from miniature swine at intervals before and during the feeding of 625 $\mu\text{Ci/day}$ of ^{90}Sr .

Observations

Peripheral leukocyte cultures were made at 2-week intervals in the standard manner, using chromosome culture media with and without PHA. Cell counts were made at 72 hr in a Coulter Counter. Results from such studies on six pigs are summarized in Figure 1.

Prior to the feeding of ^{90}Sr , the leukocyte cultures responded actively to the addition of PHA by undergoing blastogenesis and mitosis. This responsiveness decreased markedly after 1 month on ^{90}Sr feeding (625 $\mu\text{Ci/day}$) and by 2 1/2 months there

FIGURE 1. Response of Peripheral Leukocytes to Phytohemagglutinin Stimulation

was no response of the cultured cells to added PHA. This lack of response has persisted after 6 months on the feeding schedule.

Conclusions

Numerous reports have indicated a relationship between the ability of peripheral lymphocytes to transform under the influence of PHA, and the ability of the body to respond normally to an injected antigen. In some spontaneous leukemias of man,

both of these factors appear to be defective. If in vitro responsiveness of peripheral lymphocytes to PHA stimulation is, in fact, related to immunocompetence, it appears that exposure to ^{90}Sr has a marked effect on this antibody-producing mechanism. One may speculate that this lack of ability to respond in a normal immunologic manner, either to viral agents or to transformed neoplastic cells, is a critical factor in the leukemogenic process.

POSSIBLE VIRAL ASSOCIATION IN ^{90}Sr -INDUCED PORCINE LEUKEMIA

Electron microscopic examination of hemopoietic neoplastic tissue and high-speed plasma pellets from ^{90}Sr -fed miniature swine revealed particles with the size and morphologic appearance of virus-like particles associated with spontaneous leukemias of various species. Inoculations of cell-free filtrates have given tentative indication of adaptation or biologic effect on certain cell lines.

Investigators:

E. B. Howard
W. J. Clarke
R. N. Ushijima*
M. E. Frazier*

Technical Assistance:

C. C. Jannke
Judi Lund

Fifty-eight cases of hemopoietic neoplasia have now been observed in miniature swine following chronic ingestion of ^{90}Sr . These include a predominance of myeloproliferative disorders with lesser numbers of lymphoproliferation and other variants including erythroleukemia and reticulosis. It is known that leukemo-

genic viruses may be pluripotential and produce a spectrum of leukemic disorders in avian and murine species, and that radiation induction may be involved in the pathogenesis of certain virus leukemias of mice.

Observations

During the past year a series of investigations were initiated to determine if a virus is present in

* Consultant-collaborators, Montana State University, Missoula, Montana.

neoplastic tissues induced by ^{90}Sr feeding. Tissues from several leukemic pigs were fixed and embedded for electron microscopic examination. These tissues included lymph nodes, liver, and spleen. Blood plasma was collected from these animals and subjected to high-speed centrifugation and the resulting pellets were examined in a similar manner.

Virus-like particles were observed in tumor cells from lymph node and spleen sections. The particles were frequently associated with the membrane of cytoplasmic vacuoles (Figure 1) or the outer cell membrane. The particles were 60-80 m μ in diameter and had the general morphologic appearance of "C-type" particles. An electron-dense nucleoid which is typical of this type particle was not observed. Examination of plasma pel-

lets disclosed the presence of particles of similar size and morphology to those observed in the neoplastic cells (Figure 2).

Filtrates (0.45 μ) of leukemic tissue were inoculated into various tissue culture systems to detect biological activity of these cytoplasmic particles. In newborn porcine kidney cultures the particles were retained in the cultured cells for as long as 12 passages, and could be detected by electron microscopy. Cytopathic effects were observed in fetal hamster cells, as characterized by altered growth rate, appearance of multinucleated cells, and loss of contact inhibition. Culturing of tissues from leukemic pigs has resulted in the detection of bizarre cytoplasmic inclusions in fibroblastic cells grown

56,000X

FIGURE 1. Subcellular Particles (Arrows) in Vacuoles and Budding into Vacuole in a Tumor Cell. Lymph Node Section

56,000X

FIGURE 2. Discrete Particles (Arrow), Morphologically Similar to Those in Figure 1, Observed in a High-speed Plasma Pellet from a Leukemic Pig

from the lung, kidney, and spleen. There was a marked tendency for the formation of syncytial masses containing numerous nuclei in the cultures from these tissues.

As a followup to these preliminary observations, experiments have been initiated using a broad range of cell cultures in an attempt to unmask a latent virus which may be present. Screening studies have also been initiated to determine the normal viral population of our pig herd by culture systems and specific anti-sera inactivation.

Conclusions

Early studies have revealed the presence of subcellular particles,

similar to viruses, in ^{90}Sr -induced leukemic tissue and plasma. It has not been ascertained if these are merely transport viruses involving the damaged tissue or if they are latent oncogenic viruses that are activated by the radiation. In vitro studies indicate certain features of cellular transformation. It is now necessary to relate this agent to the normal viral flora present in the pig herd, and if it is not found as a normally present agent, it must be determined if it plays a role in the pathogenesis of the ^{90}Sr -induced leukemia.

FAMILIAL PATTERNS IN THE DEVELOPMENT OF HEMATOPOIETIC DISORDERS IN ^{90}Sr -FED SWINE

No correlation was found between the incidence of hematopoietic dyscrasias and familial relationships in swine fed 25, 225, or 625 μCi ^{90}Sr daily. A higher incidence in the F_1 and F_2 generations suggests the importance of in utero and/or early postnatal ^{90}Sr exposure.

Investigators:
*Beatrice J. McClanahan
R. F. Palmer
Patricia L. Hackett*

The familial relationships of swine in our ^{90}Sr chronic feeding study on daily feeding levels of 25, 125, or 625 μCi were examined to determine if a correlation existed between the occurrence of hematopoietic neoplasia or extramedullary hematopoiesis and familial background.

Observations

Table I summarizes the frequency of hematologic dyscrasias in these swine. The animals designated "at risk" are those who were on experiment long enough for the development of a disorder. Animals killed to

TABLE I. Incidence of Hematopoietic Disorders in ^{90}Sr Swine by Family Groupings

^{90}Sr Feeding Level, $\mu\text{Ci/day}$	Family Design- nation	Original Sows			F ₁ Generation			F ₂ Generation		
		No. at Risk	No. Dead Hemato- poietic Disorder		No. at Risk	No. Dead Hemato- poietic Disorder		No. at Risk	No. Dead Hemato- poietic Disorder	
			Yes	No		Yes	No		Yes	No
25	1	2	--	--	4	1	1	6	--	--
	2	2	--	--	3	--	--	2	--	1
	3	1	--	--	2	1	--	--	--	--
	4	4	1	--	3	--	--	--	--	--
	5	3	--	--	5	1	--	7	1	--
125	6	3	2	1	5	3	2	9	6	3
	7	1	--	--	3	3	--	--	--	--
	8	2	--	--	4	2	1	--	--	--
	9	2	2	--	10	9	1	--	--	--
	10	2	--	--	3	--	2	--	--	--
	11	1	1	--	1	1	--	--	--	--
625	12	1	--	1	6	5	1	--	--	--
	13	3	2	1	15	11	4	--	--	--
	14	1	--	1	3	2	1	--	--	--
	15	1	--	1	4	1	2	--	--	--

obtain ^{90}Sr tissue distribution data are not included in these figures. A "family" consists of littermate sows and their descendants.

In the 25 $\mu\text{Ci/day}$ group, there is an insufficient incidence of hematologic dyscrasias to allow any inference concerning familial pattern. As the incidence increases with time, a trend may become apparent. In the 125 $\mu\text{Ci/day}$ group, relatively few animals have died without showing hematopoietic disorders so that, again, no familial trend is apparent. In the 625 $\mu\text{Ci/day}$ group, there was a uniform high incidence in the F₁ generation which was fed ^{90}Sr for

only 6 weeks after weaning. No F₂ animals were obtained at this feeding level.

Conclusions

No relationship between familial background and incidence of hematopoietic disorders are as yet apparent in the animals of this study. The higher incidence seen in the F₁ and F₂ generations, as compared to the parent sows, suggests that in utero and/or early postnatal exposure to ^{90}Sr may be a significant factor in the development of these disorders.

RADIOSTRONTIUM RETENTION ON SWINE TEETH

Orally administered ^{85}Sr was not retained on the teeth of healthy miniature swine; therefore, periodic teeth cleaning is not warranted in the chronic strontium feeding program.

Investigator:
C. R. Watson

Many of the swine on chronic ^{90}Sr feeding develop lesions in the skull or mandible. If this were due to increased radiation dose from ^{90}Sr trapped on teeth, extrapolation from swine to man would be questionable, because humans generally practice better oral hygiene than swine. The object of this experiment was to evaluate the extent of teeth contamination following oral radiostrontium administration.

Observations

Five Hanford Miniature Swine were fed 7 μCi of ^{85}Sr in a single, spiked food pellet. Three were given the same dose intravenously. External gamma-ray counts over the head were

taken at intervals for 24 hr. Only 2% of the administered dose was detected in the head of swine following oral administration. This increased to 3% after 24 hr. About 10% of the intravenously administered dose was in the head region throughout the 24 hr period.

Conclusions

It would appear that little or no particulate matter is retained in the mouths of healthy pigs. Externally contaminated teeth which have sometimes been observed at necropsy may result from the general lack of activity (especially vigorous chewing) which is a prelude to death.

INHALATION STUDIES

This is the largest section of our Annual Report and includes only a portion of the total effort on inhalation problems. Four reports in the Space Nuclear Systems Studies Section are concerned with $^{238}\text{PuO}_2$ inhalation and several reports in the Radioelement Removal Section relate to the removal of inhaled radionuclides.

This year's report reflects a rather heavy emphasis on plutonium problems. In addition, single reports describe the culmination of long-term studies on cerium-praseodymium and promethium inhalation studies. A single report on uranium ore inhalation constitutes the vanguard of a major effort in this area which will no doubt be heavily represented in reports over the next few years. Our continuing interest in basic lung biochemistry is reflected by two reports on lipid synthesis in the lung. The final report in this section bears no necessary relation to inhalation studies, but describes a very useful anesthetization and blood sampling procedure that was developed in connection with our small animal inhalation experiments.

While 14 dogs out of 40 initially exposed still survive in the ninth year of our long-term $^{239}\text{PuO}_2$ inhalation study, the pattern of results is by now fairly apparent. Data from this and other PuO_2 inhalation experiments have been combined to establish a computer-derived model which seems to offer many advantages in summarizing and extrapolating. As more data become available from these plutonium oxide inhalation experiments, we are made increasingly aware of the need for information on other chemical forms of plutonium and information at lower dose levels. A report in this section describes results of an acute toxicity inhalation study with plutonium nitrate in beagles, which represents a start in this direction. Low-level exposures are, of course, of the most critical interest, and also, unfortunately, are the most expensive in time, facilities, and personnel.

Several reports are concerned with the constancy of plutonium-ameridium ratios in exposed animals, which relates to the problem of estimating plutonium content from external counting of the ^{141}Am contaminant present in nearly all samples of plutonium. It would appear that considerable reliance can be placed on such a procedure when plutonium is inhaled, as the oxide, but that this

may not be true for more soluble forms of plutonium. In this connection, attention should be called to a report in the Terrestrial Ecology Section of this document, which points out the very markedly different behavior of plutonium and americium in certain plant-soil situations.

PUBLICATIONS

Bair, W. J. and J. V. Dilley. "Pulmonary Clearance of $^{59}\text{Fe}_2\text{O}_3$ and $^{51}\text{Cr}_2\text{O}_3$ in Rats and Dogs Exposed to Cigarette Smoke," In: Inhaled Particles and Vapours II, edited by C. N. Davies. Pergamon Press, New York, 1967. pp. 251-271.

Park, J. F., W. J. Bair, W. J. Clarke, and E. B. Howard. "Chronic Effects of Inhaled Plutonium Dioxide in Dogs," Radiation Res., vol. 31, p. 553. 1967. (Abstr.)

Stuart, B. O. "Inhalation of Promethium Oxide by Beagle Dogs," Health Physics, vol. 13, p. 929. 1967. (Abstr.)

Stuart, B. O. and T. M. Beasley. "Non-Equilibrium Tissue Distributions of Uranium and Thorium Following Inhalation of Uranium Ore by Rats," In: Inhaled Particles and Vapours II, edited by C. N. Davies. Pergamon Press, New York, 1967. pp. 291-298.

Swinth, K. L. "Dependence of Plutonium X-Ray Counting on Isotopic Composition," Health Physics, vol. 13, p. 930. 1967. (Abstr.)

Swinth, K. L., B. I. Griffin, and J. F. Park. "Whole Body Counting of Inhaled Plutonium in Dogs," Health Physics, vol. 13, pp. 1223-1231. 1967.

Tombropoulos, E. G. "Biochemical Changes in Lung Tissues After Thoracic X-Irradiation," In: Proceedings of Seventh International Congress of Biochemistry, Tokyo. Pergamon, Oxford, 1967. p. 1039. (Abstr.)

Tombropoulos, E. G. "Fatty Acid Synthesis by Subcellular Fractions of Lung Tissue," In: Inhaled Particles and Vapours II, edited by C. N. Davies. Pergamon Press, New York. 1967. pp. 17-23.

Tombropoulos, E. G. "Synthesis of Long-Chain Fatty Acids by the Mitochondrial Fraction of Lung Tissue," In: Progress in Biochemical Pharmacology, edited by D. Kritchevsky, R. Paoletti, and D. Steinberg. S. Karger, Basel, Switzerland, 1967. vol. 3, pp. 23-39.

CHRONIC EFFECTS OF INHALED $^{239}\text{PuO}_2$ IN BEAGLES

Data thus far available indicate that a level of ^{239}Pu which would not cause life shortening in dogs is that amount which would result in a lung burden of less than about 2 nCi/g lung 15 years after exposure.

Investigators:
J. F. Park
W. J. Clarke
W. J. Bair

A long-term study of the biological effects of inhaled $^{239}\text{PuO}_2$ in dogs is in its ninth year, and the results of this study have been reported annually. Of 40 dogs exposed, 20 have died or were sacrificed when death was imminent, while five were sacrificed to obtain tissue distribution data. Twelve of the 20 dogs have had primary pulmonary tumors. Since the last Annual Report, one additional dog was sacrificed at 67 months postexposure, with a primary pulmonary tumor. Several of the 14 surviving dogs show radiographic evidence of lung tumors. Most show lymphopenia, which has been a consistent finding in this study.

The tissue distribution of plutonium and the pathologic findings were summarized in last year's report. Analyses of the tissues from the last tumor dog sacrificed are

incomplete. This report summarizes all of the mortality and tumor data obtained to date from several experiments with dogs which inhaled $^{239}\text{PuO}_2$.

Observations

Figure 1 shows the relationship between the concentration of plutonium in the lung at death and the survival time after exposure for dogs that died or were sacrificed when death was imminent due to respiratory insufficiency. The data were fitted by least squares analysis. Seven of these dogs had primary pulmonary neoplasia in addition to severe pulmonary fibrosis and metaplasia, but the neoplasia was an incidental finding at necropsy or observed in thoracic radiographs after severe respiratory insufficiency developed.

FIGURE 1. Relationship Between Quantity of ^{239}Pu in Lung at Death and Survival Time of Dogs (Dashed lines indicate 95% confidence limits)

Thus, the relationship expressed is concerned with death due to pulmonary insufficiency caused primarily by pulmonary fibrosis and metaplasia. Extrapolating these data to 15 years, the near maximum life span of the dog, we estimate that a dog would not

die of plutonium-induced nonneoplastic pulmonary lesions with a lung burden less than about 2 nCi/g lung. However, life span could be shortened by the development of a lung tumor. The data from five dogs sacrificed when pulmonary neoplasia was observed in the thoracic radiographs without the occurrence of severe respiratory insufficiency are also plotted in Figure 1, but were not included in fitting the curve to data for pulmonary deaths. It appears that lung neoplasia may occur at levels of plutonium below those that would eventually produce a pulmonary death. Further information on this point is expected from the dogs remaining on this experiment.

Conclusions

From a compilation of all mortality data on dogs that inhaled $^{239}\text{PuO}_2$, it is concluded that lung burdens less than 2 nCi/g will not cause life shortening due to respiratory insufficiency, but may cause life shortening due to pulmonary carcinogenesis.

DYNAMIC SIMULATION OF RETENTION AND TRANSLOCATION OF INHALED
PLUTONIUM OXIDE IN BEAGLE DOGS

Short- and long-term analog computer simulation models were developed for inhaled plutonium. The long-term model predicts that 15 and 60% of the amount deposited in the deep lung remains in the deep lung and lymph nodes, respectively, 25 years after exposure.

Investigators:
B. O. Stuart
P. J. Dionne*

A dynamic simulation model for inhaled plutonium oxide was constructed using hybrid computer facilities. Blood, tissue, and excretion data collected from more than 50 beagle dogs up to 7 years after inhaling $^{239}\text{PuO}_2$ were incorporated into a program for predicting long-term retention and translocation of inhaled insoluble plutonium.

Observations

The dynamic simulation model for inhaled plutonium oxide (Figure 1) consists of 13 compartments or pools. Deposition of $^{239}\text{PuO}_2$ is split among four compartments: 50% in a combined nasopharynx-tracheobronchial compartment having an effective removal rate or half-time of 8 min, 20% in a fast pulmonary compartment with a 1 day half-time, 10% in a slow pulmonary compartment with a continually varying half-time, and 20% in another slow pulmonary compartment with a long constant half-time of 3-1/2 years. The arrows in Figure 1 indicate the important pathways by

which a given percentage of plutonium leaves these four compartments. The biological half-times shown in parentheses for the various compartments are from the ICRP Task Group on Lung Dynamics for the fast pulmonary and tracheobronchial lymph nodes compartments,⁽¹⁾ Eve's values for the GI tract,⁽²⁾ and the ICRP-suggested values for the systemic organs.⁽³⁾

Our recent dog data suggest that long-term whole-body retention of plutonium follows a logarithmic relationship with time after exposure. For data from five long-term dogs, single regression lines were obtained with slopes varying from -0.023 to -0.037. This logarithmic decrease of the whole-body burden with time fits the concept of two slowly clearing deep lung compartments, one of which feeds the lymph node compartment at a low, but constant rate. Our dog data suggest that the half-time may be as long as 42 months. Material leaving the second slow pulmonary compartment enters the blood, lymph nodes, or GI tract. The biological half-time of this compartment appears to be continually increasing, thus matching long-term

* *Applied Physics and Electronics
Department*

FIGURE 1. Model for the Retention and Translocation of Inhaled $^{239}\text{PuO}_2$ in Beagle Dogs

whole-body data; i.e., the initial half-time may be as short as 6 months, doubling every 6 months. The material from the fast pulmonary compartment feeds into the GI tract.

Figure 2 shows the computer results for the short-term inhalation model. The simulation is set up to predict the amounts deposited in the various compartments of the lung, given the whole-body and fecal data for the first 2 weeks after exposure. This is done by matching the model to the data for each animal. This model also shows the amounts of the initi-

ally soluble portion of the inhaled $^{239}\text{PuO}_2$ that reach the systemic organs. These values are then incorporated into the long-term inhalation model which predicts the long-term burdens for the deep lung, lymph nodes, and systemic organs after a single inhalation exposure. Figure 3 shows typical results from the long-term simulation, as well as the experimental points to which the model was fitted.

The model has served to point out areas where additional data are needed. For example, experimental

FIGURE 2. Short-Term Inhalation Model Predictions

FIGURE 3. Long-Term Inhalation Model Predictions

data are unavailable on the dynamics of plutonium entering the blood from the lung. Additional long-term data are needed to test compartment retention times and to verify the model.

Conclusions

The long-term model for retention and translocation of inhaled plutonium oxide predicts that 15 and 60% of the plutonium deposited in the deep lung will remain in the deep lung and lymph nodes, respectively, 15 years after exposure, and that the

whole-body burden decreases less than 10% after the initial rapid clearance which occurs during the first 2 weeks after exposure.

References

1. *Task Group on Lung Dynamics. Health Physics, vol. 12, pp. 173-207. 1966.*
2. *Eve, I.S. Health Physics, vol. 12, pp. 131-161. 1966.*
3. *Report of ICRP Committee 2 on Permissible Dose for Internal Radiation (1959), Health Physics, vol. 3, 1960.*

USE OF IN VIVO COUNTING TO DETERMINE RETENTION OF INHALED ^{239}Pu IN DOGS

Whole-body burdens of ^{239}Pu in dogs determined by in vivo counting of the 17 keV X ray from ^{239}Pu and the 60 keV γ ray from ^{241}Am compared closely with values obtained by alpha analyses of the total tissues.

Investigators:
K. L. Swinh*
W. J. Bair

Technical Assistance:
W. Skinner

Many attempts have been made to utilize in vivo counting methods to determine the retention of inhaled ^{239}Pu in dogs. The purpose was not only to facilitate the objectives of the particular experiment, but also to obtain information which might be

applicable to the development of in vivo counting procedures for use in human exposures. In a recent experiment, 12 dogs were given a single exposure to $^{239}\text{PuO}_2$ aerosols. The tissue distribution and excretion data were reported previously (Annual Report, 1966). This report summarizes results of in vivo counting of the dogs.

* *Applied Physics and Electronics Department*

INHALATION STUDIES

Observations

After exposure to the plutonium aerosol, dogs were cleaned of external contamination, wrapped in a thin polyethylene sheet, and counted. Counting started within 1 hr after exposure and continued on a daily schedule for 2 weeks, then weekly, and finally, biweekly after 2 months. The dogs were counted unanesthetized and placed on their backs in the center of the four plutonium counter units.⁽¹⁾ One unit was below the back, one above the chest, and one on each side of the chest. Counting was performed in a cave made of old steel and lined with lead. Since the dogs were not anesthetized, it was necessary for a handler to remain with them in the counter.

Two energy bands were used for analysis of the counting results. One band covered the 17 keV X ray from ^{239}Pu and the other covered the 60 keV γ ray from ^{241}Am . Results from a typical dog are shown in Figure 1. Count rates were high and the backgrounds of approximately 100 cpm in the X-ray region and 200 cpm in the y-ray region, posed no problem. Controlling the horizontal position of the dog was a major difficulty and no doubt accounts for a major portion of the variability seen in the data of Figure 1.

Half-times for retention of the rapidly eliminated component were calculated for each dog. The ratio of initial count rate to that achieved after the elimination rate leveled off was also determined. These values are shown in Table I.

FIGURE 1. Total Body Count Rate Following Inhalation of $^{239}\text{PuO}_2$ as Measured Using 17 keV X Ray or 60 keV Gamma

Average values of 1.00 ± 0.62 days and 1.07 ± 0.85 days were obtained for the initial retention half-times as deduced from the X-ray and y-ray counting results, respectively. Average values for the ratios of initial to final deposition were 6.0 ± 5.1 and 6.4 ± 4.7 , respectively. The large standard deviations attest to the extreme individual animal variability in these parameters.

No attempt was made to estimate the long-term retention half-time from the in vivo counting data. Half-time values calculated from excretion analysis ranged from 950-3440 days (Annual Report, 1966).

Final in vivo counts were made on anesthetized animals shortly before they were sacrificed. Calibration

TABLE I. Results of In Vivo Counting of Inhaled $^{239}\text{PuO}_2$: Comparison of Values Based on X-Ray and Gamma Counting and Tissue Radioanalysis

Dog Number	Ratio: Initial Count Final Count		Initial Retention Half- Time, days		Final Body Burden, μCi		
	X Ray	Gamma	X Ray	Gamma	X Ray	Gamma	Tissue Analysis
306	5.4	5.2	0.90	0.85	1.5	1.8	1.8
311	2.9	3.8	0.85	0.86	3.7	3.9	3.2
313	2.9	4.1	1.0	0.95	7.3	7.1	6.0
307	2.4	3.5	1.0	0.95	81	62	45
308	2.8	3.2	1.4	1.5	22	23	20
316	1.0	1.0	∞	∞	6.5	6.4	6.1
339	4.7	5.3	1.2	1.1	22	27	29
317	2.4	3.1	0.76	0.79	8.6	7.8	7.5
323	13	13	0.31	0.31	6.2	5.5	5.1
318	18	18	2.7	3.2	1.6	1.5	1.3
334	10	8.0	0.48	0.69	7.9	7.4	7.1
352	5.4	7.2	0.44	0.53	25	23	20
Averages	6.0 $\pm$ 5.1	6.4 $\pm$ 4.7	1.00 $\pm$ 0.62	1.07 $\pm$ 0.85			

factors were obtained from measurements with a phantom. Body burdens estimated from these counts are compared in Table I with values determined from plutonium analysis of the tissues. Agreement was, in general, quite satisfactory, with the gamma counting showing somewhat closer correlation to the tissue analyses than the X-ray counting.

Conclusions

In vivo counting of dogs following inhalation of $^{239}\text{PuO}_2$ provided estimates of the body burdens which agreed closely with values obtained by alpha analyses of the tissues. However,

daily variations in whole-body counts, particularly when made on unanesthetized animals, were too great for reliable estimates of the long-term retention half-times. The results were not conclusive in demonstrating the superiority of counting the gammas from ^{241}Am rather than the low energy X rays from ^{239}Pu .

Reference

1. Swinth, K. L. and B. I. Briffin. *Hanford Radiological Sciences Research and Development Annual Report for 1964*, pp. 2.27-2.31, BNWL-36. Pacific Northwest Laboratory, Richland, Washington, January 1965.

THE DETERMINATION OF PLUTONIUM TO AMERICIUM RATIOS IN BIOLOGICAL SPECIMENS

Techniques were developed to determine the plutonium-to-ameridium ratios in organs of animals which had inhaled a mixed plutonium-ameridium oxide. These methods employed alpha pulse height or photon spectrometry techniques.

Investigators:
Y. M. Jones*
P. O. Jackson^x

For evaluating the relative behavior of plutonium and ameridium in exposed animals, a very precise method of measurement is required. Two techniques developed for this purpose are described in this report. Both allow for simultaneous measurement by direct counting techniques, eliminating the necessity for chemical separation of the two radionuclides which could result in preferential loss of one or the other.

Observations

In the first method, following wet ashing of the tissue, all salts are converted to chlorides and the plutonium and ameridium electroplated from an ammonium chloride electrolyte. Typical plating recoveries exceed 99% in the absence of interfering trivalent elements. Following electroplating, alpha pulse height analysis was used to measure the ratio of 5.15 MeV alphas to the 5.48 MeV alphas (Figure 1). The 5.15 MeV alphas are emitted by ^{239}Pu and ^{240}Pu while the 5.48 MeV alphas are emitted by both

^{241}Am and ^{238}Pu . The additional peak at 4.18 is due to ^{238}U which is added as a carrier for electrodeposition. To use this method for plutonium-ameridium analysis, it is necessary that the ratios of the plutonium isotopes be known.

Photon spectrometry is employed in the second method. Plutonium X rays and the low energy ameridium gamma rays are measured with a recently developed lithium-drifted silicon

FIGURE 1. Alpha Spectra of ^{239}Pu and ^{241}Am in a Biological Sample

* Environmental and Radiological Sciences Department

diode having approximately 1 keV resolution (Figure 2). The greater penetration of these photon emissions allows direct counting of wet-ashed samples if corrections for self-absorption effects are made. These effects depend on the sample mounting technique and a very uniform mount must be used.

Conclusions

Where samples have been analyzed by both of these methods, good agreement was obtained. The photon spectrometric method is preferred because it can be applied directly to wet-ashed samples. However, because present silicon diode detectors are small and the X-ray fluorescence yields for ^{239}Pu are low, the overall counting efficiency is lower than that which can be achieved with alpha analysis.

FIGURE 2. Photon Spectra of ^{239}Pu and ^{241}Am in a Biological Sample (Lithium-drifted silicon diode detector)

PLUTONIUM-AMERICIUM RATIOS IN DOGS AFTER INHALATION OF $^{239}\text{PuO}_2$

Americium and plutonium showed a different biological behavior in dogs after inhalation of $\text{Pu}(\text{NO}_3)_4$, but not after inhalation of PuO_2 .

Investigators:
W. J. Bair
R. W. Perkins*

The relative retention, translocation, and excretion of plutonium and americium are being studied in dogs

that inhaled ^{239}Pu aerosols containing ^{241}Am present as a contaminant. The purpose of these studies is to determine whether ^{241}Am , a gamma emitter, is a valid tracer for ^{239}Pu in whole-body monitoring of

* *Environmental and Radiological Sciences Department*

humans. Tissues from dogs that had inhaled plutonium were analyzed for ^{239}Pu and ^{241}Am by the methods described in the previous paper. All ratios and percentages are expressed on a radioactivity basis.

Observations

Preliminary results have been obtained from three types of plutonium exposures. Table I shows ^{239}Pu - ^{241}Am ratios in tissues of dogs sacrificed 3 months after inhalation of one of four different preparations of $^{239}\text{PuO}_2$. These ratios are compared with the ratios in a sample of the

aerosol collected at the time of exposure. In dogs that inhaled the oxide prepared by heating the metal to 450 °C, there was a slightly increased ^{239}Pu - ^{241}Am ratio in the lungs and the lymph nodes, suggesting a relatively greater clearance of ^{241}Am . Greatly increased ratios were observed in the lymph nodes of one dog inhaling oxalate calcined at 1000 °C; however, this was not observed in a second dog. Samples from a third dog are being analyzed.

Data obtained at much longer times following inhalation of $^{239}\text{PuO}_2$, prepared by calcining the oxalate at 350 °C, are shown in Table II. The

TABLE I. ^{239}Pu - ^{241}Am Ratios in Dog Tissues 3 Months After Inhalation of $^{239}\text{PuO}_2$ Formed From the Oxalate or Pu Metal

Dog Number	$^{239}\text{PuO}_2$ Form of Inhaled	^{239}Pu - ^{241}Am Ratios (a)						
		Aerosol	Lung	(b)				
				Lymph Nodes				
				M	R		L	
311	Oxalate calcined at 1000 °C	17.4 ± 2.2	11.6 ± 0.7	36.4 ± 3.4	28 ± 4		57 ± 9	
313		13.8 ± 0.3	13.4 ± 0.3	11.6 ± 0.6				
307	Oxalate calcined at 350 °C	12.0 ± 0.2	13.0 ± 1.2	14.2 ± 0.1				
316		13.4 ± 0.7	10.8 ± 1.2	13.4 ± 0.9	16.2 ± 1.0		17.2 ± 2.3	
309	Metal oxidized at 450 °C	31.0 ± 1.9	31.5 ± 3.2	33.5 ± 2.6	32.6 ± 2.0		30.3 ± 2.5	
317		31.0 ± 1.9	37.8 ± 1.5	36.1 ± 1.2				
323		31.0 ± 1.9	39.6 ± 4.1	49.6 ± 3.0	33.6 ± 4.0		52.7 ± 5.0	
352	Metal oxidized at 123 °C	31.0 ± 0.6	31.5 ± 1.0	30.0 ± 0.9				

(a) ± Values are standard deviations associated with the measurements

(b) M = media2 bifurcation
R = right bifurcation
L = left bifurcation

TABLE II. ^{239}Pu - ^{241}Am Ratios in Dog Tissues at Long Intervals After Inhalation of $^{239}\text{PuO}_2$

Dog Number	Time After Exposure, months	^{239}Pu - ^{241}Am Ratios(a)			
		Aerosol	Lung	Lymph Nodes ^(b)	Liver
268	40	12.6 ± 0.2	12.8 ± 0.2	10.5 ± 0.3	
257	44	13.0 ± 1.0	15.8 ± 1.3	17.2 ± 0.8	
259	54	13.6 ± 0.2	13.4 ± 0.4	11.4 ± 0.4	
246	54	13.1 ± 0.3	11.8 ± 0.4	9.8 ± 0.2	
216	60	13.0 ± 1.0	15.4 ± 1.3	15.6 ± 0.8	7.5 ± 1.0
85	66	(c)	15.4 ± 0.8		10.6 ± 1.5
86	67	(c)	6.3 ± 0.4	13.3 ± 0.9	
81	73	(c)	11.8 ± 0.9	14.3 ± 1.0	
212	78	13.2 ± 0.3	14.3 ± 0.8	9.0 ± 0.3	

(a) ± Values are standard deviations associated with the measurements

(b) Medial bifurcation

(c) Inhaled same $^{239}\text{PuO}_2$ as other dogs in this group

^{239}Pu - ^{241}Am ratios in the tissues were not greatly different from those in the inhaled aerosol. The limited liver data suggest a possibly greater accumulation and/or retention of ^{241}Am .

The results of a $^{239}\text{Pu}(\text{NO}_3)_4$ inhalation experiment are given in Table III, which shows that all of these dogs died within 138 days from pulmonary damage. All dogs analyzed to date show an increased ^{239}Pu - ^{241}Am ratio in lungs and lymph nodes and a decreased ratio in liver relative to the aerosol inhaled. This is tentatively interpreted as being due to relatively greater translocation of

^{241}Am to the liver. The high ratios in the lymph nodes could result from preferential translocation of ^{241}Am from the lymph nodes, or the disproportionation may have occurred before accumulation in the lymph nodes. There was evidence that a small fraction of the ^{241}Am was leached from the liver samples by the formaldehyde in which they were fixed. Therefore, the liver ratios may actually be lower than indicated.

Conclusions

There is little indication that ^{239}Pu and ^{241}Am are significantly

TABLE III. ^{239}Pu - ^{241}Am Ratios in Dog Tissue After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

Dog Number	Time After Exposure, days	^{239}Pu - ^{241}Am Ratios (a)			
		Aerosol	Lung	Lymph Nodes ^(b)	Liver
424	76	37.5 ± 2.8	73.2 ± 16.4		
420	89		68.6 ± 4.0	49.4 ± 2.5	11.7 ± 0.8
388	96	28.7 ± 1.5	49.4 ± 4.5	54.2 ± 3.2	13.0 ± 1.0
396	103	35.4 ± 1.4	56.9 ± 4.2	45.5 ± 3.2	13.7 ± 1.3
458	109	37.6 ± 2.8	61.4 ± 3.9	56.8 ± 4.0	20.6 ± 1.5
340	117	37.5 ± 2.0		66.3 ± 3.5	19.7 ± 1.3
376	118	31.8 ± 1.4		64.4 ± 3.5	17.1 ± 1.5
419	138	37.4 ± 3.0		81.2 ± 4.9	11.7 ± 0.8

(a) ± Values are standard deviations associated with the measurements

(b) Medial bifurcation

separated in the body after inhalation of PuO_2 containing trace amounts of ^{241}Am . However, since ^{241}Am was clearly translocated to the liver

more rapidly than ^{239}Pu after inhalation of $\text{Pu}(\text{NO}_3)_4$, it cannot be concluded that americium is a valid tracer for plutonium in all cases.

RADIONUCLIDE SCANNING FACILITY FOR DOGS

A dog scanning apparatus has been evolved which allows simultaneous scanning for ^{239}Pu and ^{241}Am .

Investigator:
C. R. Watson

In experiments with inhaled radionuclides it is often desirable to follow the distribution of activity by

external scanning. In most cases, it is sufficient to determine whether or not the activity is in the lungs; thus,

the goal of scanning is reliability, rather than high spatial resolution. As a result of continuing refinement of the apparatus since 1960, it is now possible to scan for two radio-nuclides simultaneously.

The scanning facility is a tunnel 2 feet long, the walls of which are 6 in. steel. A platform driven by a worm-gear passes through the tunnel

at constant speed. Plexiglass boxes, which comfortably restrain the beagles, fit onto the platform. Mounted in the tunnel are three 2 x 2 in. NaI crystals, and two 1 mm x 2 in. crystals. The signal from either array of detectors is displayed on a strip chart recorder after amplification and discrimination in a single-channel analyzer (Figure 1).

FIGURE 1. Block Diagram of Radionuclide Scanner

This apparatus was utilized in an experiment to determine whether inhaled ^{239}Pu and ^{241}Am follow the same path within the body. Rather than scan each animal twice, the Picker Twin Prober, normally used for kidney function tests, was utilized. A simple interface unit was constructed which allowed operation of the single channel recorder simultaneously with the dual-channel instrument. The scans thus produced (Figure 2) were easily interpreted and produced at a considerable saving in counting time.

FIGURE 2. Typical Scan of Beagle Following Inhalation of ^{241}Am -Enriched ^{239}Pu

HETEROPHILE ANTIBODY FORMATION IN RATS AND BEAGLES AFTER $^{239}\text{PuO}_2$ INHALATION

The ability of rats and beagle dogs to form heterophile antibodies against sheep red blood cells was impaired following inhalation of $^{239}\text{PuO}_2$. Impaired antibody formation occurred in rats prior to the onset of lymphopenia. This suggests that those lymphoid cells concerned with antibody formation may be the most sensitive to radiation damage.

Investigator:
J. V. Dilley

Technical Assistance:
K. E. McDonald

Lymphopenia is the first detectable symptom of toxicity from inhaled $^{239}\text{PuO}_2$ in beagle dogs and rats. Respiratory distress and/or lung tumors appear from a few months to years later. The purpose of these experiments was to determine whether the onset of lymphopenia is accom-

panied by a loss of heterophile antibody-forming capacity.

Observations

Thirty-five female Sprague-Dawley rats were exposed to an aerosol of $^{239}\text{PuO}_2$. One group of five rats was

immediately injected with 10^6 sheep red cells. On each succeeding week another group of five was injected. Two or three unexposed animals were also injected with sheep red cells each week and served as controls for lymphocyte counts and heterophile antibody titers. One week after red cell injection each group was sacrificed and blood was taken for cell counts, antibody titers, serum electrophoresis, and ^{239}Pu analysis of lung tissues.

Figure 1A shows the rapid decline of antibody formation in the exposed rats following an apparent initial stimulation. The lymphocyte counts remained within or above the range of the control animals. There was no

change in the serum electrophoretic patterns. At the time of sacrifice, these animals had a $^{239}\text{PuO}_2$ lung burden of $0.05 \pm 0.017 \mu\text{Ci}$.

This experiment was repeated at higher exposure levels, resulting in lung burdens of $0.32 \pm 0.03 \mu\text{Ci}$. Under these conditions, antibody formation was almost completely inhibited and a lymphopenia was also produced (Figure 1B).

Six young adult beagle dogs were injected with sheep red cells and assayed for heterophile antibodies at 1, 5, and 7 weeks. These data are shown in Figure 2 and indicate a half-life of about 4 weeks for the heterophile antibody.

FIGURE 1. Lymphocyte Counts and Heterophile Antibody Titers in Rats After Inhaling $^{239}\text{PuO}_2$

FIGURE 2. Persistence of Heterophile Antibodies in Six Beagle Dogs

Several "old" dogs from the $^{239}\text{PuO}_2$ toxicity study were also tested for antibody titers. Two exposed dogs, which had a marked lymphopenia and showed pulmonary tumors radiographically, and four control dogs were assayed for heterophile antibodies 1 week and 3 weeks after the initial sensitization to sheep red blood cells, and again a week after re-sensitization. The ^{239}Pu -exposed dog with the most severe lymphopenia had an antibody titer equal to the controls, while the other exposed

dog, with a less severe lymphopenia, had a low antibody titer (Table I). This suggests that the ability to form antibodies is not directly related to the number of circulating lymphocytes.

Finally, heterophile antibodies were measured in three beagle dogs that showed respiratory distress after inhaling $^{238}\text{PuO}_2$. All of these animals demonstrated a severe lymphopenia. Their antibody titers were measured at 1 and 3 weeks after an initial sensitization to sheep red cells and all had low titers (Table II).

TABLE II. Heterophile Antibody Titers in Beagle Dogs in Respiratory Distress From Inhaled $^{238}\text{PuO}_2$

Dog Number	Highest Serum Dilution for Positive Antibody Response	
	1 Week	3 Weeks
422	32	--
427	2	2
464	64	32
Control range	256	128

TABLE I. Heterophile Antibody Titers in Beagle Dogs 5 Years After Inhaling $^{239}\text{PuO}_2$

Dog Number	Age, months	Exposure Status	Highest Serum Dilution for Antibody Response			Lymphocyte Counts per $\text{mm}^3 \times 10^3$
			1 Week	3 weeks	Resensitized	
1	109	$^{239}\text{PuO}_2$	128	64	128	0.95
249	86	$^{239}\text{PuO}_2$	32	8	32	1.78
24	99	Control	256	64	128	2.84
193	112	Control	128	64	64	3.68
251	86	Control	128	64	64	2.80
297	72	Control	128	64	128	2.38

Conclusions

These experiments demonstrate a new and distinct toxic response to inhaled plutonium which may have important immunological consequences. There has been much recent interest in the mechanism of "immune surveil-

lance" as a control for cancer induction. These findings are especially interesting from this point of view. Studies relating loss of heterophile antibody synthesis and tumor induction are planned for the future.

BILIARY EXCRETION OF PLUTONIUM AFTER PULMONARY DEPOSITION IN RATS

Biliary excretion of plutonium was measured in rats after intratracheal injection and inhalation of several plutonium compounds. Maximum biliary excretion occurred after inhalation of $\text{Pu}(\text{NO}_3)_4$. This amounted to about 35% of the total plutonium excreted between the seventh and thirty-eighth day after exposure.

Investigators:

*N. Matsusaka**
W. J. Bair

Technical Assistance:

M. D. Snyder
W. W. Roberts
K. E. McDonald

The work of Ballou and Sullivan (Annual Report, 1963), demonstrating the excretion of plutonium in bile after intravenous injection, suggested that the biliary route may contribute to the appearance of plutonium in the feces after an inhalation exposure. The evaluation of this possibility is of some practical significance, since fecal excretion of plutonium following an inhalation exposure has frequently been considered to represent plutonium cleared from the lung via the bronchiotracheal route.

Observations

Plutonium compounds were injected intratracheally immediately following cannulation of the common bile duct under Nembutal anesthesia. Bile was collected for a 2 hr period. The summarized results of this experiment are shown in Table I.

Detectable amounts of plutonium were excreted in the bile with larger amounts present in the case of the more soluble forms.

In a second experiment, 16 unanesthetized rats were exposed to an aerosol of $^{239}\text{Pu}(\text{NO}_3)_4$. The common bile duct was cannulated and 2-hr bile samples collected under anesthesia on postexposure days 1, 3, 7,

* *Japanese Research Fellow: National Institute of Radiological Sciences, Anagawa, Chiba, Japan.*

and 38. Following bile collection, the animals were killed and total plutonium content (excluding skin) determined. Results of this experiment are shown in Table II. From the bile collection data a curve for daily excretion of plutonium into the bile was constructed (Figure 1), assuming that the 2-hr bile collection under anesthesia accurately

reflected the rate of biliary plutonium loss.

The mean body burden decreased from 12.2 μCi on the first day to 2.2 μCi on the thirty-eighth day. From the area under the curve of Figure 1, it can be estimated that biliary excretion accounted for only about 2.5% of the total plutonium excreted during the 38 days. However, if the first week's data are

TABLE I. Biliary Excretion of ^{239}Pu After Intratracheal Administration to Rats

Compounds	Injected Form	Injected Dose, μCi	Percent of Dose in 2-hr Bile Collection ^(a)
$\text{Pu}(\text{NO}_3)_4$	In 0.14N HNO_3	5.0	0.0003 (0.0001-0.0004)
PuO_2	In water (particle size 0.1-0.5 μ)	7.5	0.00004 (0.00002-0.00006)
PuF_4	In 0.13N HNO_3	5.2	0.01 (0.001-0.018)
PuF_4	In water	4.4	0.0003 (0.0002-0.0004)

(a) Average values from four rats with range in parentheses

TABLE II. Biliary Excretion of Inhaled Plutonium Nitrate by Rats

Days After Exposure	No. of Rats	Mean Body Burden, μCi	Percent of Body Burden in 2-hr Bile Collection ^(a)
1	4	12	0.002 (0.001-0.004)
3	5	5.2	0.016 (0.002-0.042)
7	3	2.9	0.027 (0.016-0.045)
38	4	2.2	0.005 (0.001-0.007)

(a) Mean % of body burden at time of collection with range in parentheses

omitted from consideration, about 35% of the plutonium excreted from the seventh to thirty-eighth day is excreted via the bile.

Conclusions

These results show that biliary excretion of plutonium after an inhalation exposure can, under some conditions, represent a significant fraction of the plutonium appearing in the feces. This is particularly true for the more soluble forms of deposited plutonium and for periods subsequent to the initial clearance of the upper respiratory and nasal passages. Caution must therefore be exercised in equating lung clearance rates to the rate of excretion of plutonium in feces.

FIGURE 1. Daily Biliary Excretion of Inhaled $\text{Pu}(\text{NO}_3)_4$

ACUTE TOXICITY OF INHALED PLUTONIUM-239 NITRATE IN BEAGLES

Beagle dogs died of pulmonary insufficiency 3-10 months after inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$. Body burden at death ranged from 5-65 μCi , of which approximately half was present in the lungs.

Investigators:

J. F. Park
W. J. Bair
E. B. Howard

Technical Assistance:

L. R. Richardson

We now have considerable information on the toxic effects of inhaled "insoluble" plutonium, i.e., $^{239}\text{PuO}_2$, which implicates the lung as "critical organ." Much less information is available on the toxic effects of

inhaled "soluble" plutonium. It has been quite generally assumed that such soluble plutonium will be largely translocated to the skeleton, and that bone will be the critical organ. The experiment described in this report

was an effort to obtain experimental data on the effect of relatively large doses of inhaled $^{239}\text{Pu}(\text{NO}_3)_4$ in beagle dogs.

Observations

Twelve beagle dogs were exposed for 10-60 min to $^{239}\text{Pu}(\text{NO}_3)_4$ aerosols generated from 0.27N HNO_3 solution. The concentration of ^{239}Pu in the aerosol ranged from 0.1-10 nCi/ml. The count median diameter of the particles was 0.12 μ , with a standard geometric deviation of 2.1. Six dogs were maintained as controls.

Eleven dogs died or were sacrificed when clinical signs indicated death

was imminent, 3-10 months postexposure. One dog was sacrificed 3 months postexposure to compare plutonium distribution and histopathology in an asymptomatic dog.

Plutonium body burdens at death ranged from 5-65 μCi , with 41-63% in the lungs, 17-37% in the skeleton, 9-27% in the liver, and 2-5% in the tracheobronchial and mediastinal lymph nodes (Table I). The highest concentrations of plutonium occurred in the tracheobronchial lymph nodes followed in descending order by lungs, mediastinal lymph nodes, liver, other lymph nodes, and skeleton (Table II).

The first indication of clinical changes in the dogs was panleucopenia

TABLE I. Dog Mortality and Tissue Distribution Following Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

Dog Number	Time of Death, days postexposure	Plutonium Content, μCi			Distribution of Plutonium, % of body burden			
		Initial Deposition		Total Body at Death	Lymph Nodes ^(b)			
		Total	Alveolar		Lungs	Liver	Skeleton	
424	75	251	54	47	60	2	10	25
420	89	175	53	49	59	5	10	24
396	103	152	74	65	51	5	9	33
458	109	29	24	20	56	2	9	30
340	117	35	32	27	63	3	11	19
376	119	99	46	41	50	2	9	36
419	138	32	27	23	46	2	11	37
421	172	12	11	8	47	2	14	33
423	198	7	6	5	41	2	27	27
461	236	12	11	9	46	3	17	31
447	303	11	10	8	41	2	16	37
388 ^(a)	96	7	6	5	63	2	15	17

(a) Dog 388 was sacrificed for comparison of tissue distribution of plutonium in an asymptomatic animal. All other dogs died or were sacrificed when death was imminent.

(b) Tracheobronchial and mediastinal lymph nodes.

TABLE II. Concentration of Plutonium in Tissues of Beagle Dogs Following Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$ (nCt/g wet tissue)

Dog Number	Lymph Nodes			Lungs ^(b)	Liver	Skeleton
	Tracheobronchial	Mediastinal	Others ^(a)			
424	2000	80	170	420	20	13
420	7200	720	190	450	44	14
396	3900	310	29	390	31	16
458	420	29	6	140	6	5
340	1900	100	64	240	19	5
376	1400	480	18	240	22	12
419	780	110	17	150	15	8
421	340	12	5	63	8	2
423	140	13	3	41	8	2
461	280	19	2	50	4	2
447	160	9	2	36	5	2
388	160	15	2	51	4	1

(a) *The highest concentration occurred in the axillary, prescapular, suprapharyngeal or mandibular lymph nodes.*

(b) *Lungs were 2-3 times normal weight due to pathology; therefore, calculated normal weights were used to determine concentration.*

appearing the second week postexposure. Leucocyte counts dropped to 25% of values for control dogs (Figure 1). Erythrocyte, hemoglobin, and hematocrit values were normal. Respiration rates gradually increased from a normal of 20-40 respirations per minute to over 100 per minute the week prior to death. The pO_2 in arterial blood was significantly decreased from 60 days postexposure until death. During the week prior to death, pCO_2 of arterial blood was significantly elevated and pH decreased. As respiratory distress increased, the dogs showed intermittent anorexia and loss of 30-50% of body weight.

Pathology in the lungs consisted primarily of severe fibroplasia, alveolar cell hyperplasia, bronchiolar hyperplasia and metaplasia, various stages of vascular damage, flooding of the alveoli with proteinaceous fluid and blood, and organized fibrinoid plugs in the alveoli and terminal bronchioles.

The tracheobronchial lymph nodes were depleted of normal lymphoid elements which were replaced with fibrinous connective tissue. Vascular damage was severe with hemorrhage and proliferation of hemosiderin-filled macrophages. The mediastinal lymph nodes had less severe lesions of a similar nature. The remainder

FIGURE 1. Leucocyte Counts in Dogs After Inhalation of $^{239}\text{Pu}(\text{NO}_3)_4$

of the lymph nodes appeared to be undergoing a reactive hyperplastic response.

The livers showed congestion, dilated sinusoids, and degenerative processes primarily related to passive congestion due to lung pathology.

The trabecular bone showed areas of necrosis, degeneration, osteoclastic activity, and fibroplasia associated with plutonium deposition. Marrow cells were depleted at the higher dose levels.

Conclusions

Death was caused by lesions in the lung. These lesions were similar to those seen in dogs dying within a year after inhalation of $^{239}\text{PuO}_2$ and the lethal dose on a $\mu\text{Ci/g}$ of lung at death basis was also similar. About 50% of the plutonium was translocated to the liver and skeleton causing lesions in trabecular bone and a leucopenic response. Inhaled $^{239}\text{Pu}(\text{NO}_3)_4$ is considerably more toxic on a $\mu\text{Ci/kg}$ basis than intravenously injected plutonium. Comparable levels of intravenously injected plutonium-239 citrate killed dogs, at the

University of Utah, after approximately 50 months.

Additional experiments at lower dose levels are needed to define the long-term toxicity of inhaled "soluble" plutonium. Assuming that inhalation of smaller amounts of $^{239}\text{Pu}(\text{NO}_3)_4$ will cause pulmonary tumors in dogs similar to those caused by inhaled $^{239}\text{PuO}_2$, and that plutonium translocated to bone will cause skeletal tumors similar to those resulting from intravenously injected plutonium-239 citrate, the critical organ will be determined by the dynamics of translocation and the relative sensitivity of the tissues exposed. Liver and lymph nodes should also not be overlooked as possible critical organs.

Inhalation experiments with $^{239}\text{Pu}(\text{NO}_3)_4$ are of particular interest because they do result in the distribution of plutonium among many organ systems in a pattern such as might be expected to result from continuing occupational exposure to a variety of forms of plutonium via different routes of entry. The long-term effects of such exposure patterns would be of great practical interest to those concerned with hazard evaluation and control.

LONG-TERM STUDIES OF INHALED ^{144}Ce - $^{144}\text{PrO}_2$ IN BEAGLES

Beagle dogs which inhaled calcined ^{144}Ce - $^{144}\text{PrO}_2$ aerosols, 3-1/2 years previously, retained about half the initially deposited material, with 50-60% of the body burden at death in the liver and 25-30% in the skeleton. Dogs treated with DTPA subsequent to exposure retained only about 15-20% of the initially deposited material at 5 years postexposure with about 25% of the total body burden in the liver and about 50% in the skeleton. Dogs depositing more than 100 μCi (total β) showed a significant lymphopenia.

Investigator:

B. O. Stuart

Technical Assistance:

J. C. Gaven

The deposition, translocation, elimination, and biological effects of inhaled ^{144}Ce - $^{144}\text{PrO}_2$ particles have been studied in beagle dogs over a period of 5 years. Previous reports discussed whole-body retentions with biological half-times up to several years, and indicated longer pulmonary retention of the calcined oxide than the peroxide-prepared oxide. Both forms of the oxide showed translocation principally to the liver and skeleton in dogs sacrificed at 8, 16, and 30 months after exposure (Annual Report, 1964, 1966). This report describes the tissue distribution and long-term effects in dogs killed 3-1/2 and 5 years after exposure.

Observations

Table I shows the tissue distribution of ^{144}Ce - ^{144}Pr in two beagles sacrificed 3-1/2 years after inhalation of submicronic particles of the calcined oxide, and in two beagles sacrificed 5 years after inhalation of the peroxide-prepared oxide. These

data for the dogs exposed to the calcined oxide do not differ greatly from results previously reported at 8, 16, and 30 months after exposure. At 3-1/2 years, about one-half of the ^{144}Ce - ^{144}Pr initially deposited in the alveolar lung remained in the total body.

Dogs 205 and 220 (Table I) were treated intravenously with DTPA (diethylenetriaminepentaacetic acid) from 64 to 189 days and from 9 to 189 days, respectively, after exposure to low levels of peroxide-prepared ^{144}Ce - $^{144}\text{PrO}_2$. In contrast to the tissue distribution patterns of all other dogs exposed to either form of cerium oxide, these dogs showed about 50% of the total-body burden in the skeleton and about 25% in the liver. From 15-20% of the cerium initially deposited in the alveolar lung remained in the total body after 5 years.

Figure 1 shows the effects of inhaled ^{144}Ce - $^{144}\text{PrO}_2$ on lymphocyte counts. At initial deposition levels of 320 μCi total beta, a marked lymphopenia occurred immediately after exposure, which became less

TABLE I. Tissue Distribution of ^{144}Ce - ^{144}Pr in Beagle Dogs After Inhalation of Cerium Oxide Particles

Tissue	Percent of Total-Body Burden at Sacrifice			
	Calcined Oxide 3-1/2 Years After Exposure		Peroxide-Prepared Oxide 5 Years After Exposure ^(a)	
	Dog 299	Dog 319	Dog 205	Dog 220
Tracheobronchial lymph nodes	3.6	2.7	2.1	0.6
Lungs	6.8	7.6	16	5.1
Liver	57	53	23	23
Skeleton	26	31	50	54
Spleen	0.04	0.02	0.1	0.1
Kidneys	0.1	0.5	0.9	1.2
Muscle			4.2	7.7
Pelt			0.4	4.1

(a) Dog 205 received DIPA treatments beginning 64 days after exposure, continuing until 189 days. Dog 220 received DIPA treatments beginning 9 days after exposure, continuing until 189 days.

FIGURE 1. Lymphocyte Counts After Inhalation of ^{144}Ce - $^{144}\text{PrO}_2$ by Beagle Dogs (Initial alveolar deposition, in μCi , indicated for each animal)

pronounced as control lymphocyte levels gradually decreased over the ensuing 2 years. Neutrophil levels showed little change. No lymphopenia was observed in dogs that deposited 20-50 μCi ^{144}Ce - ^{144}Pr .

Conclusions

Sacrifice of the two dogs remaining in the calcined ^{144}Ce - $^{144}\text{PrO}_2$ inhalation experiment showed tissue distributions at 3-1/2 years which were similar to those seen at 8, 16, and 30 months after exposure to either calcined or peroxide-prepared oxides. About one-half of the retained body burden was in the liver and about one-fourth in the skeleton. The calcined oxide showed little change in lung percent of total-body burden between 2-1/2 and 3-1/2 years

after exposure. Although the two forms of the oxide were earlier shown to exhibit different pulmonary retentions, the distribution patterns of translocated material are clearly similar. The dogs which had received DIPA treatments subsequent to exposure showed a distinctly different distribution pattern with twice as much deposited in the skeleton as in the liver. This is presumably due to selective removal of the radionuclide from the latter organ as was indicated in earlier short-term studies with DIPA (Annual Report, 1964). As has been shown to be the case with inhaled $^{239}\text{PuO}_2$, an early and persistent decrease in circulating lymphocyte levels appears to be the most sensitive indicator of damage due to inhaled ^{144}Ce - $^{144}\text{PrO}_2$.

INHALATION STUDIES WITH PROMETHIUM OXIDE IN BEAGLE DOGS

At 5 months and 1 year after inhalation of Pm_2O_3 , the liver and skeleton each contained about one-half of the system's burden. Rate of translocation from the lung was influenced by the physicochemical form of the Pm_2O_3 inhaled. Initial deposition of approximately 1 mCi ^{147}Pm caused a marked drop in circulating lymphocytes.

Investigator:
B. O. Stuart
Technical Assistance:
J. C. Gaven

In previously reported experiments, we have exposed dogs to aerosols of promethium perchlorate (Annual Report, 1963), calcined promethium oxide (Annual Report, 1965), and recalcined

promethium oxide (Annual Report, 1966). The earlier study with calcined Pm_2O_3 was repeated with Pm_2O_3 recalcined after neutron irradiation, because of the unusual apparent in

vivo solubility of the material used in the initial experiment. The neutron irradiation converts a portion of the ^{147}Pm to ^{148}Pm , a 42 day half-life gamma emitter that allows external monitoring. In this report, we compare some of the longer term data from the two Pm_2O_3 experiments.

Observations

Figure 1 compares excretion data for dogs that inhaled calcined Pm_2O_3 (Dogs 5 and 6), and recalcined Pm_2O_3 (Dogs 7 and 8). All dogs showed the high intercept and rapidly diminishing rates of fecal excretion which distinguish inhaled insoluble substances. Urinary curves for Dogs 7 and 8 show the typically low urinary excretion for insoluble substances--initially more than two orders of magnitude below fecal excretion rates. Urinary excretion was considerably greater in Dogs 5 and 6. It is suspected that neutron irradiation of the calcined Pm_2O_3 caused changes in the particles which resulted in greater solubilization in vivo.

Table I shows the tissue distribution of promethium in four dogs (two from the calcined and two from the recalcined group) at about 5 months and 1 year after exposure. As found in our studies with promethium perchlorate (Annual Report, 1963), liver and skeleton were the major reservoirs of ^{147}Pm translocated from the lungs. The 8 and 3% burdens in the lungs of dogs exposed to the calcined (then neutron-irradiated) Pm_2O_3 indicate a 4-5 month pulmonary retention half-time. The 45 and 35%

FIGURE 1. Excretion of Promethium Following Inhalation by Beagle Dogs (Dogs 5 and 6 inhaled the original calcined Pm_2O_3 ; Dogs 7 and 8 inhaled the recalcined Pm_2O_3 . The shaded areas show typical 95% confidence intervals.)

lung burdens for dogs exposed to recalcined Pm_2O_3 suggest retention half-times of the order of a year or longer, similar to those previously reported from longitudinal scanning data (Annual Report, 1966). If the percentages in liver and bone are calculated on the basis of "systemic burden" (i.e., excluding lung burdens), they are nearly the same for the perchlorate exposures and both oxide exposures.

Figure 2 shows the effect of approximately 1 mCi (initial alveolar deposition) of ^{147}Pm on circulating lymphocyte levels in a representative

TABLE I. Distribution of Promethium in Tissues of Beagle Dogs After Inhalation of Pm_{203}

Tissue	Percent of Body Burden at Sacrifice			
	Calcined Pm_{203}		Recalcined Pm_{203}	
	Dog 6, 5 Months Postexposure	Dog 5, 15 Months Postexposure	Dog 10, 5 Months Postexposure	Dog 9, 12 Months Postexposure
Tracheobronchial lymph nodes	0.08	0.06	3.5	7.5
Lungs	8.0	2.7	45.0	35.0
Spleen	0.13	0.07	0.02	0.03
Kidneys	0.85	0.69	0.65	0.60
Liver	56.0	46.0	22.0	25.0
Skeleton	30.0	44.0	25.0	27.0
GI tract	0.66	0.35	0.36	0.23
Muscle	2.0	4.9	1.7	1.5
Skin	1.8	0.58	0.86	0.70
Other	0.48	0.65	0.91	2.4

FIGURE 2. Lymphocyte Counts in Beagle Dogs Following Inhalation of Pm_{203} (Dog 5 had an initial alveolar deposition of $920 \mu Ci$ ^{147}Pm + $40 \mu Ci$ ^{148m}Pm . Dog 8 had an initial alveolar deposition of $1190 \mu Ci$ ^{147}Pm + $28 \mu Ci$ ^{148m}Pm .)

dog from each group (calcined and recalcined). A marked drop in lymphocyte levels occurred immediately after exposure in both cases and a general downward trend has continued since. Neutrophil levels dropped 40%.

Conclusions

Pulmonary retention half-times of inhaled recalcined promethium oxide were roughly 1 year or longer, based on both longitudinal scanning and tissue radioanalysis. Neutron-activated nonrecalcined oxide was translocated from the lung twice as rapidly, indicating greater in vivo solubiliza-

tion; higher levels of urinary excretion support this interpretation. Subtracting lung burdens of promethium from the total-body burden for both forms of the oxide and the perchlorate, results in very similar liver and skeletal percentages, suggesting nearly identical translocation patterns for any inhaled promethium after it passes from the pulmonary lung into the circulation. Initial pulmonary deposition of approximately 1 mCi $^{147}\text{Pm}_2\text{O}_3$ caused severe depression of circulating lymphocytes comparable to that observed after alveolar deposition in beagles of similar levels (total β) of $^{144}\text{Ce}-^{144}\text{PrO}_2$ (Annual Report, 1964).

IN VIVO SEPARATION OF URANIUM AND THORIUM AFTER INHALATION OF URANIUM ORE BY BEAGLES

Four beagle dogs inhaled uranium ore dust daily for 8 days. Two dogs sacrificed 2 weeks after the last exposure showed separation of ^{238}U , ^{230}Th , and ^{210}Po in the lungs, tracheobronchial lymph nodes, liver, kidneys, spleen, and urine.

Investigators:
B. O. Stuart
Y. M. Jones*

Technical Assistance:
J. C. Gaven

In previous experiments we showed early marked separation of ^{238}U from its daughter, ^{230}Th , in the lungs, tracheobronchial lymph nodes, liver, spleen, skeleton, and kidneys of rats

after daily inhalation of pitchblende ore dust. Thorium levels remained high in all organs except kidneys, with up to 50 times more ^{230}Th than ^{238}U 4 months after cessation of exposures (Annual Report, 1964).

These observations were extended to dogs in the present study.

* *Environmental and Radiological Sciences Department*

Observations

Four beagle dogs were exposed to ore dust aerosols (approximately 1 mg/liter; 26% U_3O_8) for 8 days, at which point exposures were terminated because of severe renal pathology. Two animals were sacrificed at 13 and 16 days following the last exposures and representative tissue and excreta samples were analyzed for ^{238}U , ^{230}Th , and ^{210}Po . Table I shows the accumulation of uranium in tissues.

TABLE I. Distribution of Uranium 2 Weeks After Inhalation of Uranium Ore Dust by Beagle Dogs (mg/organ)

Organ	Dog #414	Dog 8415
Lungs	7.0	7.9
Middle tracheo-bronchial lymph node	0.24	0.42
Kidneys	0.80	0.82
Spleen	0.02	0.01

Although most of the deposited uranium was still in the lungs, there was significant translocation to the tracheobronchial lymph nodes and kidneys.

Table II reports ^{238}U , ^{230}Th , and ^{210}Po concentrations in several organs from the same animals. Since the inhaled pitchblende ore was in secular equilibrium, the same disintegration rates for the uranium and its daughters would be expected if they were translocated and excreted without fractionation. Ratios of $^{230}Th/^{238}U$ ranged from 1.4-2.5 in the middle tracheobronchial lymph node and lungs, and from 1.2-2.5 in the kidneys and spleen, indicating separation of these radionuclides after only a week's inhalation exposure. Ratios of $^{230}Th/^{210}Po$ varied between 0.9 and 1.6 for kidneys, lungs, and the middle tracheobronchial lymph node.

Excretion of uranium in the urine of all four dogs ranged from 8-100

TABLE II. Concentrations of ^{238}U , ^{230}Th , and ^{210}Po in Organs of Beagle Dogs 2 Weeks After Inhalation of Uranium Ore Dust (Disintegrations/min/g wet tissue)

Organ	Dog #414			Dog #415		
	^{238}U	^{230}Th	^{210}Po	^{238}U	^{230}Th	^{210}Po
Lungs	85	210	140	69	160	130
Middle tracheo-bronchial lymph node	440	620	540	460	800	510
Kidneys	19	23	26	17	26	25
Spleen	1.8	4.7	2.0	0.6	0.7	3.4

times higher than that of ^{230}Th during the week of exposures, with mean levels of 29, 14, 48, and 34 times for the four dogs. Fecal samples during the same period showed approximately two times more ^{230}Th than ^{238}U . Polonium-210 levels were 30-80% those of ^{230}Th . From 0.05-0.4 mg of ore were eliminated in the feces each day during exposure.

Conclusions

Separation of both ^{230}Th and ^{210}Po from the parent uranium was observed in all tissues and excreta from dogs sacrificed 2 weeks after inhaling uranium ore dust. These results suggest that in vivo solubilization of uranium was greater than that of ^{230}Th and ^{210}Po . These findings agree with the results of earlier studies with rats.

EFFECTS OF X-RADIATION ON LIPID SYNTHESIS BY LUNG TISSUE

Mitochondria from thoracic X-irradiated rats incorporated twice as much ^{14}C -palmitate into lipids as did mitochondria from unirradiated controls. The relative distribution of the ^{14}C among different lipid classes was the same for irradiated and control animals.

Investigator:
E. G. Tombropoulos
Technical Assistance:
W. G. Morrow

Previous observations had indicated that the lipid content of rat lungs increased during the first 2 days following 800 R X-irradiation of the thorax (Annual Report, 1966). This report presents the results of studies on the incorporation of palmitate and acetate by lung mitochondria following irradiation of the thorax.

Observations

Forty-day-old female Sprague-Dawley rats were exposed to 800 R thoracic X-irradiation. The rats were starved

beginning 2 hr before exposure. At 22 hr postexposure, the animals were killed and the lung mitochondrial fraction isolated and homogenates incubated with acetate-1- ^{14}C and palmitate-1- ^{14}C by methods previously described. Results shown in Table I indicate that lung mitochondria from X-irradiated animals incorporated twice as much ^{14}C from palmitate into lipids as did the mitochondria from control animals. Because fasting depresses fatty acid synthesis from acetate, the results with acetate were inconclusive.

TABLE I. Total Incorporation of ^{14}C -Labeled Acetate and Palmitate Into Lipids by Lung Mitochondria

Substrate	Nanomoles Incorporated/ mg Protein ^(a)	
	X-Irradiated Rats	Control Rats
Acetate-1- ^{14}C	0.05	0.04
Palmitate-1- ^{14}C	14	7.6

(a) Values are means from three experiments; 10-13 animals per group in each experiment

The lipids synthesized from palmitate by the irradiated and control mitochondria are compared in Table II.

Conclusions

Although thoracic X-irradiation increased the incorporation of palmitate into lipids by the lung mitochondrial

TABLE II. Relative Incorporation of Palmitate-1- ^{14}C Into Different Lipids by Lung Mitochondria

Lipid Fraction	Percent of Total ^{14}C in Lipid Fractions Examined ^(a)	
	X-Irradiated Rats	Control Rats
Cholesterol esters	1.6	2.3
Triglycerides	47	53
Diglycerides	25	20
Monoglycerides	5.1	5.3
Lecithin	21	19

(a) Mean values from 10-13 animals

fraction at 22 hr postexposure, it did not alter the relative distribution of the incorporated palmitate among the various lipid classes examined.

INCORPORATION OF PALMITATE INTO LIPIDS BY SUBCELLULAR FRACTIONS OF LUNG TISSUE

The microsomal fraction from lung tissue was most active in the esterification of palmitate, followed, in order of decreasing activity, by the mitochondria and supernatant fractions. The individual lipid classes in which palmitate was incorporated were also examined.

Investigator:
E. G. Tombropoulos
Technical Assistance:
W. G. Morrow

This is a continuing study of biosynthetic and regulatory mechanisms pertaining to lung surfactants. We

previously reported that the mitochondrial fraction was most active in the synthesis of long-chain fatty acid

constituents of lung surfactants (Annual Report, 1965). This report describes further results on the esterification of long-chain acids and the biosynthesis of lecithin. the most active component of lung surfactants.

Observations

Lung tissue from Sprague-Dawley female rats was homogenized and sub-cellular fractions separated by standard centrifugation techniques. These fractions were incubated for 40 min with 0.5 mmole 1- 14 C-palmitate and 5 mmole DL- α -glycerophosphate. Their ability to esterify palmitate is shown in Table I. The synthesized lipids were fractionated and isolated by column and thin-layer chromatography with results as shown in Table II. For all fractions, the major products of palmitate esterification were the mono- and diglycerides. These may represent merely a stage in the ultimate synthesis of triglycerides and phospholipids. The microsomal and mitochondrial fractions showed very similar patterns of palmitate incorporation, with substantial production of triglycerides, cholesterol esters, and lecithin. The supernatant fraction was clearly different, with a greater incorporation into cholesterol esters, and very little into lecithin.

Conclusions

Adding to the earlier observation that long-chain fatty acids are

TABLE I. Esterification of Palmitate-1- 14 C by Lung Subcellular Fractions (40 min incubation)

Fraction Tested	Palmitate-1- 14 C Esterified Nanomoles/mg Protein
Microsomal	5.9
Mitochondria rich	2.9
Supernatant	1.5

TABLE II. Relative Distribution Among Different Lipid Classes of 14 C Incorporated from Palmitate by Lung Subcellular Fractions

Lipid	Percent of the Total Lipid Fractions Tested		
	Micro- somal	Mito- chondrial	Super- natant
Cholesterol			
esters	8.1	9	19
Triglycerides	25	21	11
Mono- and diglycerides	59	62	68
Ethanolamine	0.62	0.92	0.29
Lecithin	7.5	7.3	1.4

synthesized mainly by the mitochondrial fractions, we can now state that esterification and lecithin formation is a product mainly of microsomal, and to a lesser degree mitochondrial, activity.

THE HEMOGRAM OF RATS ANESTHETIZED WITH METHOXYFLURANE DURING BLOOD SAMPLING

Methoxyflurane anesthesia, used to restrain rats during collection of blood samples from the ophthalmic venous plexus, did not significantly alter the hemogram.

Investigators:
L. R. Richardson
J. F. Park
R. J. Olson*

Multiple consecutive blood samples from the ophthalmic venous plexus of rats have, in our experience, produced more reliable hemograms than blood collected by other techniques. Rats are difficult to restrain during collection of blood by this technique, and particularly when dealing with radioactively contaminated animals, the procedure can be quite hazardous to the investigator.

Methoxyflurane is a nonexplosive (at room temperature) inhalation anesthetic that can quite effectively minimize this hazard, but its effect on the rat's hemogram was unknown.

Observations

Ten, 10-week-old, female rats were immobilized with methoxyflurane during blood sampling and their hemograms were compared with those of ten manually restrained, unanesthetized rats of the same sex, strain, and age maintained in the same environment. Five blood samples were collected at weekly intervals from each rat, followed by a sixth sample during the seventh week. Blood samples were collected from the ophthalmic venous plexus with a modified disposable capillary pipette, using K-EDTA as an antico-

agulant. Anesthesia was induced in a modified desiccator jar, through which an aerosol containing 30 ppm methoxyflurane was recirculated at a flow rate of 15 liters/min. Rats were removed from the jar for blood sampling as soon as the tactile hairs on the rats' nose stopped moving. This coincided with approximately stage 2 anesthesia.

The mean induction time was 66 sec, and the mean time for recovery to the standing position was 75 sec. The rats did not move during blood sampling if the sample was collected within 60 sec. One to 2 ml of blood was collected at each sampling.

There were no significant changes in the hemogram of the anesthetized group compared to the unanesthetized group (Figure 1).

Conclusions

Methoxyflurane anesthesia effectively restrained rats during collection of blood from the ophthalmic venous plexus. Hemograms obtained from such anesthetized rats were not significantly different from those of unanesthetized controls. This procedure has proved very effective where multiple consecutive blood samples must be obtained from rats.

* *Mathematics Department*

FIGURE 1. Hemogram Derived From Blood Sampled From the Ophthalmic Venous Plexus of Unanesthetized Rats and Rats Anesthetized with Methoxyflurane (Mean values from ten animals with 95% confidence interval)

SPACE NUCLEAR SYSTEMS STUDIES

Three principal aspects of our program on the hazards associated with radionuclides in space are covered in this section.

Four reports are concerned with the inhalation of ^{238}Pu . Although $^{238}\text{PuO}_2$ microspheres of 50 μ diameter were inhaled by dogs and small numbers were deposited in the nasal pharynx region, there was no indication, among 22 exposed dogs, of deposition or retention in the deep lung. Preliminary results from inhalation studies with crushed $^{238}\text{PuO}_2$ microspheres in dogs indicate a pattern of mortality and clinical effects quite similar to those observed previously with $^{239}\text{PuO}_2$. Comparative studies of the toxicity of inhaled $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ in rats have also shown no significant difference in mortality on a microcurie basis. However, experiments on the phagocytosis of $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ particles, reported in the section of this Annual Report devoted to Radioelement Removal Studies, show distinct differences in the behavior of the two isotopes. There are also indications, not yet sufficiently established to appear in a report, which suggest that inhaled ^{238}Pu is much more widely translocated, systemically, than ^{239}Pu .

The gastrointestinal passage time of $^{238}\text{PuO}_2$ microspheres in miniature swine was reported last year to be considerably prolonged, as compared to normal food passage. In further work reported this year, it is shown that the passage time of $^{239}\text{PuO}_2$ microspheres is similarly prolonged. This would seem to eliminate the possibility that alpha radiation or temperature effects are responsible for this delay, in view of the great difference in specific activity of the two isotopes. Measurements of absorption and systemic deposition of plutonium from these microspheres were quite variable, but a maximum of less than one-millionth of the fed dose was absorbed.

The final three reports are about the problem of foliar deposition and retention of $^{238}\text{PuO}_2$ microspheres or other radioactive particulates.

PUBLICATIONS

Smith, V. H., H. A. Ragan, and
J. L. Beamer. "Absorption of ^{238}Pu
from Ceramic, Plutonium Oxide Par-
ticles Fed to Swine," Health
Physics, vol. 13, p. 938. 1967.
(Abstr.)

Willard, D. H. "Inhalation and
Retention of ^{238}Pu Microspheres in
Beagle Dogs," Health Physics,
vol. 13, p. 929. 1967. (Abstr.)

PULMONARY DEPOSITION AND RETENTION OF $^{238}\text{PuO}_2$ MICROSPHERES IN BEAGLES

Although 50- μ $^{238}\text{PuO}_2$ particles were inhaled by dogs, none were deposited and retained in the lower respiratory tract. Two dogs have retained single 120- to 300- μ $^{238}\text{PuO}_2$ particles in the lung over 1 year after deposition by pulmonary intubation.

Investigator:

D. H. Willard

B. O. Stuart

Technical Assistance:

W. Skinner

The purpose of this study was to obtain experimental data relating to the possible inhalation, deposition, and retention of $^{238}\text{PuO}_2$ microspheres that might be dispersed in air as a result of their use in space nuclear auxiliary power (SNAP) systems. Some preliminary results of these investigations were reported last year (Annual Report, 1966). Two series of experiments were performed. The first involved inhalation of 50- μ $^{238}\text{PuO}_2$ microspheres dispersed in air; the second involved pulmonary intubation of 50-, 120-, or 300- μ microspheres under sodium Pentothal anesthesia. Whole-body longitudinal scanning techniques were used to follow the course of the particles in the dogs.

Observations

Twenty-two dogs were exposed for 10 min to 50- μ microspheres dispersed in air. Because of the density of the particles and their somewhat heterogeneous dispersion in the chamber, it was difficult to specify a value for aerosol concentration with which to relate deposition in the dogs. Approximately 12-85 mCi or 4,000-29,000 microspheres were passed through the chamber during an exposure. Amounts deposited in the dogs as estimated from whole-body counts varied from 10-900 μCi or from 3-300 microspheres.

Figure 1 shows scans made at several intervals following inhalation of 50- μ microspheres. A significant fraction of the plutonium was

*FIGURE 1. Longitudinal Scans of Dog
After Inhaling 50- μ $^{238}\text{PuO}_2$
Microspheres*

initially deposited in the nasal pharynx region. Subsequent scans show the passage of the plutonium through the animal. Comparison of such scans with those from a dog phantom with $^{238}\text{PuO}_2$ microspheres in the lung region indicates that any microspheres deposited in the lung

were cleared very rapidly. Complete elimination of microspheres occurred in all dogs by 16 days after exposure. One dog killed 20 min post-exposure contained 83% of the retained microspheres in the stomach and 17% on the tongue. Another dog sacrificed 2-1/2 min postexposure

contained 80.4% in the stomach, 9.7% in the oral cavity, 4.4% in the esophagus, 3.2% in the nasal cavity, 1.3% elsewhere in the head, and 0.8% in the trachea.

Results of the intubation experiments are summarized in Table I. Most of the microspheres were cleared within 8 days, although 12 were retained for 2 months or longer. Two 50- μ microspheres are still retained after more than 6 months; a 120- μ

and a 300- μ microsphere are retained after more than a year. All dogs retaining microspheres for more than 2 weeks were examined regularly for clinical symptoms of radiation injury. Chest radiographs have shown no areas of pathology. In other plutonium inhalation studies, the earliest and most consistent effect observed has been a chronic lymphopenia. Lymphocyte counts for the two dogs that have retained single microspheres for

TABLE I. Lung Retention of Intubated $^{238}\text{PuO}_2$ Microspheres

Dog No.	Approx. Particle Size, μ	Number of Particles	Retention Time															
			Days										Months					
			1	2	3	4	5	6	7	8	9	10	2	3	4	5	6	Years >1
365	300	1	_____	1														
360		1	_____	1														
365		1	_____			1												
360		1	_____	1														
365		1	_____															1 *
346	120	1	_____	1														
331		1	_____															1 *
346		1	_____											1				
368		1	_____										1					
417		3	_____	2				1										
430		3	_____	1						2								
431		8	_____	2		2		1		2			1					
432		1	_____														1 *	
443		4	_____			2		2										
444		1	_____					2										
454		1	_____	1														
346		1	_____					1										
346		3	_____	1		1				1								
454		2	_____				1			2								
439		2	_____				1			1								
417		2	_____			1		1										
443		2	_____							2								
444		1	_____									1						
368	50	4	_____		1								2				1 *	
439		5	_____					3					2					
444		3	_____	1		2												
417		3	_____		1		1			1								
346		3	_____			2				1								
443		1	_____											1				

* Microsphere still in the lung

more than a year are shown in Figure 2. In both cases a reduction of circulating lymphocytes occurred after administration of the particle. In one case, however, the lymphocyte level returned to the dog's normal range after 8 months. The lymphocyte level continues to decrease in the dog with the 300- μ microsphere. In experiments with inhaled fine particles of $^{239}\text{PuO}_2$, we have observed chronic lymphopenia in dogs with lung burdens as small as 0.5 μCi . The dog with the 120- μ microsphere has a lung burden of nearly 100 μCi , yet it does not show a chronic lymphopenia. Therefore, so far as lymphopenia is concerned, a single plutonium particle in the lung is considerably less damaging than much

smaller quantities of plutonium dispersed throughout the lung.

Conclusions

From inhalation experiments with 22 dogs, there is no evidence that 50- μ $^{238}\text{PuO}_2$ microspheres will be deposited in the lower respiratory tract. When such large particles are deposited, by intubation, they may be retained, in a small proportion of cases, for periods in excess of 1 year. Such deposited microspheres are much less effective in producing a reduction in circulating lymphocytes than would be an equivalent quantity of plutonium distributed more uniformly throughout the lung

FIGURE 2. Effect of Single $^{238}\text{PuO}_2$ Microsphere in Lung on Circulating Lymphocyte Levels in Dogs

SPACE NUCLEAR SYSTEMS STUDIES

A CONSTANT-FEED, UNIFORM DISPERSION DEVICE FOR LARGE PARTICLE INHALATION STUDIES

An instrument was developed to uniformly disperse small quantities of large particles at a constant rate into an inhalation exposure chamber.

Investigators:
B. O. Stuart
M. D. Snyder

A series of experiments to measure the inhalation, deposition, and clearance of large particles in beagle dogs required the development of an instrument to uniformly disperse these particles throughout an exposure chamber. It was necessary to evenly distribute a few milligrams of 50- μ diam $^{238}\text{PuO}_2$ spheres (density >10) over a 7 in. diam cross-sectional area at a constant rate during a 10-15 min exposure period. The high terminal settling velocity of these spheres (71 cm/sec) precluded the use of our standard aerosol generators.

Observations

The device which was developed after investigation of many different approaches is shown schematically in Figure 1. A small quantity of the spheres to be suspended are in a 1 in. diam cup near the top of the exposure chamber. These spheres are dispersed with a high velocity air jet. The dashed lines in Figure 1 describe the trajectory of the particles. The high velocity air tube, A, has seven 0.007 in. holes, B, arranged about its circumference, 3/16 in. from the bottom, as well as five 0.007 in. diameter jets, C, in

the bottom plate. These deflect the upward traveling particles radially to further improve the distribution within the chamber. A constant feed of particles over the 10-15 min exposure period is provided by the feed-drive assembly, as shown. The funnel-shaped cavity of the particle reservoir facilitates loading. The assembly consists of a 1/3 rpm clock motor which slowly advances a steel plunger through a modified automatic

FIGURE 1. Constant-Feed, Uniform Dispersion Device

pencil body. The plunger continuously propels fresh particles into the high velocity air stream for dispersion into the chamber. Figure 2 shows the uniform deposition of particles at the bottom of the exposure chamber at the beginning and end of a typical 10 min exposure period.

Conclusions

Careful manipulation of air pressure, jet diameters, and reflector

cup position of this device produced uniform and continuous dispersion of large, heavy particles in an inhalation exposure chamber designed for deposition and clearance studies in large animals. Subsequent experiments were made with this instrument by using 50- μ $^{238}\text{PuO}_2$ microspheres in inhalation exposures of beagle dogs. The device performed well when care was taken to insure that particles were well cleaned before loading into the feed-drive assembly

2A. Initial 2 min collection of 50- μ lead particles during a 10 min test exposure.

2B. Final 2 min collection of 50- μ lead particles during a 10 min test exposure.

FIGURE 2. Small Areas of 7 in. diam Inhalation Exposure Chamber Base, Showing Uniformly Dispersed 50- μ Lead Particles During Typical Test

ACUTE TOXICITY OF INHALED CRUSHED $^{238}\text{PuO}_2$ MICROSPHERES IN BEAGLES

Death occurred in six beagle dogs 50-106 days after alveolar deposition of 30-160 μCi of $^{238}\text{PuO}_2$. Death was due to respiratory insufficiency and was preceded by lymphopenia and other changes similar to those seen after inhalation of acute Lethal levels of $^{239}\text{PuO}_2$.

Investigator:
D. H. Willard
Technical Assistance
W. Skinner

The purpose of this study was to determine the acute biological effects in dogs exposed to high concentrations of $^{238}\text{PuO}_2$ aerosols. The $^{238}\text{PuO}_2$ employed was prepared by crushing plasma torch-produced microspheres manufactured for use in radionuclide power sources.

Observations

Six female, 33-month old beagle dogs were exposed for 10 min to $^{238}\text{PuO}_2$ aerosols at concentrations of 0.016-0.039 $\mu\text{Ci}/\text{cm}^3$. The count median diameter of the aerosol was 0.52 μ (og = 1.66). Alveolar deposition ranged from 30-160 μCi . Lymphopenia was the first clinical response noted (Figure 1). Increased respiratory rates were observed in the highest level dogs after 20 days (Figure 2). Minute volumes generally decreased despite the increased respiratory rates. All dogs showed increased pCO_2 and decreased pO_2 in

arterial blood beginning a week or so before death.

The earliest death occurred after 50 days and all dogs were dead by 106 days after exposure. Death was due to respiratory insufficiency in all cases. Tissue distribution of ^{238}Pu at time of death is shown in Table I. The lungs and tracheal-bronchial lymph nodes contained about 98% of the body burden which ranged from 33-160 μCi . The skeleton contained about 1% and the liver <1%.

Gross pathology was observed only in lung and lymph nodes. Histology studies are not completed.

Conclusions

Alveolar deposition of 30-160 μCi $^{238}\text{PuO}_2$ in beagle dogs caused death in all six dogs 50-106 days after exposure. The clinical changes and mode of death, respiratory insufficiency, were similar to those seen after inhalation of $^{239}\text{PuO}_2$.

FIGURE 1. Lymphocyte Response to Inhaled $^{238}\text{PuO}_2$ (Body burden at death, in μCi , shown by each curve)

FIGURE 2. Respiratory Effects of Inhaled $^{238}\text{PuO}_2$ (Body burden at death, in μCi , shown by each curve)

TABLE I. Distribution of Inhaled $^{238}\text{PuO}_2$ in Dogs at Death

Dog Number	Survival Postexposure, days	Total ^{238}Pu Burden, μCi	Percentage Distribution of $^{238}\text{PuO}_2$			
			Lungs	Tracheal-bronchial Lymph Nodes	Bone	All Other Tissue
427	50	74	96	2	1.1	0.97
466	60	110	97	2	0.78	0.48
446	61	56	90	6.6	1.5	1.5
464	63	160	95	3.2	0.82	0.48
422	81	79	95	3	1.0	0.64
437	106	33	87	9.4	1.2	2.7

COMPARATIVE TOXICITY OF INHALED $^{238}\text{PuO}_2$ AND $^{239}\text{PuO}_2$ IN RATS*

Inhaled $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ were equally effective, on a radioactive basis, in causing acute lethality in rats.

Investigators:
B. O. Stuart
W. J. Bair

Technical Assistance:
J. C. Gaven

Approximately 250 rats, in groups of 12, inhaled aerosols of either $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$ to determine the relative acute lethal toxicities of these radionuclides. The count median diameter for both materials was $0.1\ \mu$. Preliminary results from this experiment were reported last year, and seemed to indicate a greater early toxicity for $^{238}\text{PuO}_2$ (Annual Report, 1966). The more complete data now available would seem to negate this earlier conclusion.

pected for the product of alveolar-deposited plutonium and survival time. However, $\mu\text{Ci-day}$ values for rats which inhaled $^{239}\text{PuO}_2$ aerosols were 170 and 57 for rats averaging initial alveolar burdens of $8\ \mu\text{Ci/g}$ and $0.2\ \mu\text{Ci/g}$, respectively. Corresponding $\mu\text{Ci-day}$ values for $^{238}\text{PuO}_2$ rats were 180 and 70 for initial alveolar burdens of $9\ \mu\text{Ci/g}$ and $0.2\ \mu\text{Ci/g}$, respectively. These results showed that a higher total accumulated alpha

Observations

Figure 1 shows logarithmic relationships between lung burdens of plutonium at death and survival time for both isotopes, based on tissue analyses. The two regression curves are not statistically different, indicating that the two isotopes of plutonium were equally effective in causing short-term deaths.

If lethality depended only on the total accumulated alpha dose to the lungs, a constant value would be ex-

FIGURE 1. Comparative Effects of Inhaled $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ on Survival Time of Rats

* This study was supported in part by U.S. Air Force Contract Purchase Request No. 65-5710-335.

dose was necessary to kill within 1 or 2 weeks than was required for death after several months.

The highest levels of either isotope caused massive serum infiltration of the lungs within a few days. At lower doses, this was seen at later times. Lungs also showed increased proliferation of alveolar or terminal bronchiolar epithelium, alveolar wall thickening, and fibrillous organization. Rats which inhaled the highest levels of plutonium lost up to one-third of their pre-exposure weights before death at 6-8 days after exposure.

Conclusions

Because the particle size distributions of the $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ aerosols were essentially the same,

equal μCi alveolar burdens comprised approximately 270 times fewer particles of $^{238}\text{PuO}_2$, due to its much higher specific activity. This caused a greatly different microscopic distribution of particles and radiation dose in the lungs for the two isotopes. However, since the acute lethal doses were similar for the two isotopes, the difference in microscopic distribution of the absorbed radiation from the two isotopes was apparently inconsequential.

Rats which have survived more than 1 year after inhalation of the lowest levels of $^{238}\text{PuO}_2$ (initial alveolar burdens were $<0.3 \mu\text{Ci}$) are being observed for long-term effects, particularly for possible tumor formation, and the effects of translocation of plutonium to other organs.

GASTROINTESTINAL PASSAGE TIME AND ABSORPTION OF $^{238}\text{PuO}_2$ AND $^{239}\text{PuO}_2$ MICROSPHERES IN MINIATURE SWINE

Both $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ microspheres were delayed in their passage through the intestinal tract of miniature swine. Times for 50 or 99% elimination of the two radio-nuclides were similar, but the total excretion pattern was quite different. Absorption varied widely among different animals but was generally greater for ^{239}Pu than for ^{238}Pu .

Investigators:

V. H. Smith
M. T. Karagianes
C. R. Watson

Technical Assistance

J. L. Beamer

In a previous study with $^{238}\text{PuO}_2$ microspheres, it was established that their gastrointestinal passage time

in Hanford Miniature Swine was delayed several days beyond that considered normal for the passage of

food in other swine breeds (Annual Report, 1966). In an attempt to establish if that function was influenced by the radiation from the ^{238}Pu , a very similar material, $^{239}\text{PuO}_2$, was fed to pigs and both the passage time and absorption were determined. As a basis for comparison, normal passage time was determined by labeling the food with a poorly absorbed, water soluble ^{60}Co chelate.

Observations

A summary of the passage time data is shown in Figure 1, which includes the earlier data for $^{238}\text{PuO}_2$ microspheres as well as the new data on $^{239}\text{PuO}_2$ microspheres and the data obtained with the soluble nonabsorbed tracer.

The $^{239}\text{PuO}_2$ microspheres were similar in manufacture* and particle size to the $^{238}\text{PuO}_2$ microspheres previously used (Annual Report, 1966). They were fed to three pigs in quantities equivalent masswise to the earlier $^{238}\text{PuO}_2$ feedings and by the methods utilized in the earlier experiments. Due to differences in the specific activity of the two radionuclides, about 280 times less alpha radioactivity was fed in the ^{239}Pu experiments. Thus, it was anticipated that effects due to alpha radiation or thermal heat would show up as differences in the two experiments.

* Supplied by Mound Laboratory,
Miamisburg, Ohio

FIGURE 1. Time of Cumulative Excretion of Specified Fraction of Fed Radionuclide

From the data of Figure 1, one could deduce no difference in the behavior of the ^{238}Pu and ^{239}Pu microspheres. Both moved more slowly than the soluble food marker, a ^{60}Co chelate of triethylenetetraminehexaacetic acid ($^{60}\text{Co}_2\text{NaHTTHA}$). The food marker was 99% eliminated in 4-5 days, a passage time in agreement with values generally accepted for other swine.

Although not apparent in Figure 1, the pattern of $^{239}\text{PuO}_2$ microsphere excretion was quite different from that of ^{238}Pu . No more than about 45% of the ^{238}Pu was excreted during any 24 hr collection period, while over 70% of the ^{239}Pu in all three pigs was passed during a single day (Figure 2). Thus, there was a greater tendency for the $^{239}\text{PuO}_2$ microspheres to travel in a group. This was also evidenced by the fact that, though both groups showed an

FIGURE 2. Frequency Distribution of Animals Excreting a Given Percentage of Radionuclide on a Given Day

equal tendency (two-thirds of the animals) to retain particles until necropsy on the fourteenth day post-feeding, no $^{239}\text{PuO}_2$ microspheres were found above the spiral colon, while $^{238}\text{PuO}_2$ microspheres were often found in the small intestine.

The amount of ^{239}Pu absorbed from the passage of the microspheres through the gastrointestinal tract and still present in major tissues on the fourteenth day is shown in Table I. Despite the extreme variability in both this and the earlier ^{238}Pu data, one can make some comparisons. Adding the average liver and skeleton content

gives a value of about $0.15 \times 10^{-5}\%$ for absorption of ^{239}Pu from ingested microspheres, which seems quite reasonable when compared to the figure of $3 \times 10^{-3}\%$ which is frequently quoted for the absorption of $^{239}\text{Pu}(\text{NO}_3)_4$. Data reported last year for the absorption of ^{238}Pu from ingested $^{238}\text{PuO}_2$ microspheres was extremely variable and consistently lower than the presently reported results for ^{239}Pu . However, the highest measured value for ^{238}Pu absorption, $0.08 \times 10^{-3}\%$, was only a factor of two lower than the average value for ^{239}Pu .

TABLE I. Plutonium Distribution in Tissues of Miniature Swine 14 Days After the Ingestion of $^{239}\text{PuO}_2$ Microspheres

Pig Number	^{239}Pu Fed, mCi	PuO_2 Fed, mg	Distribution of Fed Dose, $10^{-6}\%$		
			Liver	Kidneys	Skeleton
2422	3.4	62	3.6	1.2	48
2469	4.9	89	0.75	2.2	53
2500	2.6	47	120	2.1	120

The choice of $^{60}\text{Co}_2\text{NaHTTHA}$ as a food tracer was based on its high stability constant, 28, and the consequent low likelihood of exchange for body metals. Only about 0.013% of the 130- μCi dose was found in the liver at necropsy on the fourteenth day. The relatively large amount of ^{60}Co was fed to be certain of seeing any tailing effects in the fecal output. Lithium fluoride powder dosimeters attached to the serosal surface of the stomach, duodenum, jejunum, ileum, cecum, spiral colon, liver, and spleen showed that the highest dose, 1.3 R, was received at the ileocecal junction. This is the location that showed the highest dose for the passage of 1 Ci of $^{238}\text{PuO}_2$ microspheres (Annual Report, 1966).

Conclusions

Both $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ microspheres exhibited a substantial delay in passage time, as compared to the rate of passage of a soluble food marker. However, there was a difference in the excretion pattern of the two radionuclides. Whether this is due to the greater radioactivity of the ^{238}Pu , to thermal properties, or to surface differences, is not known. While highly variable results were obtained for the absorption of both ^{239}Pu and ^{238}Pu from ingested microspheres, the maximum figures obtained would suggest that no more than one-millionth of the ingested material would be absorbed and deposited in liver and skeleton.

IN VIVO THERMOLUMINESCENCE DOSIMETRY OF INGESTED ^{60}Co IN SWINE

Lithium fluoride (LiF) thermoluminescence dosimeters were implanted in swine prior to feeding them a meal tagged with ^{60}Co . The dose distribution was similar to that measured in earlier studies with $^{238}\text{PuO}_2$ microspheres.

Investigators:
C. R. Watson
M. F. Gillis
M. T. Karagianes
V. H. Smith

This study was prompted by the difficulties involved in calculating radiation dose to the intestine and other visceral organs from ingested radioactive material passing through the gut. Earlier studies with $^{238}\text{PuO}_2$ microspheres were described in last year's Annual Report.

Observations

Lithium fluoride thermoluminescence dosimeter capsules were surgically implanted in the abdominal cavities of three Hanford Miniature Swine 4 months prior to oral administration of 130 μCi of a poorly assimilable water-soluble chelate of ^{60}Co (see preceding report). The pigs were killed 14 days later and the dosimeters were recovered.

The dosimeters used in one of the pigs were teflon capsules containing about 60 mg of LiF powder. There was some tissue reaction to these

dosimeters, and over half of them leaked so that the powder did not flow freely at readout. The results from this pig are not reported. Such leakage was not encountered in the other two pigs, where dosimeters were heat-sealed into 2 cm segments of polyethylene tubing.

Dosimeters were read on our TLD system which is modified for low-dose measurement and calibrated for ^{60}Co . The measured values are shown in Figure 1. Highest readings were obtained at the ileocecal junction which is the most constricted portion of the gut. A similar result was obtained last year in the ^{238}Pu study.

Conclusions

This study has shown the ease of direct measurement possible with LiF dosimeters in a situation that does not lend itself to theoretical solution.

FIGURE 1. Cumulative Radiation Dose to Various Locations in Swine 14 Days After Ingestion of Nonassimilable ^{60}Co

THE SOLUBILITY OF $^{238}\text{PuO}_2$ MICROSPHERES IN SIMULATED GASTRIC JUICE

Due to the extreme variation among microspheres tested, values given for their solubility behavior can only be considered as "examples." An initial, more rapid rate of solution was followed after 1-2 hr by a much slower rate.

Investigator:
V. H. Smith

Normally insoluble forms of radio-nuclides may be transformed under the acid condition prevailing in the stomach to a form more readily absorbed from the alimentary canal. A simulated gastric juice (Table I) was employed in studies of the solubility kinetics of individual $^{238}\text{PuO}_2$ microspheres.

Observations

The solubility of three microspheres in stirred, simulated gastric

juice at body temperature is shown in Figure 1. These microspheres had been isolated from the feces of a pig after they had passed through the gastrointestinal tract and should, therefore, have been thoroughly surface decontaminated. They derived from the same manufacturing run; were of about the same size; were relatively smooth, microscopically; and were of a high degree of sphericity.

Reruns on individual microspheres were in reasonable agreement (Figure 1), indicating that the method was

TABLE I. Composition of Simulated Gastric Juice

Gastric mucin	360 mg
Pepsin (3x crystallized)	1 mg (~3200 Hb units)
Ascorbic acid	1 mg
Urea	3 mg
Uric acid	1 mg
KCl	150 mg
$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$	40 mg
NaCl	280 mg
Sialic acid (25.5% assay)	29.2 mg (7.4 mg sialic acid)
Sodium glucuronate	2.2 mg
HCl	7.8 meq (to give pH of 1.0)
H_2O	To total volume of 100 ml

Mixed with cooling and ultrasonic agitation. The low pH was chosen to maximize solubility effects.

FIGURE 1. Solubility of $^{238}\text{PuO}_2$ Microspheres in Simulated Gastric Juice at 37 °C (Diameters of particles are noted on curves)

reliable. Since the first run with a particle usually gave a higher solubility than the second, there may be residual surface effects which influence solubility. No changes in microsphere diameter were noted using a microphotographic technique capable of measuring changes of about 0.5 μ .

The results shown in Figure 1 are in no sense "representative" of

$^{238}\text{PuO}_2$ microspheres, generally. In similar experiments with other microspheres, results were obtained which varied by as much as 100-fold.

Conclusions

In simulated gastric juice, $^{238}\text{PuO}_2$ microspheres exhibit an early rapid rate of dissolution followed after 1-2 hr by a constant and relatively slower rate. Assuming the stomach would normally empty within 1-4 hr, the faster early solution rate would dominate hazard considerations.

The values reported are valid only for the particular microspheres employed in this experiment and should not be considered to represent the solubility of an average SNAP fuel particle. Solubility may be expected to vary widely, depending on the production conditions, surface variation, cleaning procedure, and the general past history of the microsphere. To quantitate the effect of these many variables would require a very major effort which scarcely seems justified in view of the very low absorption measured in swine which ingested such particles (Annual Report, 1966).

EFFECTS OF ^{238}Pu MICROSPHERES LODGED ON LEAF SURFACES

Localized damage to leaf surfaces was observed following exposure to ^{238}Pu microspheres. Such damage increased the tendency for retention of the microspheres on the leaf surface.

Investigator:
J. J. C. Hsieh

The tendency for retention of particles on leaf surfaces is of consequence in evaluating the probability that such particles may ultimately be ingested by animals or man. In the case of intensely radioactive particles, there is a possibility that damage to the leaf surface may modify particle retention. In this study, we examined the gross and detailed morphological changes in leaf surfaces due to lodged $^{238}\text{PuO}_2$ microspheres and related these changes to observed effects on particle retention.

Observations

Acetone- or alcohol-washed $^{238}\text{PuO}_2$ microspheres (100 to 300 μ diameter) were placed on leaves of intact begonia and African violet plants. In some instances, the microspheres were placed directly on the leaf and in others they were held just short of touching the leaf surface on the pointed tip of a glass rod.

Damage was observed within 2-3 hr on the waxy surface of begonia leaves. By 10 days the entire central cellular portion of the leaf was destroyed and the surface layers had sunk into a concave configuration. Dislodgement of microspheres was easily accomplished, immediately after deposi-

tion, by turning the leaf on edge or upside down. However, once damage became obvious, turning the leaf over was no longer effective in dislodging particles.

Similar results were obtained with African violet leaves which have a very hairy surface. Grossly visible damage appeared early and frequently developed as a break in the cuticle layer of the leaf. One microsphere dropped completely through the leaf by the ninth day. Dislodgement, from the moment of deposition, was more difficult than in the case of begonia leaves, but became increasingly difficult as damage became apparent.

In all cases some very small fraction of residual activity (of the order of tens of pCi) remained on the leaf following removal of the microspheres.

Microscopic changes were studied with silicone rubber and plastic replicas of the damaged area. Sub-microscopic changes were studied in the electron microscope with chromium-shadowed carbon replicas. Light microscopy showed not only the surface depression but also gave an impression of sagging in the cells beneath the surface and a rough appearance in the surface film suggesting a melting of the wax of the

cuticle (Figure 1). Somewhat to our surprise, the electron microscope showed no significant ultrastructural differences between exposed and control areas.

Conclusions

Measurements reported elsewhere in this report indicate that effective temperatures of about 350-400 °F exist at surfaces adjacent to $^{238}\text{PuO}_2$ microspheres. Whether the damage observed is primarily due to a simple temperature effect, or to other manifestations of the intense alpha flux, cannot be stated. In any case, there is a resulting tendency for increased retention of the microsphere on the leaf surface, which must result in some increased probability of ingestion by animals or man.

80X

FIGURE 1. Replica of the Border Zone Between Normal (Above) and Damaged (Below) Upper Leaf Surface of a Begonia Leaf 3 hr After a 150- μ $^{238}\text{PuO}_2$ Particle Was Placed in Contact with the Leaf

ELECTRON MICROSCOPIC DETERMINATION OF SPECIFIC LEAF SURFACE AREA

An estimate of specific Zeaf surface area can be obtained by comparing the contour of the surface with the linear distance of the observed thin cross sections of a Zeaf replica. The first approximation of the specific surface area of a begonia Zeaf sample was about 2.3.

Investigators:
J. J. C. Hsieh
J. M. Thomas*

Leaf surface area is often assumed to be equal to the planar surface. For some purposes, such as particulate deposition and comparison of gas dif-

fusion through stomate or nonstomate areas, a value for the total surface is required. The present study is directed toward a quantitative determination of specific surface area, i.e., the ratio of the actual or

*Mathematics Department

total surface area to its normally projected planar surface. If the specific surface area was determined, then the total surface area of a leaf sample is equal to the product of the average specific surface area and the planar surface area.

The difficulty in measuring the three-dimensional actual contour area can be circumvented by electron microscopic examination of a surface sectioned vertically to the surface plane. The specific surface area can be calculated from the measurements of the contour and the corresponding planar length of the leaf replica sections.

Observations

A small section of begonia leaf was placed under vacuum in a metal evaporating unit. The leaf surface replica was formed by shadowing the leaf with chromium from two opposite directions. The replica was then coated with a relatively thick carbon film followed with 10% parlodian in amyl acetate and the coating air dried. After applying a 5% gelatin solution, the entire surface replica unit was peeled from the leaf. The replica was cut into 2-mm squares, which have enough mechanical strength to maintain the shape of the section contour. The carbon replica was floated from the gelatin film as it was suspended in amyl acetate solvent. The shadowed carbon replica was embedded in Epon and sectioned vertically at about 500 Å.

From each electron microphotograph, the contour length of the chromium

layer and its linear normal distance were measured. A tentative average specific surface area of about 2.3 was calculated for the begonia leaf by using the equation,

$$S_{\text{sample, symmetric}} \cong \frac{\sum_{i=1}^n \left(\frac{L_{ci}}{L_{ni}} \right)^2}{n}$$

where L_{ci} is the linear length of the section contour, L_{ni} is the normal resolute of L_{ci} for the i^{th} section (Figure 1), and n is the number of examined sections.

The validity of this equation is dependent on the assumptions that the observed sections are so thin that the projected microgeometry or the linear contour lengths of the two adjacent cross sections are essentially identical, and that the small sample considered has symmetric morphology. The technique employed and the morphology of the begonia leaf reasonably meet the above constraints.

FIGURE 1. Diagrammatic Thin Section of a Leaf Sample (See text for explanation)

When the assumption of symmetry is not reasonable, the following equation could be employed to approximate S:

$$S_{\text{sample, asymmetric}} = \frac{\sum_{i=1}^n \left[\left(\frac{L_{ci}}{L_{ni}} \right) \left(\frac{L'_{ci}}{L'_{ni}} \right) \right]}{n}$$

where L'_{ci} and L'_{ni} are the respective section contour and its projected planar linear length for a thin section which is cut perpendicularly to L_{ci} and L_{ni} (Figure 1).

Conclusions

Specific leaf surface area measurements can be obtained from thin cross sections of leaf replica embedded in Epon. Improvements on the methods used should include use of a planimeter to measure the length of the chromium replica and use of a revolving specimen platform to obtain a more uniform and thin coating of chromium. The method is applicable to any surface on which the sub-microscopic detail is of interest.

TEMPERATURE OF SURFACES IN CONTACT WITH $^{238}\text{PuO}_2$ MICROSPHERES

The temperature of a well-ventilated surface in contact with a 200 μ diameter $^{238}\text{PuO}_2$ particle may rise to 350-400 °F as measured by heat-sensing "ThermaTab" strips.

Investigators:
J. J. C. Hsieh
F. P. Hungate
W. C. Roesch^x

In the course of observing effects of $^{238}\text{PuO}_2$ microspheres on leaf surfaces, the question arose as to the relative importance of ionization versus simple heat effects. Efforts were therefore made to measure the temperature of a surface in contact with $^{238}\text{PuO}_2$ microspheres.

Observations

The heat-sensitive sheets, "ThermaTab Jr."** were employed in

these measurements. The normal surface of the ThermaTab is white. When the temperature rises above the critical point for the specific ThermaTab used, the color changes to black--presumably by the melting of metal organic crystals on the surface which exposes a black backing. ThermaTabs indicating temperatures at 10 °F intervals from 200-450 °F were used.

Microspheres of diameters 150-250 μ were either placed directly on the ThermaTab strips or attached to the pointed tip of a glass rod and placed over, and almost touching, the crystal surface. The experiments were conducted in an open petri dish

* *Environmental and Radiological Sciences Department*

** *Camwil, Inc., 11821 West Pico Blvd., Los Angeles, California*

subjected to a rapid flow of air in an exhaust hood.

No obvious change was observed after 2 days' exposure of 440 °F ThermaTab strips. A yellow coloration developed beneath particles on the 400 °F strip. An area of about 2-mm diameter turned black under particles on ThermaTabs with a critical temperature of 350 °F or lower. The reactions of a ThermaTab to a given $^{238}\text{PuO}_2$ microsphere were about the same whether the particle was attached to the glass rod or placed directly on the crystal surface. There were significant differences among similarly sized particles in producing thermal effects. An agglomeration of glassy microscopic bubbles on certain microspheres appeared to exert an insulating effect.

Conclusions

From the above observations it may be concluded that surfaces apposed to a 200 μ diameter $^{238}\text{PuO}_2$ microsphere may reach temperatures of at least 350-400 °F. Higher temperatures might have been reached if air movement had been more restricted. This rather high temperature recalls Primak's dis-

cussion on the thermal spike in radiation damage.⁽¹⁾ The thermal spike encompasses about 10^4 atoms which may experience a temperature rise of several hundred degrees in an intermediate conductor such as quartz. In a poor conductor the corresponding temperature rises could be much greater. Tuck⁽²⁾ favored the thermal spike hypothesis to explain the surface flaking damage of a glass container exposed to alpha particle bombardment. The possibility that leaf damage from ^{238}Pu microspheres could be due to thermal effects was heightened by observing that touching the tip of a hot glass rod to leaf surfaces produced damage visibly comparable to that from the ^{238}Pu . On the other hand, it is not certain that the melting and blackening of ThermaTab may not be a consequence of ionization-induced breakdown of lattice structure rather than due to purely thermal effects.

References

1. Primak, W. *Phys. Rev.*, vol. 98, pp. 1854-1855. 1955.
2. Tuck, D. G. *Int. Jour. of Applied Radiation and Isotopes*, vol. 15, pp. 49-57. 1964.

RADIOELEMENT TOXICITY STUDIES

Included in this section are a miscellaneous group of radioelement toxicity studies which are not associated with the major programs covered in the previous sections. The first report describes recent observations in our 17 year old radioiodine toxicity program. Aside from a few sheep that remain on long-term observation, the only activity left in this program is a study in miniature swine, which will allow comparison of long-term radioiodine effects in sheep and swine.

Four reports are concerned with metabolic studies in rats, involving radioisotopes of plutonium, iodine, and cesium. Three of these reports deal with effects of age. Particularly in the case of plutonium, we are accumulating a body of data which attests to the extreme complexity of the dose-effect relationships as influenced by such factors as age, route of administration, and physicochemical form of the administered radionuclide. Some rather puzzling differences in the acute toxicity of ^{238}Pu and ^{239}Pu now seem to be reasonably explained in terms of physicochemical state as influenced by the greatly different specific activity of the two isotopes.

The past year saw the initiation of radionuclide distribution studies in human volunteers. A study employing the photon-emitting ^{143}Pm was jointly undertaken by Battelle-Northwest and the Hanford Environmental Health Foundation. Data from this experiment were not in shape for publication at year end, but the techniques of whole-body counting which can be employed with such radionuclides would seem to offer great promise for the future. Two reports in this section are concerned with this effort. One describes preliminary tests of the ^{143}Pm injectant in a miniature swine; the other describes preliminary investigations of the potentialities of the isotope ^{237}Pu for human studies.

PUBLICATIONS

Ballou, J. E., R. C. Thompson, W. J. Clarke, and J. L. Palotay. "Comparative Toxicity of Plutonium-238 and Plutonium-239 in the Rat," Health Physics, vol. 13, pp. 1087-1092. 1967.

Eberhardt, L. L. "Relationship of Cesium-137 Half-life in Humans to Body Weight," Health Physics, vol. 13, pp. 88-90. 1967.

Mahlum, D. D. "Effect of Hepato-carcinogens on the Metabolism of Some Radionuclides by Rats," Toxicol. Appl. Pharmacol., vol. 10, p. 381. 1961. (Abstr.)

Mahlum, D. D. "Neptunium-237 Toxicity in the Rat. II. Intracellular Distribution of Neptunium and Cerium in Rat Liver," Toxicol. Appl. Pharmacol., vol. 11, pp. 264-271. 1967.

Mahlum, D. D. and M. R. Sikov. "Effect of Age on Cesium Metabolism in the Rat," Federation Proc., vol. 26, p. 307. 1967. (Abstr.)

Muth, O. H., H. W. Pendell, C. R. Watson, J. E. Oldfield and P. H. Weswig. "Uptake and Retention of Parenterally Administered ⁷⁵Se in Ewes on Different Selenium Regimens," Am. J. Vet. Res., vol. 28, pp. 1397-1406. 1967.

Sikov, M. R. "Effect of Age on the Avidity for Radioiodine and the Radiation Sensitivity of the Rat Thyroid," Radiation Res., vol. 31, p. 589. 1967. (Abstr.)

Sikov, M. R., J. M. Thomas, and D. D. Mahlum. "Mathematical Analysis of Passage Through the Gastrointestinal Tract," Physics in Medicine and Biology, vol. 12, p. 15. 1967. (Abstr.)

Thompson, R. C. "Biological Factors," vol. 2, chapter 23. In: O. J. Wick (ed.) Plutonium Handbook; A Guide to the Technology. Gordon and Breach, N.Y. 1967.

Thompson, R. C. and P. L. Hackett. "Urinary/Fecal Excretion Ratios of Strontium and Calcium in the Rat," In: J.M.A. Lenihan, J. F. Loutit, and J. H. Martin (eds.) Strontium Metabolism, pp. 250-260. Academic Press, Inc. 1967.

Watson, C. R., and R. O. McClellan. "In Vivo Thermoluminescence Dosimetry of Gamma Rays from Ingested Cesium-137 in Sheep," pp. 393-401. In: Frank H. Attix (ed.) Luminescence Dosimetry. Proceedings of International Conference held at Stanford University, June 21-23, 1965. U.S. AEC Symposium Series No. 8, 1967.

EFFECTS OF RADIOIODINE IN SHEEP AND SWINE

Thyroid glands of eight miniature swine fed 2000 μCi ^{131}I , a year previously, retained less of an ingested tracer dose of ^{131}I than did four control swine. Two thyroid adenomas and a chromophobe adenoma of the pituitary were observed in sheep previously exposed to ^{131}I .

Investigators:

M. T. Karagianes
J. L. Beamer
M. F. Gillis
J. L. Palotay

A study to determine the long-term effects of ^{131}I in miniature swine, for comparison with past studies in sheep, was initiated in 1966 (Annual Report, 1966). Eight 5-month-old females were given an oral dose of 2000 μCi . A year subsequent to this treatment a tracer dose of 10 μCi ^{131}I was administered to determine if partial ablation of the thyroid had occurred.

Observations

A year after oral administration of the 2000- μCi dose of ^{131}I , all swine appeared physically healthy with no external signs of hypothyroidism. Results of the oral administration of a 10- μCi tracer dose are shown in Figure 1. Thyroid deposition of the initial 2000- μCi

FIGURE 1. Thyroid Uptake of ^{131}I Following Ingestion of a 10 μCi Tracer Dose by Four Control Swine and eight Swine Fed 2000 μCi ^{131}I One Year Previously (Mean values $\pm$ std. dev.)

dose reached a peak at 45 hr with retention of approximately 25% of the fed ^{131}I . The tracer dose fed a year later to the same animals, which were 30-45 kg heavier, exhibited a maximum uptake time averaging 26 hr for controls and 31 hr for the previously treated swine. The maximum uptake averaged 13.8% for controls and 10.8% for the previously treated swine. Damage to the thyroids was evident not only in terms of reduced maximum uptake, but more impressively in terms of retention beyond the second day.

Loss of thyroid function in these animals will be determined annually. At 4 years following the initial insult, effects of hypothyroidism on reproductive ability will be evaluated and compared with similar data available on sheep.

A few animals remain on long-term observation from the extensive studies on ^{131}I effects in sheep which have been conducted at this laboratory over the past 17 years. Four of these animals died during the past year. All had received ^{131}I exposure during gestation and

from the dam's milk until weaning. Their dams were receiving 5 μCi orally per day. Histologic examination of the thyroids revealed one to be normal (8 years old) and two (7 and 11 years old) to have small localized proliferation of acinar epithelial cells constituting small adenomas. Most of these cells were nonfunctional, although some tended to form small acini with colloid formation. One of these sheep had a concurrent large chromophobe adenoma of the pituitary gland. Data are not yet available on the fourth animal.

Conclusions

Comparative studies of long-term effects of ^{131}I in miniature swine are still in the early stages. Any attempt at comparison with the extensive data available on sheep would be premature at this time. It is clear, however, that the 2000- μCi ^{131}I dose did partially ablate the thyroid glands of the treated swine, and further evidence of damage may be anticipated with continued observation.

PHYSICOCHEMICAL FORM AS A DETERMINANT OF THE TOXICITY OF ^{238}Pu IN THE RAT

The acute toxicity of ^{238}Pu is related to the physicochemical form injected, with the polymeric form being nearly twice as toxic as the monomer. The polymeric form produced earlier deaths, and was preferentially deposited in soft tissues, while the monomer was preferentially deposited in the skeleton.

Investigators:

D. D. Mahlum

M. R. Sikov

Technical Assistance:

Joan O. Hess

Nancy L. Lukens

Although ^{238}Pu and ^{239}Pu are both alpha emitters of similar energies, the $\text{LD}_{50/30}$ value for ^{238}Pu is about twice that for ^{239}Pu . Ballou et al. (1) suggested, on the basis of tissue distribution data, that this difference in toxicity is due to differences in the physicochemical state of the isotopes resulting from the 270-fold difference in specific activity. This led us to study directly the influence of physicochemical state on the acute toxic properties of ^{238}Pu .

Observations

Monomeric ^{238}Pu was prepared by mixing sodium citrate (>1000-fold molar excess) with an acid solution of the nuclide and adjusting the pH to 4.5 with anhydrous Na_2CO_3 . The polymeric form was prepared by adjusting an acid solution of ^{238}Pu to pH 4.5 in the absence of citrate.

Female rats of the Charles River CD strain, 3-4 months old, were injected intravenously with 54, 108, or 162 $\mu\text{Ci/kg}$ of the monomer, or 50, 100, or 150 $\mu\text{Ci/kg}$ of the polymer. These animals were kept for determination of survival times with

results as summarized in Table I.

In a second experiment rats were injected with 54 or 161 $\mu\text{Ci/kg}$ of the monomer, or 50 or 100 $\mu\text{Ci/kg}$ of the polymer, and groups killed at 1, 7, and 33 days postinjection for tissue radioanalysis. Results from this experiment are summarized in Table II.

Polymeric ^{238}Pu was significantly more toxic than the monomer. This was true whether 30- or 60-day survival figures or the median survival times were considered. Weight gain was inhibited in all of the plutonium-injected animals, but the greater toxicity of the polymer was again evident. Figure 1 shows growth curves for the groups with a substantial number of survivors beyond 60 days.

As shown in Table II, there was a substantial difference in the distribution of the monomer and the polymer. Nearly twice as much monomer, as polymer, was deposited in bone; while a substantially greater proportion of polymer was deposited in liver. The fraction in the liver decreased very little between 1 and 7 days, but was markedly reduced in the monomer-injected animals after 33 days. Femur

TABLE I. Mortality Following Intravenous Injection of Monomeric or Polymeric ^{238}Pu

Physicochemical Form	Dose Administered, $\mu\text{Ci/kg}$	Mortality ^(a)		Median Survival Time, days
		At 30 Days	At 60 Days	
Monomer	0	0/12	0/12	>224
[LD _{50/30} >162 $\mu\text{Ci/kg}$]	54	0/12	0/12	>224
[LD _{50/60} 124 $\mu\text{Ci/kg}$]	108	0/12	6/12	62
[(91-156) ^(b)]	162	1/12	9/12	38
Polymer	50	0/12	2/12	77
[LD _{50/30} 98 $\mu\text{Ci/kg}$]	100	7/12	10/12	24
[(67-144) ^(b)]	150	10/12	12/12	20
[LD _{50/60} 75 $\mu\text{Ci/kg}$]				
[(56-156) ^(b)]				

(a) *Number dead/number injected*

(b) *95% Confidence interval*

TABLE II. Distribution Among Tissues of Intravenously Injected Monomeric or Polymeric ^{238}Pu ^(a)

Days After Injection	Physico-chemical Form	Dose, $\mu\text{Ci/kg}$	Organ Content, % of administered dose				
			Liver	Spleen	Kidney	Adrenal	Femur
1	Monomer	54	21	0.2	0.47	0.007	1.5
		161	24	0.2	0.77	0.011	3.0
	Polymer	50	35	0.8	0.32	0.003	1.0
7	Monomer	54	14	0.2	0.40	0.006	2.8
		161	21	0.2	0.36	0.006	3.9
	Polymer	50	30	4.2	0.24	0.005	1.5
		100	28	2.1	0.32	0.004	1.5
33	Monomer	54	2	0.2	0.23	0.004	2.5
		161	2	0.2	0.16	0.005	3.1

(a) *Average values from two to three rats*

FIGURE 1. Effect of ^{238}Pu on the Weight of Female Rats from Groups with a Substantial Number of Survivors Beyond 60 Days

and spleen values increased beyond the first day postinjection, with particularly high spleen values attained in the polymer-injected animals.

Adrenal weights were increased at both the 50 and 100 $\mu\text{Ci/kg}$ levels of polymer, whether considered on an absolute basis or as a percent of body weight. Spleen weights, on the other hand, were decreased in both monomer- and polymer-injected animals. No remarkable changes were noted in weights of other organs.

Conclusions

The acute toxicity of ^{238}Pu , like that of ^{239}Pu , is related to the degree of polymerization of the injected material. This effect seems most reasonably attributable to differences in distribution of monomeric and polymeric forms, with the enhanced soft-tissue deposition of the polymer favoring acute lethality.

References

1. Ballou, J. E., R. C. Thompson, W. J. Clarke, and J. L. Palotay. *Health Phys.*, vol. 13, pp. 1087-1092. 1967.

EFFECT OF AGE AND PHYSICOCHEMICAL FORM ON PLUTONIUM TOXICITY
AND METABOLISM IN THE RAT

The distribution and toxic effects of monomeric and polymeric plutonium in neonatal, weanling, and adult rats were compared. The polymer was more acutely toxic than the monomer to weanlings and adults, but there was little difference between the two forms in newborn rats. These differences in acute lethality relate to marked age-dependent differences in distribution of the two forms, which also leads to age-dependent alterations in organs such as liver, spleen, and skeleton.

Investigators:

M. R. Sikov
D. D. Mahlum

Technical Assistance:

D. L. Catt
Joan O. Hess
Nancy L. Lukens

Liver and bone are the principal sites of deposition of injected plutonium. In the adult, the partition between these two tissues, as well as the resulting late effects, has been shown to be dependent on the state of the plutonium administered.(1) These tissues undergo profound alterations between birth and adulthood, but it is not known how these changes affect the metabolism of plutonium. The present studies represent the beginnings of an effort to fill this void.

Observations

Monomeric plutonium was prepared by adding a solution of $^{239}\text{Pu}(\text{NO}_3)_4$ to sufficient sodium citrate to provide a 100-fold molar excess of citrate. The pH was then adjusted to 4.5. The polymeric form was prepared by adjusting the pH of the nitrate to 4.5 in the absence of citrate. These solutions were injected intravenously into rats of the Charles River CD strain at birth,

weaning (21 days), or as young adults, at levels ranging from 6-90 $\mu\text{Ci/kg}$ body weight. Animals from selected age and dose groups were sacrificed at 1, 3, 7, 30, and 90 days for radioanalysis and determination of pathology. Other previously selected animals were observed over a 60-day period for gross mortality. Still other animals were kept for sacrifice and observation of late effects at 9 months.

Acute lethality data are summarized in Table I. The polymer was more toxic to weanlings and adults than was the monomer, but no such difference was evident in the newborn animals.

Weight gains were suppressed in animals of all age groups by ^{239}Pu administration. Figure 1 illustrates the results with a 60 $\mu\text{Ci/kg}$ dose in female rats of the three ages studied. At each dose level the weight gains of the newborns were less severely affected than those of the weanlings or adults.

TABLE I. Acute Lethal Effect of ^{239}Pu Administered as Monomer or Polymer to Rats of Different Ages (Average values from 12 animals)

Age at Administration	Form Administered	Median Survival Times. days for various injected doses ^(a)			LD ₅₀ $\mu\text{Ci/kg}$ ^(b)	
		30 μCi	60 μCi	90 μCi	30 Days	60 Days
Newborn	Monomer	>60	28	20	74 (61-90)	53 (40-66)
	Polymer	>60	>60	14	66 (48-107)	63 (45-96)
Weanling	Monomer	>60	>60	>60	>90 ^(c)	104 ^(c)
	Polymer	>60	20	13	47 (32-60)	43 (31-54)
Adult	Monomer	>60	>60	48	97 ^(c)	76 (66-87)
	Polymer	>60	21	13	47 (36-58)	38 (29-47)

(a) Administered dose levels below 30 $\mu\text{Ci/kg}$ showed no acute mortality

(b) 95% Confidence interval shown in parentheses

(c) Estimated by extrapolation

Monomeric plutonium was deposited in the skeleton to a greater extent than in the liver, while, with the polymer, liver deposition predominated. From the detailed data on tissue deposition and retention, average tissue radiation dose rates were calculated as a function of age, form administered, and time since administration. Figures 2 and 3 illustrate these findings for liver and femur, and serve to explain many of the observed pathologic effects.

The most obvious changes in the femurs consisted of a decreased osteoblastic activity and extreme disorganization of the epiphyseal

plate. In the weanling, this resulted in fracture of the shaft with abnormal callous formation. Breaking strength (fracture force per unit cross-sectional area) of the femur was determined at 9 months postexposure; the results are summarized in Table II. Administration of the monomer produced a dose-dependent decrease in strength in the weanling and adult groups, but had little effect in the newborn group; the polymer was less effective.

Gross and histological damage was noted in the livers, particularly in the animals receiving the polymer, but also evident in those receiving

FIGURE 1. Weight Changes of Rats After Treatment as Newborns, Weanlings, or Adults with 60 $\mu\text{Ci/kg}$ of Monomeric or Polymeric ^{239}Pu

the monomer. The changes were most severe in those exposed as weanlings, less in those treated at birth, and least in the adults.

The rate of clearance of rose-bengal by the liver was determined in the animals sacrificed at 9 months after exposure to the three lowest dose levels (6, 12, 18 $\mu\text{Ci/kg}$). The major effect was noted in the adult group treated with 18 $\mu\text{Ci/kg}$ of the polymer. A limited number of animals

receiving the other levels of plutonium showed a decreased clearance rate.

Neurologic function was measured at 9 months after plutonium administration to determine if the developing central nervous system, which is highly radiosensitive, had been damaged by plutonium incorporated into the skeletal elements surrounding it. The ability to learn a water maze was found to be unaltered. The data

FIGURE 2. Calculated Average Radiation Dose to the Liver Normalized to Administration of $60 \mu\text{Ci/kg}$ of Monomeric or Polymeric ^{239}Pu to Newborn, Weanling, or Adult Rats

shown in Figure 4 indicate a general tendency toward a dose-dependent increase in the susceptibility to audiogenic seizures after exposure of the weanlings to the monomer. The rats exposed at birth were the only group to show increased seizure incidence after exposure to the polymer. Similar studies were made after Metrazol administration which should lower the threshold for audiogenic seizures. Results were less consistent, with some indication

that ^{239}Pu may inhibit the usual threshold-depressing action of Metrazol.

The weights of several organs, in relation to total body weight, were altered by exposure to plutonium. The direction and magnitude of these changes related differently to the three variables, age, physicochemical form, and dose, as well as to time postexposure. This created a highly complex matrix which has not yet been completely analyzed. As an

FIGURE 3. *Calculated Average Radiation Dose to the Femur Normalized to Administration of 60 $\mu\text{Ci}/\text{kg}$ of Monomeric or Polymeric ^{239}Pu to Newborn, Weanling, or Adult Rats*

example of the results obtained, the relative weights of the spleens at 3 months after exposure are shown in Table III. The splenic weight of the rats injected at birth with the highest doses of the monomer tended to be increased while those from animals which received the polymer were largely unchanged. The spleens of animals exposed as weanlings or adults, on the other hand, were less responsive to the monomer, but were greatly decreased in size at 3 months after injection of the polymer.

Conclusions

Most of the parameters studied in these investigations were differently affected by the two physicochemical forms of plutonium and the effects varied as a function of the age of the animal studied. These parameters included radionuclide distribution and retention, growth, acute and chronic toxicity, and damaging effects on a number of specific organ systems. Although it is not yet

FIGURE 4. Percent Incidence of Audiogenic Seizures 9 Months After Exposure to ^{239}Pu (Shaded bars indicate severe seizures; open bars, total seizures. Statistically significant differences from controls are indicated by an asterisk. Numbers of animals tested are given in parentheses.)

TABLE II. Femoral Breaking Strength 9 Months After Administration of Monomeric or Polymeric ^{239}Pu to Newborn, Weanling, or Adult Rats

Age at Administration	Dose, $\mu\text{Ci/kg}$	Breaking Strength, g/mm ² (a)	
		Monomer	Polymer
Newborn	0	286 $\pm$ 22 ^(b) (16) ^(c)	
	6	237 $\pm$ 20 (4)	283 $\pm$ 48 (4)
	12	261 $\pm$ 20 (3)	283 $\pm$ 50 (4)
	18	254 (2)	306 $\pm$ 95 (4)
Weanling	0	269 $\pm$ 26 (8)	
	6	226 $\pm$ 36 (6)	205 $\pm$ 9 (5)
	12	227 $\pm$ 22 (8)	315 $\pm$ 13 (7)
	18	184 $\pm$ 21 (5)	257 $\pm$ 20 (6)
Adult	0	296 $\pm$ 20 (8)	
	6	240 $\pm$ 80 (8)	250 $\pm$ 34 (8)
	12	232 $\pm$ 73 (5)	275 $\pm$ 19 (6)
	18	195 $\pm$ 81 (4)	181 $\pm$ 22 (7)

(a) Uncorrected for mechanical advantage of system

(b) Standard error

(c) Number of animals

TABLE III. Relative Spleen Weight ($\frac{\text{Spleen Weight}}{\text{Body Weight}}$) at 3 Months After Intravenous Injection of ^{239}Pu (Average values from three or more animals)

Form Injected	Dose, μCi	Age at Injection		
		Newborn	Weanling	Adult
Control		0.16	0.17	0.15
Monomer	6	0.15	0.22	0.23
	12	0.14	0.14	0.26
	18	0.21	0.17	0.15
	30	0.14	0.13	0.084
	60	0.27	0.25	0.21
	90	0.39	0.25	0.18
Polymer	6	0.14	0.12	0.06
	12	0.12	0.17	0.06
	18	0.14	0.06	0.02
	30	0.14	0.018	0.051
	60	0.14	0.024	0.039
	90	0.26	0.017	0.073

possible to synthesize these observations into a unified cause-effect picture, the damaging effects seem generally to correlate with radiation dose as estimated from distribution data, and the variations in distribution seem reasonably explainable in chemical and physiological terms.

Reference

1. Rosenthal, M. W. and A. Lindenbaum. *Radiation Res.*, vol. 31, pp. 506-521. 1967.

RADIOELEMENT TOXICITY STUDIES

AGE DEPENDENCE OF RADIOIODINE METABOLISM AND EFFECT IN RATS

A Low-iodine diet increased the uptake of ^{131}I and altered its retention. Quantitative and qualitative differences were seen between prenatal, newborn, weanling, and adult rats. Incorporation and retention were altered when greater than minimal tracer levels of ^{131}I were administered.

Investigators:

*M. R. Sikov
D. D. Mahlum*

Technical Assistance:

*Joan O. Hess
Barbara J. Schulz
Nancy L. Lukens*

This is a continuation of studies described last year on the age-related metabolism and radiosensitivity of the thyroid gland (Annual Report, 1966). No systematic data are available on the changes in iodine metabolism which must occur between the early stages of thyroid development and the adult state. Though it is well recognized that an iodine deficient diet will increase the percentage of a tracer dose incorporated in the thyroid, there are no data relating this effect to age. These factors and their interrelations were investigated in these studies.

Observations

Gravid female rats, after 19 or 20 days of gestation, and normal females of the same age, were injected intraperitoneally with 1, 10, or 25 μCi of carrier-free ^{131}I as sodium iodine. Half of the animals were placed on a low-iodine diet 1 week prior to radioiodine injection and maintained on the diet until sacrifice. This resulted in six dose-diet groups with four or five normal females and eight gravid females

(combined 19- and 20-day gestation) within each group. All rats were sacrificed 24 hr after exposure for determination of thyroidal ^{131}I .

Results are presented in Table I. As expected, both pregnant and non-pregnant females on the low-iodine diet showed a greater incorporation than did the comparable animals on a stock diet. The incorporation of ^{131}I in the thyroid gland of the pregnant rats was significantly less than in the nongravid females. This effect of pregnancy was more marked on a normal diet than on the low-iodine diet. Raising the administered dose above 1 μCi produced a statistically significant decrease in uptake only in the gravid females receiving the low-iodine diet.

The ability of the fetal thyroid to incorporate radioiodine was significantly greater in the 23 hr period between 20 and 21 days of gestation than it was in the preceding 24 hr period. The effect of diet was also more evident in the older fetuses. Doses larger than 1 μCi had a substantial effect on iodine uptake by the fetal thyroid, particularly when the mothers were on a low-iodine diet.

TABLE I. Effect of Pregnancy, Size of Dose, Iodine Content of Diet, and Stage of Gestation on Incorporation of Intraperitoneally Administered ^{131}I

Diet	^{131}I Dose. μCi	Percent of Administered ^{131}I in Thyroid			
		Normal Females	Gravid Females	19-20 Day Fetus	20-21 Day Fetus
Stock	1	34.6 $\pm$ 3.8 ^(a)	17.7 $\pm$ 5.1	0.25 $\pm$ 0.03	0.62 $\pm$ 0.10
	10	26.3 $\pm$ 7.5	15.9 $\pm$ 1.4	0.17 $\pm$ 0.02	0.55 $\pm$ 0.18
	25	33.1 $\pm$ 11.9	15.5 $\pm$ 1.3	0.15 $\pm$ 0.02	0.45 $\pm$ 0.05
Low-Iodine	1	56.1 $\pm$ 11.5	49.6 $\pm$ 6.0	0.41 $\pm$ 0.07	1.09 $\pm$ 0.18
	10	53.9 $\pm$ 6.1	31.7 $\pm$ 4.8	0.18 $\pm$ 0.04	0.86 $\pm$ 0.06
	25	43.1 $\pm$ 3.6	34.4 $\pm$ 5.8	0.17 $\pm$ 0.02	0.77 $\pm$ 0.11

(a) Standard error

To further define these various relations of age and dietary iodine content, radioiodine was administered intraperitoneally at levels of 0.01 $\mu\text{Ci/g}$ body weight to 19-day fetuses (via the mother), newborns, weanlings (21 days), young adults (3 months), and older adults (9-11 months). Half of the animals were placed on a low-iodine diet 1 week prior to radioiodine injection and maintained on this diet until sacrifice. Three to five animals of each age-diet group were killed at intervals to 20 days for tissue radioanalysis. Thyroid incorporation data are shown in Figure 1. The percentage uptake by animals exposed prenatally is calculated on the assumption of uniform partition of radioiodine between dam and fetus at the time of administration. The retention curves for the prenatal animals are distorted by continuing intake of ^{131}I supplied in

the mother's milk. The low-iodine diet increased the iodine content of the thyroid most markedly in the animals exposed prenatally and in the young adults. The weanling animals showed a substantially lower uptake of radioiodine than adults and were relatively unaffected by a low-iodine diet.

Comparable studies were performed at substantially higher dose levels (0.5, 1, and 3 $\mu\text{Ci/g}$) in animals on a low-iodine diet. Thyroid uptake and retention curves for these animals are shown in Figure 2. In this case, the uptake by the animals exposed prenatally is presented in terms of percent of the total dose administered to the dam. Age-related differences in level of incorporation were similar to those observed at the lower dose level (Figure 1). However, the shape of the retention curves are clearly altered by the radiation

FIGURE 1. Interrelations of Age and Dietary Iodine on the Incorporation and Retention of Intra-peritoneally Injected ^{131}I

FIGURE 2. Influence of Dose on Uptake and Retention of Injected ^{131}I by Rats of Various Ages

effect produced at higher levels of ^{131}I incorporation.

Conclusions

A low-iodine diet alters the metabolism of administered radioiodine at all ages, and to a degree which

varies markedly with age. Pregnancy tends to decrease the incorporation of radioiodine in the thyroid gland. Administration of thyroid-damaging levels of radioiodine alters incorporation and retention in a manner which is complexly related to age and radiation dose.

AGE-RELATED DIFFERENCES IN THE METABOLISM OF ^{137}Cs BY THE RAT

The whole-body retention and tissue distribution of ^{137}Cs was measured over a 30-day period in newborn and adult rats. The rate of loss was initially slower in the newborn but accelerated after the second week. Other age-dependent dissimilarities were noted in distribution among various tissues.

Investigators:
D. D. Mahlum
M. R. Sikov

Technical Assistance:
Joan O. Hess

We previously reported that ^{137}Cs does not pass freely from the maternal circulation to the fetus and that the distribution of ^{137}Cs differs in the fetus, newborn and adult (Annual Report, 1966). In a continuation of these studies we have compared the retention and subsequent redistribution of ^{137}Cs in the neonatal and adult rat hoping to clarify the changes in cesium metabolism which must occur during the neonatal period.

Observations

Newborn and adult rats were injected intraperitoneally with 7.5 μCi of ^{137}Cs . The whole-body radio-

activity was assayed immediately after injection and semi-weekly thereafter for a 30-day period. Other newborn and adult rats were injected with 10 μCi of ^{137}Cs and randomly selected animals killed at 1, 3, 9, or 23 days postinjection for analysis of radioactivity in selected tissues.

Newborn rats initially retained a somewhat larger fraction of the administered dose than did adults (Figure 1). However, at approximately 2 weeks postinjection the body burden of the neonates began to decrease more rapidly so that by the end of the 30-day experimental period the adults had twice the ^{137}Cs content of the neonates.

FIGURE 1. Retention of Intra-peritoneally Injected ^{137}Cs by Adult and Neonatal Rats

The concentrations of ^{137}Cs in several tissues of the neonate and adult at 1, 3, 9, and 23 days after injection are shown in Table I. To facilitate comparison, values have been normalized to the concentration in striated muscle for the given age and time of sacrifice. At 24 hr postinjection, the neonates show a generally more uniform distribution than do the adults. These differences are less apparent by 9 days postinjection, as a result of more rapid elimination from some adult tissues. By 23 days postinjection, the values were similar for most tissues in both age groups, with the notable exception of blood and most

TABLE I. Relative Concentrations of Tissue ^{137}Cs in Adult and Newborn Rats (Tissue Conc./Muscle Conc.) at Various Times After Injection

Tissues	Newborn ^(a)				Adult ^(b)		
	1 Day	3 Day	9 Day	23 Day	1 Day	9 Day	23 Day
Blood	0.16	0.21	0.19	0.13	0.27	0.095	0.076
Diaphragm	1.51	1.26	1.01	0.57	2.36	1.02	0.67
Kidney	1.65	1.53	1.41	0.79	2.76	0.75	0.57
Liver	1.26	1.36	1.23	0.50	3.00	0.62	0.50
Heart	1.08	0.95	0.83	0.45	2.25	0.54	0.44
Trachea	0.61	0.71	0.33	0.26	0.78	0.36	0.21
Larynx	0.90	0.86	0.56	0.33	1.82	0.53	0.42
Sternal Rib	1.01	1.09	0.78	0.64	1.30	0.83	0.57
Vertebral Rib	0.77	0.93	0.56	0.32	0.92	0.40	0.12
Skull	0.40	0.47	0.38	0.14	0.21	0.07	0.04
Xiphoid	1.61	0.98	0.41	0.29	0.81	0.10	0.13
Femur	0.61	0.63	0.49	0.30	0.46	0.20	0.15

(a) Average values from 3 animals

(b) Average values from 2 animals

skeletal tissues which remained relatively higher in the young animals than in the adults.

Conclusions

The rate of loss of ^{137}Cs from neonatal rats was slower than from adults during the first 2 weeks following administration of the nuclide

as indicated by both total body counting and tissue analyses. Thereafter, the rate of loss from the neonate increased, resulting at 30 days postinjection in a distribution pattern more similar to that of the adult and a total ^{137}Cs burden which was half that of the adult.

METABOLISM OF ^{143}Pm IN A MINATURE SWINE

At 100 times the projected dose in human studies, ^{143}Pm caused no toxic effects in a male pig. The isotope is an easy-to-use tracer for ^{147}Pm , simplifying whole-body counting and radioanalyses of blood, urine, and feces.

Investigators:
V. H. Smith
R. L. Amster
C. R. Watson

Technical Assistance:
Alison T. Clark

As a precaution before using ^{143}Pm in studies with human volunteers (to be jointly undertaken by Battelle-Northwest and the Hanford Environmental Health Foundation), a male 71.3 kg Hanford Miniature Swine was intravenously injected with 9.56 μCi of $^{143}\text{PmCl}_3$ in 8 ml of pH 3.5 solution. This was 100 times the projected human dose. The animal was observed for any signs of toxicity, blood and excreta were collected for radioanalysis, and periodic total-body counts were made. Results are available for the first 28 days postinjection.

Observations

No pyrogenicity or other sign of toxicity from the injectant was noted. After 28 days about 0.027% of the administered radionuclide is in the circulating blood. The disappearance of ^{143}Pm from the blood appears to follow a power function (Figure 1) where concentration as percent of injected dose per gram blood is given by the expression

$$c = 59 \times 10^{-5} t^{-0.58}$$

FIGURE 1. Disappearance of ^{143}Pm from the Blood of a Pig

This expression will be more precisely defined as more data become available.

Excretion data are shown in Figure 2. About 10% of the injected promethium is accounted for in the excreta up to the twenty-eighth day

Urinary excretion was unusual in that this pig did not urinate for the first 3 days of the experiment.

Whole-body counting data, as shown in Figure 3, are not in good agreement with the excretion data, indicating nearly 25% removal by the

FIGURE 2. Daily Excretion of Intra-venously Injected ^{143}Pm (No samples were collected between 14 and 21 days, and between 21 and 28 days.)

FIGURE 3. Retention of Intravenously Injected ^{143}Pm in a Pig as Determined by Whole-Body Counting

twenty-eighth day. The first day value for total-body count was no doubt influenced by the unusual excretory behavior of this pig. Otherwise, the data agree well with those of Stuart for retention of intravenously injected $^{148\text{m}}\text{Pm}$ in dogs (Annual Report, 1963).

Conclusions

The injectant elicited no pyrogenicity and from that standpoint

appears safe for human use. No other toxic effects have been noted. This particular isotope appears well suited for fairly long-term metabolism studies, although the blood disappearance is rapid enough that radioanalyses become difficult after the first week, even with doses of $0.13 \mu\text{Ci/kg}$. Based on measurements taken thus far, the $10\text{-}\mu\text{Ci}$ dose of ^{143}Pm appears to be a good choice for further animal metabolism studies.

PRODUCTION AND DOSIMETRY OF EXPERIMENTAL QUANTITIES OF ^{237}Pu

Plutonium-237, an electron capture isotope which emits a 60 keV gamma ray and $K\alpha$ and $K\beta$ X rays of ^{237}Np , was produced in experimental quantities. This material is being investigated for use in plutonium metabolic studies in animals with possible extension to use in human testing.

Investigators:

*I. C. Nelson**
*A. J. Haverfield**
*W. W. Schultz***

Plutonium-237 has been demonstrated as a tracer in animal studies of plutonium metabolism and excretion.⁽¹⁾ Its potential usefulness in human studies is suggested by the fact that its decay is accompanied by photon emissions easily measured from outside of the body, when injected at levels which would result in a very small radiation dose to the body. In practice, however, the production of ^{237}Pu is accompanied by the production of contaminating, long-lived, alpha-emitting isotopes. To investigate the suitability of ^{237}Pu for animal and human studies, a small amount of the material was produced for testing and evaluation.

Observations

The ^{237}Pu was formed by the ^{235}U (a, 2n) ^{237}Pu reaction produced by irradiating a 93% enriched ^{235}U foil with 27.5 MeV helium ions in the 88 in. cyclotron at the Lawrence Radiation Laboratory, Berkeley, California.

The bombardment consisted of 84.0 μA hr at a rate of 10 $\mu\text{A/hr}$.

The solution of the irradiated target in HNO_3 was subjected to two cycles of anion resin purification. The final product, on August 31, 1967, consisted of 5.1 μCi ^{237}Pu and 1.1 nCi alpha-emitting contaminants contained in 1.6 ml of nitrate solution. The contaminants are identified in Table I.

The photon spectrum of the purified product, as obtained using a Ge(Li) detector, is shown in Figure 1. All of the peaks shown were identified as the ^{237}Pu gamma or as X rays from the ^{237}Np daughter. No higher energy peaks were observed. The absolute emission rate of the photon radiation, calculated from the work of Hoffman and Dropesky,⁽²⁾ is given in Table II. The half-life was previously established as 45.6 days.⁽³⁾

The decay of ^{237}Pu is also accomplished by emission of an alpha particle, 3.3×10^{-6} of the time, to ^{233}U , which has a half-life of 1.6×10^5 years. This mode of decay is sufficiently infrequent to be neglected in dose calculations. The energy Q of the electron capture mode of decay is 0.22 MeV.

* *Biophysical Sciences Section,
 Environmental and Radiological
 Sciences Department*

** *Materials and Process Chemistry
 Section, Chemistry Department*

TABLE I. Plutonium-237 Contaminants

Radionuclide	Percent Abundance	pCi/ μ Ci ^{237}Pu
^{236}Pu	42.60	91
^{238}Pu	24.88	53
^{239}Pu and/ or ^{240}Pu	15.09	32
^{233}U and/or ^{234}U	2.96	6.3
^{228}Th	4.83	10.3
^{224}Ra	2.41	5.1
^{220}Rn	2.41	5.1
^{216}Po	2.41	5.1
^{212}Bi	0.80	1.7
^{212}Po	1.61	3.4

Because plutonium is a bone seeker, it was assumed that bone constituted the critical organ of interest and the dose to that organ was calculated. Calculations were based on standard man parameters and on an intravenous injection of $1 \mu\text{Ci } ^{237}\text{Pu}$ as it was constituted on August 31, 1967. Dose calculations were made with reference to radium wherein one maximum permissible body burden sustained delivers 0.56 rem/week to bone. The results of the dose calculations are shown in Table III. Contributions from ^{236}Pu , ^{238}Pu , ^{239}Pu , ^{233}U , and ^{228}Th in addition to ^{237}Pu were considered. The dose from the other alpha-emitting contaminants was negligible.

The total dose contributed by the ^{237}Pu itself was less than 100 mrem. The principal dose after 20 years is contributed by ^{238}Pu and ^{239}Pu .

FIGURE 1. Plutonium-237 Photon Spectrum, Ge(Li) Detector (20 cm^3)

TABLE II. Principal Photon Emission-Plutonium-237

Photon	Approximate Energy, keV	Approximate No. of Photons/100 Dis.
Neptunium L X-rays	17	30
Neptunium K X-rays	100	40
Unconverted gamma rays	60	5

TABLE III. Calculated Dose to Bone From $1 \mu\text{Ci } ^{237}\text{Pu}$ Plus Contaminants Injected Intravenously

Year, Post-injection	Dose Rate, mrem/year	Accumulated Dose, rem
1	220	0.22
2	109	0.33
3	96	0.42
4	85	0.51
5	79	0.59
10	60	0.94
15	53	1.2
20	49	1.5

Conclusions

This experiment showed that it is possible to produce ^{237}Pu in microcurie amounts without significant gamma-emitting contaminants. There are formed, however, measurable amounts of alpha-emitting contaminants. It is estimated that the longer lived of these contaminants would deliver about 50 mrem/year to bone per microcurie ^{237}Pu injected. Another irradiation will be scheduled at a lower helium ion energy, but for a longer total irradiation time,

in an effort to reduce the proportion of longer-lived alpha contaminants.

References

1. Todd, R. and R. Logan. *Intern. J. Appl. Radiation Isotopes*, vol. 17, pp. 253-255. 1966.
2. Hoffman, R. C. and B. J. Dropesky. *Phys. Rev.*, vol. 109, pp. 1282-1290. 1950.
3. Lederer, C. M., J. M. Hollander, and I. Perlman. *Table of Isotopes*, 6th Ed., John Wiley and Sons, Inc., New York. 1967.

RADIOELEMENT REMOVAL STUDIES

The first report in this section provides answers to some very practical questions concerning the treatment of plutonium-contaminated wounds. It would seem to be clearly established that irrigation or suffusion of such wound areas with DTPA is without beneficial effect. Because this approach has been widely employed, it is comforting to note that the procedure seems to have no undesirable effects on plutonium absorption and distribution.

Administered internally, DTPA was the most effective of several chelating agents tested for their effectiveness in removal of intravenously injected promethium from rats. The removal of inhaled, insoluble particulate material remains a problem without a promising approach to its solution. Like PuO_2 , inhaled PuF_4 is not responsive to chelating agents such as DTPA. A wide variety of pharmacologic agents with a potential for stimulation of natural pulmonary clearance processes were, in only a few cases, marginally effective.

Perhaps the most interesting developments reported in this section are concerned with our increasing understanding of the cellular and subcellular mechanisms of insoluble particle clearance by phagocytic processes. For reasons of technical expediency, preliminary studies have involved peritoneal phagocytic cells, but now the studies are being extended to the lung. A striking finding of these studies is the radiation resistance of the phagocyte which survives the ingestion of intensely radioactive particles with little apparent damage.

This area of our research interest was the subject of our 1967 Hanford Symposium on the Diagnosis and Treatment of Deposited Radionuclides. Held May 15-17 in Richland, and co-sponsored by the U.S. Atomic Energy Commission, Battelle Memorial Institute, and the Hanford Environmental Health Foundation, this symposium attracted 136 off-site registrants, including 28 scientists from 12 foreign countries. The complete proceedings of this symposium should soon appear as a monograph publication of the Excerpta Medica Foundation.

PUBLICATIONS

Sanders, C. L. "Phagocytosis and Translocation of $^{239}\text{PuO}_2$ Particles Deposited in the Peritoneal Cavity of Rats," J. Reticuloendothelial Soc., vol. 4, p. 432. 1967.
(Abstr.)

PLUTONIUM-CONTAMINATED WOUND STUDIES

Plutonium was not effectively removed from contaminated wounds by irrigation with DTPA, nor from intramuscular deposition sites by suffusion with DTPA. Simultaneous administration of dimethylsulfoxide (DMSO) did not increase the effectiveness of DTPA. Plutonium injected subcutaneously as the DTPA chelate was rapidly absorbed and 85% was excreted in the first day's urine.

Investigator:
Beatrice J. McClanahan
Technical Assistance:
Linda G. Smith

These experiments were designed to answer some practical questions concerning the treatment of plutonium-contaminated wounds. Surgical excision is the most effective procedure but is not always practicable and seldom complete. Irrigation of such wounds with solutions of diethylenetriaminepentaacetic acid (DTPA) to chelate the plutonium has frequently been urged as an effective means of decontamination, but questions have been raised as to whether such treatment might not increase plutonium absorption and retention.

Observations

In the first series of experiments (Table I), lacerations were made through the skin and subcutaneous

tissue of the hind leg of rats. Solutions or suspensions of the appropriate plutonium compound, containing about 5 μCi ^{239}Pu , were placed in the wounds. One-half hour later the wounds were rinsed with 500 ml of either 0.9% NaCl or 0.2% DTPA in 0.9% NaCl. Some of these animals were subsequently injected intravenously with Na_3Ca DTPA (14 mg/kg body weight) daily for a total of five injections. Animals were sacrificed after 7 days.

There was no significant difference in the effectiveness of the saline and DTPA rinses for any of the compounds studied, and little difference among the compounds, suggesting that removal was primarily a mechanical process.

TABLE I. Effect of Various Treatments on the Retention and Translocation of Plutonium Deposited in Skin Lacerations

Type of Plutonium Deposited	Type of Rinse Employed	Plutonium Removed in Rinse, % (a)	Subsequent Treatment	Translocation of Retained Plutonium, % (a)—— Residual		
				Liver	Carcass	Femur
Nitrate	Saline	4.7	None	0.57	2.7	0.088
			DTPA, iv	0.28	3.0	0.080
	DIPA	3.1	None	0.59	3.0	0.12
			DTPA, iv	0.31	2.8	0.08
Citrate	Saline	46	None	9.1	38	2.8
			DTPA, iv	2.5	22	1.3
	DIPA	37	None	7.4	32	1.7
			DTPA, iv	3.4	19	0.86
Dioxide	Saline	3.4	None	0.018	0.64	0.0063
			DTPA, iv	0.030	1.3	0.0011
	DIPA	5.5	None	0.016	2.0	0.0017
			DTPA, iv	0.012	2.3	0.0062

(a) *Average values from eight rats. Standard errors of mean ranged from 5-20% except for dioxide where standard errors were as large as 50%.*

The behavior of the citrate was exceptional. Due to its anticoagulant properties, there was seepage from the wound of blood and serum with resultant formation of a clot which contained most of the plutonium removed during irrigation.

Rinsing the wounds with DIPA versus saline had no discernible effect on the subsequent tissue distribution of plutonium. The extent of translocation was a function of the chemical form of plutonium. The retention in liver and skeleton was reduced by intravenous DIPA treatment, though less than might have been expected and not at all in the case of the dioxide.

In a second series of experiments, plutonium was injected subcutaneously as the nitrate or as the previously formed DIPA chelate. Some of these animals were subsequently treated with DIPA intravenously, at approximately daily intervals, for a total of nine treatments. Excreta were collected and animals sacrificed at intervals to 14 days. Better than 85% of the plutonium nitrate deposit remained at the site of injection after 14 days. Better than 85% of the injected plutonium-DTPA chelate was excreted in the urine during the first day, and less than 1% remained at the injection site after 14 days.

The time course of plutonium deposition and retention in liver and femur is shown in Figure 1. Despite the fact that most of the plutonium nitrate remained at the injection site, its deposition in tissues far exceeded that of the DTPA chelate. Only in the kidney was there a transiently greater deposition of the chelate than of the nitrate, which was no doubt related to the much greater urinary excretion of the chelate. Intravenous treatment with DTPA effected a reduction in plutonium content of tissues, even when the plutonium was initially injected as the DTPA chelate. This suggests

that there was some breakdown of the chelate within the body or that chelation in the injection solution was incomplete.

The pH of DTPA solutions suffused into an area of subcutaneously deposited plutonium was shown to have no effect on absorption or retention (Table II). The DTPA was administered 0.5 and 24 hr after 5 μ Ci of plutonium nitrate was injected intramuscularly. When the pH of the injected plutonium was raised from 2 to 4, there was a marked reduction in absorption from the injection site.

Dimethylsulfoxide (DMSO) is reported to be a penetrant of biological

FIGURE 1. Plutonium Content of Liver and Femur Following Subcutaneous Injection of Plutonium Nitrate or Plutonium-DTPA Chelate as Influenced by Subsequent DTPA Therapy (Average values from six rats at each sacrifice)

membranes, including the skin, and a carrier of various chemical agents. It was found to be without effect on the percutaneous absorption of DTPA, or in promoting the chelation of subcutaneously deposited plutonium by DTPA. Details of this experiment are

summarized in Table III. The plutonium content of the liver and femur was lowered by either subcutaneous or topical administration of DTPA, but the effect on plutonium retention at the injection site was therapeutically insignificant.

TABLE II. *Effect of pH on Absorption of Subcutaneously Deposited Plutonium*

pH of Injected Pu(NO ₃) ₄	pH of Suffused DTPA	Distribution of Injected Plutonium, % (a)			
		Injection Site	Residual Carcass	Liver	Femur
2	3	85	2.7	0.34	0.030
	6	81	3.4	0.50	0.018
4	3	97	0.67	0.060	0.012
	6	77	0.56	0.033	0.007

(a) *Average values from five rats. Standard deviations were as large as 100% in liver and femur values.*

TABLE III. *Effect of DTPA and DMSO on Absorption of subcutaneously Deposited Plutonium*

Treatment	Distribution of Injected Plutonium, % (a)			
	Injection Site	Residual Carcass	Liver	Femur
None	98	4.9	0.94	0.14
Subcutaneous DTPA (b)				
Without DMSO	93	3.1	0.35	0.05
With DMSO	94	3.0	0.63	0.06
Topical DTPA (c)				
Without DMSO	94	2.6	0.49	0.09
With DMSO	92	4.5	0.38	0.06

(a) *Average values from four rats sacrificed 5 days after plutonium injection.*

(b) *DTPA (0.65 mM/kg), in saline or 38% DMSO, injected 1/2 hr and 24 hr after plutonium injection.*

(c) *DTPA (0.26 g), in saline or 23% DMSO, applied to skin at injection site, three times a day for 4 days.*

Conclusions

Under the quite varied conditions employed in these experiments, DTPA was without significant therapeutic effect in the direct treatment of simulated contaminated wounds. It was also without deleterious effect,

in that no increased internal deposition resulted from its use. The fact that previously chelated plutonium is rapidly absorbed and excreted implies that the ineffectiveness of wound treatment with DTPA results from a lack of effective chelate formation in the in vivo situation.

TREATMENT OF PROMETHIUM DEPOSITION IN THE RAT

Of the chelons tested, DTPA proved superior for preventing the deposition of $^{147}\text{Pm}^{3+}$ in the rat. Treatment 1 hr after intravenous injection decreased the promethium content of the liver to 11%, and of the skeleton to 45% of the untreated controls.

Investigator:
V. H. Smith

Technical Assistance:
Alison T. Clark

Increasing interest in ^{147}Pm for a variety of scientific and industrial applications has resulted in the isolation of large quantities of this radionuclide and increased the likelihood of accidental human poisoning. A number of chelating agents were tested for their possible efficacy in the treatment of such cases.

Observations

Six groups of eight Sprague-Dawley female rats were injected intravenously with 33 μCi ^{147}Pm as the chloride. One hour later chelating agents were administered intraperitoneally to prevent the deposition and hasten the elimination of the radionuclide. Animals were sacrificed

after 7 days and tissues analyzed for ^{147}Pm . Details of the experimental design and results are shown in Table I.

The behavior of EDTA was unusual in that it effected a 40% decrease in liver ^{147}Pm , compared to controls, but had no significant effect on bone ^{147}Pm . This suggests that binding sites in bone are quite strong relative to the EDTA ligand. On the other hand, DTPA and TTHA were able to decrease the deposition in bone to 50% of the control value, and were more effective than EDTA in modifying soft tissue deposition. Since calcium gluconate-glucoheptonate was not given to the animals receiving DFA, one cannot conclude that DFA increased the ^{147}Pm content of the

TABLE I. ^{147}Pm Content of Rat Tissues 7 Days After Various Therapeutic Treatments

Group	Treatment (a)	Percent of Injected $^{147}\text{Pm} \pm$ Standard Error		
		Liver	Kidney	Skeleton
1	1 cc physiological saline	30 $\pm$ 1.2	1.1 $\pm$ 0.10	24 $\pm$ 1.0
2	EDTA (ethylenediamine-tetraacetic acid)	19 $\pm$ 0.53	0.74 $\pm$ 0.043	27 $\pm$ 0.8
3	DTPA (diethylenetriamine-pentaacetic acid)	3.2 $\pm$ 0.18	0.47 $\pm$ 0.042	11 $\pm$ 0.62
4	TTHA (triethylenetetramine-hexaacetic acid)	6.6 $\pm$ 0.44	0.51 $\pm$ 0.045	10 $\pm$ 0.52
5	DFA (deferroxamine)	32 $\pm$ 1.4	0.58 $\pm$ 0.16	30 $\pm$ 0.85
6	DFA + DTPA	2.9 $\pm$ 0.23	0.15 $\pm$ 0.041	11 $\pm$ 0.56

(a) *All chelons were administered intraperitoneally 1 hr after intravenous injection of the radionuclide as 1 cc of pH 7.0 solution containing 0.3 mmoles of the chelon. Except for Group 4, all treatments were given with 1 cc of calcium gluconate-calcium glucoheptonate, 10% w/v (Abbott). DTPA and TTHA were furnished through the courtesy of Giegy Chemical Corp. and DFA through the courtesy of CIBA, Ltd.*

skeleton. The ^{147}Pm content of the control animals was somewhat lower than expected, which may be due to the ligand associated with the injected calcium.

Conclusions

Considering that a portion of the promethium in the liver will ultimately move into the skeleton, it would appear that DTPA is the agent

of choice for preventing the deposition of ^{147}Pm in tissues. While DFA has a great ability to chelate Fe^{3+} , its specificity is such that it apparently ignores Pm^{3+} . The ability of DTPA to prevent deposition of heavy metals in the liver is again illustrated in the case of promethium and must depend on some factor other than the strength of the chelate formed since TTHA forms stronger complexes.

REMOVAL OF INHALED $^{239}\text{PuO}_2$ IN RATS

Progesterone, chlorpromazine, and certain filtration gels were the most promising of a number of agents tested for their ability to stimulate removal of inhaled $^{239}\text{PuO}_2$.

Investigators:

*J. V. Dilley
K. E. McDonald*

Chelating agents have shown little promise in removing nonionic, insoluble oxides from the lung. Consequently, our approach to this problem has been to attempt to stimulate natural physiological pulmonary clearance process with pharmacological agents.

Observations

Female Sprague-Dawley rats (150-200 g) were exposed to aerosols of $^{239}\text{PuO}_2$. After exposure the animals were randomized into groups of five animals each and treated with the test agents listed in Table I. All animals were sacrificed 18 days after exposure. The trachea, lungs, heart, and all thoracic lymphatic tissues were analyzed for ^{239}Pu content. The results of these studies, with lung burden at sacrifice expressed as percent of untreated controls, are summarized in Table I.

Agents 1 and 2, which have the effect of increasing basal metabolic rate, were not effective. Of the next three agents, which are inert dry polymers, only sephadex G-25 enhanced the removal of $^{239}\text{PuO}_2$, perhaps by binding the plutonium as it absorbed water in the lung. Of three steroids tested (6, 7, and 8), only progesterone was effective, though not at the higher dosage. Chlorpromazine was somewhat effective in removing plutonium, but not at high doses which produced sedation. Other agents were, at best, only marginally effective.

Conclusions

Though results to date have not been encouraging, this investigation will continue in an effort to find more effective agents, or combinations of agents, to remove inhaled $^{239}\text{PuO}_2$.

TABLE I. Effect of Various Pharmacological Agents on the Removal of Inhaled $^{239}\text{PuO}_2$ from Rat Lungs

Agent	Daily Treatments ^(a)	Route of Administration	Lung Burden at Sacrifice, % of controls
1 Thyroid-stimulating hormone	0.1 unit	Intraperitoneal	95
2 2,4-dinitrophenol	1 mg/kg	Oral	109
3 Carbose	10 min aerosol	Inhalation	94
4 Carbopol	10 min aerosol	Inhalation	143
5 Sephadex G-25	10 min aerosol	Inhalation	68
6 Prednisolone	5 mg/kg	Intraperitoneal	102
7 Deoxycorticosterone	5 mg/kg	Intraperitoneal	118
8 Progesterone	5 mg/kg	Intraperitoneal	82
Progesterone	5 mg/kg	Intraperitoneal	82
Progesterone	8 mg/kg	Intraperitoneal	92
Chlorpromazine + progesterone	10 mg/kg + 8 mg/kg	Oral + intraperitoneal	109
9 Chlorpromazine	10 mg/kg	Oral	69
Chlorpromazine	20 mg/kg	Oral	113
Chlorpromazine + Bayer 29493	10 mg/kg + 10 mg/kg	Oral + oral	129
10 0,0 diethyl [methylthio (m-tolyl) phosphorothioate] (Bayer 29493)	10 mg/kg	Oral	89
11 2,4-dihydroxyphenylalanine	5 mg/kg	Intraperitoneal	131
12 Tyrosine	100 mg/kg	Oral	106
13 Phenobarbital	5 mg/kg	Oral	131
14 Serotonin creatinine- PO_4	5 mg/kg	Intraperitoneal	107
15 Tryptamine HCl	10 mg/kg	Intraperitoneal	93
16 DL-Amphetamine	10 mg/kg	Oral	87
17 D-penicillamine	50 mg/kg	Oral	88
18 Phytohemagglutinin P	0.002 mg/kg	Intraperitoneal	96

(a) Oral treatments, 7 days/week; intraperitoneal injections and aerosols, 5 days/week; all animals sacrificed after 18 days.

REMOVAL OF INHALED $^{239}\text{PuF}_4$ IN BEAGLES

DTPA was not effective in removing plutonium from three dogs treated over a period of 12 weeks following inhalation of $^{239}\text{PuF}_4$.

Investigators:
J. V. Dilley
K. E. McDonald

The therapeutic effectiveness of diethylenetriaminepentaacetic acid (DTPA) is dependent on whether the plutonium in the body can be chelated. Plutonium (IV) fluoride is "relatively insoluble" in water; its behavior in the body is unknown. This experiment was designed to test the effectiveness of DTPA in removing inhaled $^{239}\text{PuF}_4$ from beagle dogs.

Observations

Six beagle dogs were exposed to dry aerosols of $^{239}\text{PuF}_4$. Immediately after the exposure, each animal was counted in the whole-body counter. Approximately 2 hr after the exposure each of three dogs was injected intraperitoneally with 0.5 g of DTPA. This treatment was repeated after 1, 2, 4, 7, 11, 16, 18, 23, 37, 44, 51, and 58 days. Whole-body counts of treated and control animals were made at frequent intervals. No effects were seen that could be clearly attributed to the DTPA treatment. Retention of $^{239}\text{PuF}_4$ at 71 days post-exposure is shown in Table I.

Conclusions

The behavior of $^{239}\text{PuF}_4$ is similar to $^{239}\text{PuO}_2$ with respect to its retention in the body and its removal by DTPA. Neither of these appear to exist in the body in a form favorable for chelation. This conclusion is based on the total-body retention data; any redistribution of $^{239}\text{PuF}_4$ which may have been effected by DTPA treatment will be detectable only after sacrifice and tissue analysis.

TABLE I. Effect of DTPA on Retention of Inhaled $^{239}\text{PuF}_4$ in Beagle Dogs

Dog Number	DTPA Treatment	Retention at 71 Days, % of initial deposition(a)
451	Treated	3.1
336	Treated	12
334	Treated	44
429	Control	13
441	Control	31
448	Control	5.9

(a) *Based on whole-body counts immediately after exposure and 71 days after exposure.*

IN VIVO AND IN VITRO CHELATION OF PLUTONIUM BY α -LIPOIC ACID AND DTPA

In leaching PuO_2 from a Sephadex column, α -lipoic acid was more effective than DTPA but was ineffective in removing plutonium administered as the nitrate to rats.

Investigator:

J. V. Dilley

Technical Assistance:

K. E. McDonald

Experiments were performed comparing the in vitro chelating ability of 6,8-epi-dithiooctenoic acid (α -lipoic acid) and diethylenetriaminepentaacetic acid (DTPA), and their in vivo applicability as therapeutic agents for plutonium removal.

Observations

Glass columns (30 cm x 1 cm) were packed with Sephadex G-25 (coarse) and loaded with 40 μCi of $^{239}\text{PuO}_2$ (0.3 μCMD). After flushing with water, the columns were treated with either 1.0 g DTPA or 0.1 g α -lipoic acid and washed with additional water until plutonium was no longer detectable in the eluate. About 0.3% of the plutonium was removed from the column with α -lipoic acid treatment; about 0.1% was removed with DTPA treatment. Treatment with α -lipoic acid following DTPA elution, or with DTPA following α -lipoic acid elution, resulted in quite negligible additional removal of plutonium from the column.

Female rats were intravenously injected with 1.0 μCi $^{239}\text{Pu}(\text{NO}_3)_4$ and then randomized into three groups of six animals each. One group received 30 mg/kg α -lipoic acid, the second group received 0.1 mM DTPA

per animal, the third group received 0.1 ml propylene glycol and served as controls. All treatments were given intraperitoneally at 3, 24, 48, and 96 hr after plutonium injection. All urine and feces were collected and the animals were sacrificed for plutonium tissue analysis on the tenth day. The results of this experiment are summarized in Table I.

It is apparent that α -lipoic acid is ineffective in promoting the excretion of plutonium. It does, however, alter the distribution of plutonium so that greater concentrations are present in liver, kidney,

TABLE I. *Distribution of Plutonium Following DTPA and α -Lipoic Acid Treatment^(a)*

	DTPA	α -Lipoic Acid	Controls
Urine	72	4	3.8
Feces	15	15	21
Kidney	0.4	2.8	0.2
Liver	0.9	12	1.3
Carcass ^(b)	11	54	72
Viscera	0.6	12	0.5

(a) *Average values from six animals expressed as percent of total plutonium recovered.*

(b) *Residual carcass excluding skin.*

and viscera at the expense of the carcass (presumably skeletal) burden.

Conclusions

a-Lipoic acid is a good chelating agent for plutonium, probably acting by the formation of a metal-sulfur bond. The in vitro experiment sug-

gested that it might be more effective than DTPA. However, the in vivo experiment demonstrated that α -lipoic acid, while capable of altering the distribution of plutonium within the body, apparently does not bind the plutonium in a form that can be eliminated from the body.

PHAGOCYTOSIS AND TRANSLOCATION OF INTRAPERITONEALLY INJECTED $^{239}\text{PuO}_2$ AND $^{238}\text{PuO}_2$ PARTICLES

Particles of $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ were phagocytized by peritoneal mononuclear phagocytes at similar rates. The $^{239}\text{PuO}_2$ was transported to the lymphatics of the omentum, resulting in necrosis of the omentum and subsequent release of ^{239}Pu back into the peritoneal cavity. The fate of the $^{238}\text{PuO}_2$ particles was distinctly different and as yet not completely resolved.

Investigator:
C. L. Sanders

Further experiments were performed to evaluate the role and response of the reticuloendothelial system with regards to plutonium particle behavior (Annual Report, 1966). Two plutonium isotopes of very different specific activity were employed in an effort to identify effects attributable to radiation.

Observations

Female rats were given intraperitoneal injections of either 1.4 μCi $^{239}\text{PuO}_2$ or 1.8 μCi $^{238}\text{PuO}_2$ particles of count median diameter 0.05 μ suspended in 2 ml saline. Animals

were selected at intervals following injection and the abdominal cavity washed twice with 10 ml saline. Peritoneal fluid cells from the wash were examined autoradiographically. Other animals were sacrificed for assay of tissues for plutonium.

Essentially all the $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ particles in the peritoneal cavity were engulfed by phagocytes within the first 2 hr. There was no difference in the rate of phagocytosis of the two isotopes (Figure 1). About 28% of all peritoneal mononuclear cells in the wash had phagocytized $^{239}\text{PuO}_2$ particles by the second hour. This value increased

FIGURE 1. Phagocytosis of $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ Particles by Peritoneal Mononuclear Phagocytes

slightly to 35% at the fourth hour and then fell rapidly to less than 5% by the seventh hour.

The ^{239}Pu content of the peritoneal wash decreased slowly from 80% of administered plutonium at 10 min to 75% at 1 hr. During this same period, $^{238}\text{PuO}_2$ was lost more rapidly, falling to 30% of administered dose within 30 min. Less than 10% of the administered dose of either isotope remained in the wash at 7 hr (Figure 2).

The disappearance of ^{239}Pu from the peritoneal wash was associated with a concomitant gain in the ^{239}Pu content of the omentum and mesentery (Figure 3). The majority of this plutonium was found in the omentum, concentrated within the lymphatic

nodules ("milky spots") scattered over the omental surface (Figure 4). Subsequent to the first few days, significant amounts of ^{239}Pu were "released" from the omentum and mesentery back into the peritoneal cavity (Figures 2 and 3). This loss was associated with the appearance of peritoneal adhesions and fibrosis of the omentum and its lymphatics at 7 days.

In contrast to the behavior of ^{239}Pu , ^{238}Pu did not accumulate in the omentum and mesentery. Transport of $^{238}\text{PuO}_2$ particles from the peritoneal cavity did occur via phagocytes, but the fate of these phagocytized $^{238}\text{PuO}_2$ particles is still unknown. There was a substantially

FIGURE 2. Plutonium Content of the Peritoneal Wash at Intervals Following Intraperitoneal Injection of $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ Particles

FIGURE 3. Plutonium Content of the Omentum and Mesentery at Intervals Following Intraperitoneal Injection of $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ Particles

greater proportion of the ^{238}Pu , as compared to ^{239}Pu , that eventually deposited in the liver (Figure 5) and other tissues.

Conclusions

The highly radioactive nature of the $^{238}\text{PuO}_2$ particles did not appear to influence the rate at which they were phagocytized, as compared to $^{239}\text{PuO}_2$ particles. The subsequent fate of the phagocytized $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ was, however, quite different. The $^{239}\text{PuO}_2$ particles were transported within phagocytes to the omentum and mesentery where they accumulated in the lymphatics. Fibrosis of the lymphatics resulted in the subsequent release of ^{239}Pu back into the peritoneal cavity. The

640X

FIGURE 4. Autoradiogram of Omentum Showing Accumulation of $^{239}\text{PuO}_2$ in the Lymphatic Nodules

FIGURE 5. Plutonium Content of the Liver at Intervals Following Intraperitoneal Injection of $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ Particles

fate of the ^{238}Pu is not clear although it was more widely disseminated systemically than was the ^{239}Pu . In any event, it can be

stated that the phagocyte plays a vital role in the clearance of particulate plutonium from the peritoneal cavity.

EFFECT OF DTPA AND CALCIUM ON INTRAPERITONEALLY INJECTED $^{239}\text{PuO}_2$ PARTICLES

The simultaneous intraperitoneal injection of ionic calcium and $^{239}\text{PuO}_2$ particles resulted in the rapid accumulation of plutonium in the omentum and mesentery, which was associated with marked adhesion of peritoneal cells and the formation of a fibrin-like material around these cells. DTPA inhibited this process, probably by chelation of ionic calcium.

Investigators:
C. L. Sanders
W. J. Bair

Phagocytosis of particles has been shown to be enhanced by the presence of calcium ions and suppressed by chelators of calcium, such as EDTA. Diethylenetriaminepentaacetic acid (UTPA), a chelator similar in structure and function to EDTA, has been administered for removal of plutonium from animals and man. The objective of this investigation was to ascertain the role of calcium, in excess and DTPA-induced deficiency, on the phagocytosis and translocation of intraperitoneally administered $^{239}\text{PuO}_2$ particles.

Observations

Female rats were given intraperitoneal injections of $1.4 \mu\text{Ci } ^{239}\text{PuO}_2$ (count median diameter, 0.12μ), with or without accompanying intraperi-

toneal injections of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, or DTPA. Smears of saline washes from the peritoneal cavity were evaluated microscopically and autoradiographically at intervals following injection.

Plutonium particles were readily phagocytized by peritoneal mononuclear cells. Over 90% of the particles in the wash were phagocytized by the second hour; about 30% of all peritoneal mononuclear cells had phagocytized particles; about 75 % of administered plutonium was phagocytized and recovered in the wash. However, after 7 hr, only ~50% of the injected plutonium was recovered in the wash, the remainder being localized in the lymphatic nodules scattered over the visceral peritoneum.

Coadministration of 0.02 mmole DTPA with plutonium particles depressed phagocytosis; only 4-5% of phagocytes engulfed particles during a 7 hr period following injection. DTPA also prevented the accumulation of particles in the visceral peritoneum (Figure 1).

Coadministration of 0.2, 0.3, and 0.5 mmoles calcium with plutonium particles resulted in the formation of peritoneal cell "clots" 1-2 mm in diameter. Electron micrographic examination showed no evidence of cell fusion but only of cell adhesion (Figure 2). Some cells were separated by only 200-400 Å; others were sur-

rounded by a fibrous material, often arranged in bundles in the extracellular space. The plutonium content of these clots was negligible.

Plutonium localized on the visceral peritoneum increased from 5% of administered dose at 10 min after injection of 0.3 or 0.5 mmole calcium to 70% at 15 min and decreased to 40% by the sixth hour. Lower concentrations of calcium were less effective in promoting the uptake of plutonium.

Administration of 0.5 mmole calcium 1 hr after plutonium injection resulted in a rapid accumulation of ^{239}Pu in the visceral peritoneum 30 min later. Administration of 0.3

FIGURE 1. Effect of Calcium and DTPA on the Uptake of ^{239}Pu by the Visceral Peritoneum

Neg 0680640-3

9200X

FIGURE 2. *Peritoneal Clot Sampled at 60 min After Intraperitoneal Administration of 0.5 mmole Calcium and $^{239}\text{PuO}_2$ Particles [A large amount of fibrin-like material (F) is found surrounding red blood cells (R) and peritoneal mononuclear cells (M). Only a few hundred A units separate the cell membranes of red blood cells and mononuclear cells (arrows).]*

mmole magnesium was without effect. Injection of 10 mg bovine fibrin caused the peritoneal uptake of 50% of administered plutonium within 1 hr, but 10 mg heparin had no significant effect.

Conclusions

These experiments showed that phagocytosis of PuO_2 particles and uptake by the peritoneal lymphatics were inhibited by DTPA and stimulated by calcium. By inference, it is suggested that the inhibitory action of DTPA is due to its chelation of calcium, an ion required for phagocytosis. It is further suggested that DTPA might play a role in the removal of plutonium other than by chelation of ionic plutonium. By preventing phagocytosis of particles, DTPA might delay their translocation from the site of deposition long enough for mechanical removal.

These experiments suggest that the action of calcium in promoting phagocytosis of plutonium particles may be related to the known role of calcium ion in fibrin formation since the administration of fibrin also stimulated the uptake of plutonium by the peritoneal lymphatics.

THE ULTRASTRUCTURE OF PERITONEAL MONONUCLEAR PHAGOCYTES EXPOSED
IN VIVO TO $^{239}\text{PuO}_2$ PARTICLES

Peritoneal phagocytes which readily engulf plutonium particles are relatively resistant to alpha irradiation emitted by $^{239}\text{PuO}_2$ particles.

Investigators:
C. L. Sanders
R. R. Adee

Elsewhere in this report we have described the phagocytosis of intraperitoneally injected $^{239}\text{PuO}_2$ particles and their transport into the visceral peritoneum within 5-7 hr after administration. The purpose of this study was to determine the subcellular effects and localization of phagocytized $^{239}\text{PuO}_2$.

Observations

Female rats given intraperitoneal injections of 1.4 μCi $^{239}\text{PuO}_2$ (count median diameter, 0.12 μ) were killed at 2, 6, 24, or 168 hr after injection and the peritoneal cavity was washed with saline. Peritoneal cell pellets were fixed in 1% glutaraldehyde, postfixed in osmium tetroxide and embedded in EPON 812. Sections were stained with uranyl acetate and lead citrate for electron microscopy. Autoradiograms of sections were prepared with Ilford L-4 emulsion.

Numerous electron-dense particles were found localized within cytoplasmic vacuoles of phagocytes (Figure 1). These were shown to be plutonium by electron microscopic autoradiography (Figure 2). Numerous, small, electron-dense particles, which appeared to be plutonium, were

Neg 0673470-4 36,800X

FIGURE 1. Engulfed $^{239}\text{PuO}_2$ Particles (Arrows) Within Cytoplasmic Vacuoles (V_1 , V_2) at 2 hr After Intraperitoneal Injection

Neg 0673470-4 14,400X

FIGURE 2. Autoradiogram of Phagocyte Showing Alpha Track Activity Emanating From Within a Cytoplasmic Vacuole (V) at the Periphery of the Cell--4-Day Exposure.

found lining the interior periphery of large lysosomal-like structures in the cytoplasm of phagocytes at 7 days. These structures were shown to be lysosomes by their positive staining reaction for acid phosphatase

Only minimal alterations in phagocytes were observed during the first 6 hr. These included an increased number of lysosomal structures and the appearance of large cytoplasmic vacuoles, filled with varying amounts of debris. Significant alterations found at 1 and 7 days included a marked swelling of phagocytes, an increased number of pseudopodial projections extending from their surface and the presence of numerous inclusion bodies within the phagocyte's cytoplasm. Obvious necrosis of phagocytes was observed at 7 days. Significant alterations occurring after the first day may be due as

much to the large amount of phagocytized cell debris as to irradiation from plutonium particles.

Lymphocytes were depleted after the first day. Necrosis of lymphocytes and their subsequent engulfment and digestion by phagocytes was frequently observed after the first day.

Conclusions

It is concluded that peritoneal mononuclear phagocytes are more resistant to alpha irradiation than are other peritoneal cells. Phagocytosis of plutonium particles during the first few hours after deposition did not result in ultrastructural damage which would appear to impair their ability to transport particles to the visceral peritoneum.

MOLECULAR AND CELLULAR LEVEL STUDIES

Our more basic studies in this area continue to focus on two principal problems: the control of RNA metabolism in Escherichia coli and the transport and synthesis of tryptophan in Neurospora crassa. Work on the RNA problem has been mainly concerned with establishing more firmly, by a variety of confirmatory experiments, the tentative conclusions set forth in some detail in last year's Annual Report. Presently, these experiments do not lend themselves to a final interpretation; therefore, no report on this work is included this year.

The Neurospora studies have proceeded on several fronts, with three reports included in this volume. Of particular interest is the progress being made toward isolation of a protein that appears to be a component of the tryptophan transport system. Some of this work as indicated by footnotes has received substantial support from the Battelle Institute. By combining the support available from the AEC Division of Biology and Medicine and the Battelle Institute, progress has been made on a broader front than could have been realized by separate efforts on behalf of the two sponsors.

Two reports are concerned with electron microscope studies. One of these derives from a cooperative study with Dr. Heller of the Pacific Northwest Research Foundation, Seattle, Washington, who has supplied us with human testicular tissue. Other applications of the electron microscope will be found elsewhere in this Annual Report, including some rather spectacular visualizations of phagocytized plutonium particles which are reported in the section on Radioelement Removal Studies.

Growing concern for the thermal pollution problem in our rivers and streams has focused attention on the effects of temperature on aquatic organisms. Two reports are concerned with such effects in fish at the biochemical level. Other biochemical studies, on lung tissue, will be found in the section on Inhalation Studies.

MOLECULAR AND CELLULAR LEVEL STUDIES

PUBLICATIONS

Berlin, J. D. "Early Irradiation Effects on the Localization of Acid Mucopolysaccharide in Intestinal Absorptive Cells," J. Cell Biol., vol. 35, p. 12A. 1967. (Abstr.)

Berlin, J. D. "The Localization of Acid Mucopolysaccharides in the Golgi Complex of Intestinal Goblet Cells," J. Cell Biol., vol. 32, pp. 760-766. 1967.

Berlin, J. D. and J. M. Dean. "Temperature-Induced Alterations in Hepatocyte Structure of Rainbow Trout (Salmo gairdneri)," J. Exptl. Zool., vol. 164, pp. 117-132. 1967.

Matchett, W. H. "Methionine and the Regulation of Ribonucleic Acid Synthesis in Escherichia coli," J. Bacteriol., vol. 93, pp. 96-97. 1967.

Matchett, W. H. "Ribosomal Precursors in Steady State Cells of Escherichia coli," Bacteriol. Proc., p. 119. 1967. (Abstr.)

Turner, J. R. and W. H. Matchett. "Derepression of Tryptophan Synthetase in Neurospora crassa," Bacteriol. Proc., p. 115. 1967. (Abstr.)

Wiley, W. R. and W. H. Matchett. "Metabolic Control of Tryptophan Transport in Neurospora," Bacteriol. Proc., p. 106. 1967. (Abstr.)

Wiley, W. R. and W. H. Matchett. "Tryptophan Transport in Neurospora crassa. I. Specificity and Kinetics," J. Bacteriol., vol. 92, pp. 1698-1705. 1967.

TRYPTOPHAN TRANSPORT IN NEUROSPORA: EFFECT OF INTRACELLULAR POOLS *

The rate of tryptophan transport in Neurospora appears to be regulated by intracellular pools of tryptophan or some product of tryptophan metabolism. A similar regulatory influence was exerted by leucine pools, but not by glutamic acid or lysine pools.

Investigator:

W. R. Wiley

Technical Assistance:

Mary M. Quentin

The regulation of biosynthetic pathways by feedback inhibition is a fairly widespread phenomenon in microorganisms. In most cases, the end product of the biosynthetic sequence exerts an inhibitory effect on the first enzyme of the pathway. Because of the enzymic nature of the tryptophan transport system in *Neurospora* (Annual Report, 1965), one may question whether the transport system exhibits patterns of feedback inhibition similar to those of biosynthetic enzymes. The experiments described here attempt to answer this question.

Observations

When cells of *Neurospora* were grown overnight in tryptophan-containing

medium, a reduction was observed in the rate of tryptophan transport relative to cells grown in minimal medium. This reduction in the rate of transport occurred without significant alteration in the rate of growth or protein synthesis. The intracellular tryptophan pool in the tryptophan-grown cells, however, was 20 μ moles/g cells dry weight as compared to only 1.8 μ moles in cells grown in minimal medium. From these observations, it was postulated that the reduction in the rate of transport was related to the higher concentration of intracellular tryptophan. To test this point, transport was measured in cells which had been preloaded with tryptophan.

Cells were grown for 9 hr in minimal medium, abruptly shifted to minimal medium supplemented with 3 mM tryptophan and incubated for an additional 15 min at 30 °C. During this

* *This study derived partial support from the Battelle Institute, a Division of Battelle Memorial Institute, Columbus, Ohio.*

15-min preloading period, the intracellular tryptophan pool increased from 2 to 40 $\mu\text{moles/g}$ cells. Control cells received the same treatment without tryptophan supplementation. The preloading process was terminated by pouring the culture onto frozen crushed minimal medium. The cells were washed twice and the time course of tryptophan uptake measured. Rates are indicated by the slopes of the curves presented in Figure 1. Preloading with tryptophan reduced the rate of transport from 3.9 to 0.76 $\mu\text{moles/min/g}$ cells.

The relationship between the intracellular tryptophan pool and the rate of tryptophan transport was further investigated by terminating the preloading process at various intervals. The ratio of postshift to preshift rates of transport are plotted in Figure 2 as a function of the duration

of the preloading process. Reduction in the rate of transport is apparent after 5-min preloading. The initial rate of uptake was reduced to about one-half the original rate after 10 min and reached a minimum after 20 min. Following the shift to preloading medium, the tryptophan pool size increased from 2 to 40 $\mu\text{moles/g}$ cells at 5 min, and to 42 $\mu\text{moles/g}$ cells at 20 min.

Previous investigations showed (Annual Report, 1966) that indole and tryptophan are transported across the cell membrane of *N. crassa* by independent transport systems. Free indole is not accumulated by the cell, but is immediately converted to tryptophan. Thus, if intracellular pool

FIGURE 1. Influence of Intracellular Pool Tryptophan on the Time Course of Tryptophan Uptake

FIGURE 2. Relationship Between the Duration of Preloading with Tryptophan and the Rate of Tryptophan Uptake

tryptophan, or a metabolite of tryptophan, is responsible for the observed reduction in rate of tryptophan transport, pools formed from indole should provide a stringent test of this contention. Figure 3 shows the effect of a shift to indole-containing medium on the rate of tryptophan uptake. The experiment was similar to that described in Figure 2 except indole replaced tryptophan in the preloading medium. The slight increase in rate of transport by control cells is unexplained. The indole-grown cells showed a reduction in rate of tryptophan transport which was quite comparable to that exhibited by tryptophan-grown cells in Figure 2. During the incubation period in indole medium, the tryptophan pool increased from 1.8 to 6.0 μ moles/g cells after 15 min, and to 10 μ moles/g cells

FIGURE 3. Effect of Endogenously Formed Pools of Tryptophan on the Rate of Tryptophan Transport

after 60 min. It would appear from these results that endogenously synthesized tryptophan is as effective as exogenously supplied tryptophan in controlling the rate of tryptophan entry.

The criterion of competitive inhibition of amino acid uptake has been used to demonstrate distinct transport groups, or families, of amino acids in *Neurospora* (Annual Report, 1966). On this basis diamino acids such as lysine and arginine constitute one family, dicarboxylic acids such as glutamic and aspartic acid comprise a second group, and a third group is comprised of neutral amino acids such as tryptophan, leucine, methionine, and phenylalanine. One may question whether the group concept of transport applies also to inhibition exerted by amino acids in intracellular pools. The results of experiments directed to this question are shown in Table I. Preloading cells with lysine or glutamic acid had no effect on the rate of tryptophan uptake. In contrast, preloading with leucine, a member of the tryptophan transport family was nearly as effective as

TABLE I. Effect of Other Amino Acid Pools on the Rate of Tryptophan Uptake

Amino Acid	Ratio	Postshift Rate Preshift Rate
L-Lysine		1.0
L-Glutamate		1.08
L-Leucine		0.13
L-Tryptophan		0.10

tryptophan itself in the inhibition of tryptophan uptake. It would appear from these results that the family concept of uptake may apply also to the regulation of transport activity.

Conclusions

The simplest explanation of these results is that intracellular pool tryptophan, or a product of tryptophan metabolism, feeds back and

either inhibits the activity or represses the biosynthesis of the transport site(s). It is possible that both processes are involved. The observation (Annual Report, 1966) that the transport system turns over at an unusually high rate makes it difficult to distinguish between these possible regulatory processes. Isolation and characterization of transport site(s) would greatly facilitate further progress and this problem is presently under attack.

TRYPTOPHAN TRANSPORT IN NEUROSPORA: TRYPTOPHAN BINDING PROTEIN RELEASED BY OSMOTIC SHOCK"

Osmotic shock decreased transport rates of tryptophan, lysine, isoleucine, and glutamic acid. In this process a proteinaceous material is released from the cell which may be active in the transport of tryptophan. The material is non-dialyzable, absorbs at 280 mμ, gives a positive reaction in the Lowry protein assay, and binds tryptophan.

Investigator:

W. R. Wiley

Technical Assistance:

Mary M. Quentin

When cells of Escherichia coli are converted to spheroplasts under hypertonic conditions by treatment with lysozyme, enzymes such as alkaline phosphatase, latent ribonucleases, and Co^{+} -requiring nucleotidase are released to the suspending medium.(1) It was suggested that these enzymes are localized on the cytoplasmic membrane or in the peroplasmic space

between the cell wall and the cell membrane. Enzymes known to reside inside the cell are not released during spheroplast formation.

Recently, it was shown that the enzymes released during spheroplast formation may also be released by osmotic shock procedures which do not alter cell viability.

Since amino acid transport systems are thought to reside either in the peroplasmic space or on the cell membrane, the osmotic shock procedure suggested itself as a possible

* This study derived partial support from the Battelle Institute, a Division of Battelle Memorial Institute, Columbus, Ohio.

MOLECULAR AND CELLULAR LEVEL STUDIES

tool for the isolation of the tryptophan transport site(s) in *Neurospora*.

Observations

Cells were subjected to osmotic shock by the following standardized procedure: In stage I (preshock treatment), cells were suspended in Tris-HCl buffer containing 20% sucrose and 0.001M ethylenediaminetetraacetic acid (EDTA) and incubated at 24 °C for 10 min. In stage II, the cells were centrifuged and immediately re-suspended (shock treatment) in distilled water. Control cells were treated as described in Table I. As shown, the rate of tryptophan transport was sharply decreased following osmotic shock treatment. Rates of transport of lysine, isoleucine, and glutamic acid were similarly decreased

As demonstrated by treatment 3 of Table I, incubation of cells with sucrose-EDTA, without shock, is slightly inhibitory. However, this effect is small relative to that observed in the osmotically shocked cells. Treatment with sucrose in stage I is an absolute requirement for osmotic shock. The degree of shock as measured by microscopic appearance of the cells is not affected by EDTX. However, if EDTA is omitted, there is an increased release of 260 mμ-absorbing material, which suggests that EDTA plays a role in the prevention of lysis and release of intracellular constituents. Similar effects were observed in the earlier work on *E. coli*.⁽²⁾

Viability does not appear to be affected by osmotic shock treatment. There is a lag of about 3 hr before

TABLE I. *Effect of Osmotic Shock on Amino Acid Transport by Neurospora*

	Treatment (a)		Rates of Amino Acid Uptake, μmoles/min/g cells (b)			
	Stage I	Stage II	Tryptophan	Lysine	Isoleucine	Glutamate
1.	Min med	Distilled water	3.96	16.1	2.10	0.578
2.	Min med	Double strength min med	3.84	--	--	--
3.	Sucrose-EDTA	Double strength min med	2.64	6.33	1.0	0.30
4.	Sucrose-EDTX	Distilled water	0.14	1.53	0.50	0.008

- (a) 1. *Unshocked distilled water control*
 2. *Unshocked double strength minimal medium control*
 3. *Unshocked sucrose-EDTA control*
 4. *Shocked cells*

(b) *Rates of uptake were measured in minimal medium containing the radioactive forms of the respective amino acids at a final concentration of $1 \times 10^{-5}M$. Radioactivity of washed cells was determined on samples removed from the reaction mixture at 1-min intervals.*

growth and protein synthesis are resumed. Normal transport rates are resumed following a lag of 3-4 hr.

If the effect of osmotic shock on amino acid transport is a result of the release of components of the transport system into the shock medium, it might be possible to recover the binding component from the medium and to measure its activity *in vitro*. To explore this possibility, a large volume of shock fluid was dialyzed for 24 hr against a large excess of dilute buffer. This process removed most of the dialyzable components from the shock fluid, which was then concentrated by lyophilization. The lyophilized material had many of the properties of proteins. It was nondialyzable, had a specific absorption at 280 m μ , and a positive reaction in the Lowry protein assay.

The tryptophan binding activity of the nondialyzable material was measured by gel filtration. The shock material was incubated for 40 min in the presence of 0.23 μ moles per ml of L-tryptophan [3^{14} C] at 22 °C, following which the mixture was layered over the top of a Sephadex G-25 column and eluted at 4 °C. This procedure permits the separation of components based on molecular size. Small molecules are retarded by the gel while large molecules pass through the column unretarded. As shown in Figure 1, a minor radioactive peak appeared in the elution profile distinctly ahead of the major peak. The minor peak was associated with the nondialyzable material as judged by the absorption at 280 m μ . These results suggest that some type of

complex is formed between tryptophan and the shock material.

Additional support for this contention was obtained through the use of equilibrium dialysis. Shock material was added to the inside of an equilibrium dialysis chamber, L-tryptophan [3^{14} C] to the outside, and the apparatus incubated at 4 °C for 16 hr. The resulting distribution of radioactivity is shown in Table II. The higher concentration of radioactive tryptophan associated with the

FIGURE 1. Demonstration of a Tryptophan-Protein Complex on Sephadex Ge 2-25

TABLE II. *Equilibrium Dialysis Measurement of the Tryptophan Binding Activity of a Protein Isolated by Osmotic Shock*

Tryptophan Concentration in Dialysis Fluid, <u>μmoles/ml</u>	Distribution <u>Ratio(a)</u>
1.0	1.55
0.25	1.30
0.023	1.03

(a) *Radioactive tryptophan concentration in chamber containing nondialyzable shock material, divided by radioactive tryptophan concentration in outer chamber, after 16 hr dialysis.*

280 m μ -absorbing material suggests again that a complex is formed. The unequal distribution of radioactivity does not appear to result from a Donnan equilibrium since the salt concentrations on both sides of the membrane were relatively high. Changes in the distribution ratio with changes in tryptophan concentration may be of significance, however, the present data do not permit an interpretation of these differences.

Conclusions

Osmotic shock was shown to markedly decrease the rates of transport of tryptophan, lysine, isoleucine, and glutamic acid. During osmotic shock, a material is released from the cells which binds tryptophan in vitro. The material released has many of the characteristics of proteins, it is nondialyzable, absorbs at 280 m μ , and reacts positively in the Lowry protein assay. These data suggest, but do not prove, that the binding component is active in the transport of tryptophan in *Neurospora*. The determination of the dissociation constant for the binding process, as well as the inhibition constants for amino acids known to compete with tryptophan for transport, in vivo, should provide evidence for or against this interpretation.

References

1. Neu, H. C. and L. A. Heppel. *J. Biol. Chem.*, vol. 240, pp. 3685-3692. 1965.
2. Anraku, Y. and L. A. Heppel. *J. Biol. Chem.*, vol. 242, pp. 2561-2569. 1967.

REGULATION OF ENZYME SYNTHESIS IN NEUROSPORA CRASSA*

Accumulation of a tryptophan precursor, indole-glycerol phosphate, or a histidine precursor, imidazoleglycerol phosphate, in Neurospora crassa interferes with the tryptophan mediated regulation of tryptophan synthetase and kynureninase. Mutants unable to form these precursors are not affected by conditions which cause aberrations in the regulatory reactions of wild type. These aberrations occur in the presence of large amounts of tryptophan and are consistent with the idea that a derivative of tryptophan rather than the free amino acid is responsible for regulation.

Investigator:

J. R. Turner

Technical Assistance:

Carla J. Farrell

Recent investigations have implicated biosynthetic intermediates as an integral part of the regulatory processes in microorganisms. Our previous studies (Annual Report, 1966) yielded results demonstrating that in Neurospora crassa the accumulation of indoleglycerol phosphate (InGP), a precursor of tryptophan, modifies the regulatory control which tryptophan exerts on the formation of tryptophan synthetase and kynureninase. Further, a histidine intermediate imidazoleglycerol phosphate (ImGP), which accumulates in appropriately blocked mutants or in response to an inhibitor, mimics the effect of InGP. This report presents results indicating that mutants unable to form these intermediates do not exhibit the regulatory responses shown by the wild-type.

Observations

Two enzymes that are regulated by tryptophan were examined: tryptophan

synthetase, a biosynthetic enzyme repressible by tryptophan, and kynureninase, a degradative enzyme inducible by tryptophan. Regulation of these proteins was assessed by measuring the differential rate of enzyme synthesis in exponentially growing cells.

Figure 1 (Panel A and B) shows the relative rate of synthesis of these two enzymes in wild-type Neurospora under conditions where the intermediates InGP and ImGP are accumulated (Panel C). Accumulation of InGP occurs (Panel C, Line 2) in the presence of indole acrylic acid (IA). The accumulation of this intermediate is accompanied by derepression of tryptophan synthetase (Panel A, Line 1). Indole acrylic acid is used without tryptophan since tryptophan inhibits anthranilic acid (AA) formation and prevents the accumulation of InGP. Imidazoleglycerol phosphate accumulates in the presence of 3-amino-1, 2,4-triazole (3AT) with or without tryptophan (Panel C, Line 1). Derepression of tryptophan synthetase (Panel A, Line 2) and inhibition of the tryptophan mediated

* This study derived partial support from the Battelle Institute, a Division of Battelle Memorial Institute, Columbus, Ohio.

FIGURE 1. Wild-Type: Relative Rates of Formation of Tryptophan Synthetase and Kynureninase and Accumulation of ImGP, InGP, and Intracellular Tryptophan, as Influenced by Indicated Supplements to Medium

induction of kynureninase (Panel B, Line 2) occur under this condition.

The effect of accumulated InGP on kynureninase was tested in a mutant (ti-yp-3) which accumulates InGP in the presence of tryptophan and anthranilic acid (AA). Figure 2 shows the relative rate of kynureninase and tryptophan synthetase formation under this condition. In the presence of tryptophan and AA, InGP accumulates

FIGURE 2. Tryp-3 Mutant: Relative Rates of Formations of Tryptophan Synthetase and Kynureninase and Accumulation of InGP, as Influenced by Indicated Supplements to Medium

(Panel C, Line 1) and tryptophan synthetase is derepressed (Panel A, Line 1), but kynureninase induction is not inhibited (Panel B). Although the formation of kynureninase appears to be independent of the accumulation of InGP, preliminary experiments indicate that at higher intracellular concentrations of the intermediate, induction of this enzyme would be inhibited.

Accumulation of ImGP and InGP in these experiments occurs in the presence of 3AT and either AA or IA, respectively. The presence of these compounds in the growth medium is slightly inhibitory to the growth of

Neurospora. However, studies with appropriately blocked mutants have shown that these compounds have no direct effect on the formation of tryptophan synthetase or kynureninase but act only to cause the accumulation of metabolic intermediates. When a hist-7 mutant (unable to form ImGP) was challenged with 3AT (Figure 3, Panel A, Line 2) derepression of tryptophan synthetase was not observed, nor was the induction of kynureninase inhibited (Panel B, Line 1). Derepression of tryptophan synthetase was observed in this strain

FIGURE 3. *Hist-7 Mutant: Relative Rates of Formation of Tryptophan Synthetase and Kynureninase in a Mutant Unable to Make ImGP, as Influenced by Indicated Supplements to Medium*

in response to indole acrylic acid (Panel A, Line 1), a response analogous to the wild-type. Similarly, a tryp-4 mutant (unable to form InGP) (Figure 4) failed to exhibit derepression of tryptophan synthetase in response to IA or AA but was responsive to 3AT.

The aberrations in tryptophan mediated regulation caused by accumulation of InGP and ImGP occur in the presence of amounts of tryptophan sufficient to maintain the usual responses, i.e., repression of tryptophan synthetase and induction of kynureninase (Figure 1, Panel D). This observation is consistent with the idea that regulation may be mediated, not by the free amino acid but by a derivative. One means by which the accumulated intermediates might affect regulation is by interference with the formation of such a derivative. Current theory of metabolic

FIGURE 4. *Tryp-4 Mutant: Relative Rate of Formation of Tryptophan Synthetase in a Mutant Unable to Make InGP, as Influenced by Indicated Supplements to Medium*

regulation proposes that sRNA derivatives of amino acids function in regulation of protein synthesis. Evidence supporting this notion has been found in bacterial systems.

If the same or an analogous mechanism is operative in *Neurospora*, we would expect these intermediates to interfere with the charging of tryptophan to its sRNA. We have tested the effect of some derivatives of indole on a cell-free tryptophanyl-sRNA forming system isolated from *Neurospora*, the results of which are shown in Table I. At a concentration of 0.5 μ moles/ml, only indole propionate and indole pyruvate have any significant effect on the charging of tryptophan to its sRNA. At a concentration of 1 μ mole/ml, 3AT has no effect on this reaction. Although there seems to be no unequivocal conclusion to be drawn from this preliminary experiment, it does indicate the desirability of isolating and testing the physiologically important intermediates.

Conclusions

These results show that the presence of 3AT, IA or AA in the growth medium of *Neurospora* has no direct effect on the formation of kynureninase and tryptophan synthetase. Derepression of tryptophan synthetase and inhibition of kynureninase induction

TABLE I. Inhibition of Tryptophan-sRNA Formation

Addition to Complete Reaction	Concentration, μ mole/ml	Percent Inhibition
None	0	0
Indole	0.5	7
Indole propionate	0.5	29
Indole lactate	0.5	14
Indole butyrate	0.5	13
Indole pyruvate	0.5	40
Indole acetate	0.5	16
3-amino-1, 2,4-triazole	1.0	0

observed in the presence of these compounds is due at least in part to their inhibition of histidine and tryptophan synthesis and the resultant accumulation of biosynthetic intermediates.

The indication that a derivative of tryptophan may be the critical regulatory compound and the observed inhibition of tryptophanyl-sRNA formation by derivative of indole point to the desirability of testing the effect of physiological intermediates on this reaction.

ACID MUCOPOLYSACCHARIDE LOCALIZATION IN INTESTINAL ABSORPTIVE
CELLS AFTER IRRADIATION

Localization of acid mucopolysaccharides (AMPS) in the intestinal absorptive cell was determined using colloidal thorium staining. Immediately after irradiation, the Golgi complex and cytoplasmic vesicles showed a markedly increased staining with thorium. A thick, filamentous surface coat, heavily stained with thorium, was not observed until 5 hr postexposure. These observations suggest that the Golgi complex is involved in the final synthesis of AMPS and that this AMPS becomes a component of the enteric surface coat.

Investigator:
J. D. Berlin
Technical Assistance:
R. R. Adee

A thick, AMPS-rich, enteric surface coat was previously reported to occur in irradiated rats, but not in normal rats (Annual Report, 1965). This report describes the sequence of subcellular events that occur in mature intestinal absorptive cells of irradiated rats which lead to the manifestation of this surface coat.

Observations

Rats of the Charles River strain were given 1000 R wholebody X-irradiation (40 R/min) and sacrificed at various time intervals up to 5 days after exposure. Tissue samples were obtained from the terminal ileum of the irradiated rats, and from unirradiated control rats, and fixed for electron microscopy in phosphate-buffered osmium tetroxide. This material was embedded in methacrylate, sectioned, and stained for AMPS with colloidal thorium at low pH. The sections were further stained with

uranyl acetate and lead citrate to enhance contrast and examined in an RCA EMU-3B electron microscope.

The Golgi cisternae, Golgi-associated vesicles, vesicles in the terminal web region, and enteric surface coat found in and on intestinal absorptive cells from normal rats had only a slight affinity for the AMPS stain (Figures 1 and 2).

At 27 min after initiation of irradiation (2 min after completion) thorium staining, indicative of AMPS, was intense in the Golgi cisternae, the Golgi-associated vesicles, and the vesicles of the terminal web (Figures 3 and 4). In contrast, at this time interval after exposure, the enteric surface coat is thin (Figure 4) and is essentially similar to that found in the normal rat.

By 5 hr after irradiation, the intensity of the AMPS stain had subsided in the Golgi region and in the terminal web area, although it was more intense than in the normal rat.

40,000X

FIGURE 1. Normal Rat Absorptive Cells
[The Golgi complex (G) and Golgi-associated vesicles (V) in adjacent absorptive cells show little affinity for thorium particles. A desmosome (D), interdigitating plasma membranes (PM), rough endoplasmic reticulum (ER), and a portion of a nucleus (N) are also shown in this micrograph.]

43,200X

FIGURE 2. Normal Rat Absorptive Cell.
[Scattered vesicles (V) in the terminal web contain only a slight reaction product when stained for AMPS. Thorium particles are found in close apposition to the external leaflet of the plasma membrane encompassing the microvilli (MV). A thick enteric surface coat is absent.]

38,400X

FIGURE 3. *Rat Absorptive Cell Immediately After Irradiation [The Golgi complex (G) and Golgi-associated vesicles (V) are heavily stained with thorium. The reaction is more intense in the Golgi cisternae at the mature face (M) than in the cisternae of the forming face (F).]*

36,800X

FIGURE 4. *Rat Absorptive Cell Immediately After Irradiation [Vesicles (V) in the terminal web region are heavily stained with thorium particles. Apparent invaginations of the plasma membrane (unlabeled arrows) show thorium particles located on the external side of the membrane. The transversely sectioned microvilli (MV) lack an enteric coat.]*

A thick, AMPS-rich, enteric surface coat was present at 5 hr after exposure and persisted throughout the observation periods of this study.

These results are summarized in Table I.

Conclusions

It is clear from the present study that the function, or perhaps the rate of function, of the Golgi complex in mature, intestinal absorptive cells is altered immediately after, if not simultaneously with, irradiation. Based on our limited knowledge of the mechanism of the thorium stain, it would appear that this altered function involved the sulfation of AMPS that are destined to become an integral part of the enteric surface coat. The surface coat, when present, is the interface between the intestinal lumen and the absorptive cells; substances entering or leaving the apical surface of these cells must traverse this coat. While the precise role of such surface coats in membrane physiology is not known, it is frequently suggested that they may influence the permeability of

TABLE I. Effect of 1000 R Whole-Body X-Irradiation on Acid Mucopolysaccharide Localization in Intestinal Absorptive Cells

	Unir- radiated Rats	Irradiated Rats (Time Postir- radiation, hr)		
		0	5	24- 120
Golgi cisternae	±	++++	++	+
Golgi vesicles	+	++++	++	+
Terminal web vesicles	±	++++	++	+
Surface coat	±	±	++++	++++
Other structures				

- No staining observed

++++ Intense stain observed

the cell surface. Whether or not the enteric surface coat, which is increased in thickness following irradiation, is responsible for, or related to, the altered transport that occurs in the intestine after irradiation remains an open and tantalizing question.

INTRANUCLEAR INCLUSIONS IN LEYDIG CELLS OF HUMAN TESTES

A morphological description is given for intranuclear inclusions found in Leydig cells of a 32-year-old man treated with human chorionic gonadotropin (HCG). Similar inclusions have been reported to occur only in men 70 or more years of age. It is suggested that the intranuclear inclusions may be a morphological expression of a mechanism common to both HCG treatment and aging.

Investigator:

J. D. Berlin

Technical Assistance:

Josephine A. Mars

Intranuclear inclusions in Leydig cells of the human testes have previously been reported to occur only in men 70 or more years of age.⁽¹⁾ The present report describes intranuclear inclusions in the Leydig cells of a man 32 years of age that had received human chorionic gonadotropin (HCG), an anterior pituitary-like hormone.

Observations

Biopsied testicular material obtained from a man that had received 4000 IU of HCG every other day for 16 weeks was prepared for electron microscopic examination. The tissue was initially fixed in glutaraldehyde, subsequently fixed in osmium tetroxide, dehydrated in ethanol, and embedded in Epon. Silver-gray sections were stained with uranyl acetate and lead citrate. Intranuclear inclusions in a Leydig cell are shown at various magnifications in Figures 1-3 and diagrammatically in Figure 4. The inclusions are clustered in regions of the nucleoplasm that are devoid of chromatin.

Each inclusion appears to have the form of three curved cylinders in a triangular arrangement (Figures 2 and 3). Each cylinder is identical in structure; they have electron opaque walls composed of five spherical subunits, each subunit being about 6 m μ in diameter. An amorphous material of moderate electron density is associated with each subunit (Figure 3). Within each cylinder is an electron transparent lumen approximately 18 m μ in diameter. The total diameter of each cylinder is, therefore, about 30 m μ and the separation between the cylinders is approximately 10 m μ .

When sectioned longitudinally, the inclusions appear as four electron opaque lines, representing the walls of two cylinders (generally only two of the three cylinders appear in longitudinal section), separated by three electron transparent regions, the two outer regions being the lumens of the cylinders and the center region being that space separating the cylinders. The longitudinally sectioned electron opaque lines exhibit a periodicity of approximately 16 m μ , resulting from a linear

array of electron opaque subunits that are $6 \text{ m}\mu$ in diameter and have a separation of $10 \text{ m}\mu$.

It has not been determined if the spherical subunits are connected or, if connected, whether they are arranged around the cylinder in a linear or a helical manner.

Inclusions as long as $130 \text{ m}\mu$ were observed; however, no significance can be attached to this figure because the sections were only $60 \text{ m}\mu$ thick. Curved cylinders of $30 \text{ m}\mu$ diameter would rarely remain in longitudinal section for any considerable distance in $60 \text{ m}\mu$ sections. For this

FIGURES 1-3. Successively Higher Magnifications of Intranuclear Inclusions in a Leydig Cell of a HCG-Treated Man [(1) A nucleus and a portion of the cytoplasm--the intranuclear inclusions are barely discernible at this magnification. (2) Numerous inclusions in different planes of section--the circular profiles, shown in cross-section, illustrate the triangular arrangement of the three cylinders (X-labeled arrows); the linear arrays result from longitudinally sectioned inclusions (unlabeled arrows). (3) Inset shows area contained within the rectangle outlined in (2)--inclusions are shown in both transverse (X-labeled arrow) and longitudinal (unlabeled arrow) section; the spherical nature of the subunits in the inclusions is slightly obscured by the associated amorphous material.]

FIGURE 4. *Diagrammatic Representation of Intranuclear Inclusions in Leydig Cells of a HCG-Treated Man (All dimensions are given in $m\mu$. Note that the diagram of longitudinally sectioned inclusions portrays only two cylinders because of the muddled appearance obtained when all three cylinders occur in a $60\text{-}m\mu$ section.)*

same reason, though the inclusions appear to be randomly oriented, it is impossible to rule out a coil-type configuration in which all of the inclusions are actually continuous.

Conclusions

The significance of these intranuclear inclusions is not known. They are strikingly similar to those observed by Yasuzumi et al., in men past 70.⁽¹⁾ Indeed, close scrutiny of the micrographs of Yasuzumi et al reveals a tubular morphology that was not reported by these workers.

HCG is described as an anterior pituitary-like hormone primarily because it has an interstitial cell-stimulating hormone (ICSH) effect. Both ICSH and the follicle-stimulating

hormone (FSH) are produced by the anterior pituitary. The administration of HCG appears to eliminate anterior pituitary function; i.e., it results in elevated testosterone levels, an expression of the ICSH effect of HCG, and decreased FSH levels (resulting in decreased spermatogenesis).

It is generally recognized that elevated gonadotropin levels appear in older men. Unfortunately, separate assays do not appear to have been performed for the FSH, ICSH, or the luteinizing hormone (LH). Comfort⁽²⁾ ambiguously suggests that pituitary FSH content of men 70 years old remains at normal adult levels or falls, occasionally, even below childhood levels. Hence, the gonadotropin levels of older men may be

similar to that found after HCG treatment, i.e., increased ICSH effect and decreased FSH levels.

It is conceivable that inclusions of the type seen in this study may be a morphological expression of a similar mechanism existing in HCG-treated subjects and in men 70 or more years of age.

References

1. Yasuzumi, G., Y. Nakai, I. Tsuba, M. Yasuda, and T. Sugioka. *Exptl. Cell Res.*, vol. 45, pp. 261-276. 1967.
2. Comfort, A. *The Biology of Senescence*, London, Routledge and Paul. 1956, p. 181.

METABOLISM OF ACETATE BY TROUT TISSUES AT HIGH TEMPERATURES

Acetate-1-¹⁴C was incubated with trout tissues at temperatures from 18-38 °C. There was a general increase in rate of oxidation of acetate with increased temperature. The production of energy is apparently not the limiting factor in thermal death of fish.

Investigator:

J. M. Dean

Technical Assistance:

Alice M. Russell

Failure of the central nervous system, denaturation of proteins, inactivation of enzymes and a decreased ability to convert metabolites into energy have been suggested as possible mechanisms of death for poikilotherms at high temperatures. We have examined the problem by measuring the conversion of a labeled intermediary metabolite, acetate-1-¹⁴C to ¹⁴CO₂ in liver, red muscle, and white muscle of trout.

Observations

Tissue homogenates from 18 °C acclimated trout were incubated with

acetate-1-¹⁴C at temperatures ranging from 18-38 °C, with the results shown in Figure 1. Also shown for comparison are the results of an earlier experiment (Annual Report, 1966) in which tissues from 18 °C acclimated trout were incubated at lower temperatures. Agreement between results from the two experiments at the overlap point of 18 °C is not too good, reflecting, perhaps, a seasonal effect, since the two experiments were conducted at a 6 month interval. There is also an unexplained dip in the white muscle curve at 22 and 26 °C, which is not present in the other tissues. However, the results

FIGURE 1. Incorporation of Acetate-1- ^{14}C into Respiratory CO_2 by Rainbow Trout Liver, Red and White Muscle Homogenates ($\pm$ standard error of mean)

show a generally increased rate of oxidation of acetate at high incubation temperatures. Certainly, there is no evidence of inhibitory effect even at temperatures far in excess of the lethal limit for this species.

Conclusions

If denaturation of proteins is a critical factor in heat death, there

should be a concomitant reduction of enzyme activity. Or, if a particular enzyme involved in energy production had a low temperature of inactivation, the oxidation of metabolites, such as acetate, should be reduced. Since this was shown not to occur, the production of energy is probably not the limiting factor in heat death.

EFFECT OF ACCLIMATION TEMPERATURE ON MITOCHONDRIAL ENZYMES IN FISH

Activity of cytochrome oxidase in homogenates from several tissues was higher in cold than in warm acclimated goldfish. The activities of cytochrome oxidase and succinate- and NADH- cytochrome c reductase were greatest in gill mitochondria from the cold adapted goldfish, but cytochrome levels were unchanged. Cold acclimated carp had higher gill homogenate cytochrome oxidase activity than warm carp. However, NADH-cytochrome c reductase activity in gill mitochondria decreased with cold acclimation.

Investigator:

R. S. Caldwell

Technical Assistance:

Alice M. Russell

Previous studies of cold acclimation in fish and other poikilotherms have frequently indicated an elevation of respiratory rates in response to low temperature. It has been suggested that greater oxygen consumption by cold adapted animals may reflect an increased dependence on extramitochondrial oxidative pathways. The present study was initiated to investigate this problem.

Observations

Goldfish (Carassius auratus) were acclimated either to 10 or 30 °C and carp (Cyprinus carpio) to either 5 or 30 °C for 2-4 weeks prior to sampling. Ten percent homogenates of goldfish tissues were prepared in 0.25M sucrose containing 0.01M Tris buffer, pH 7.5, and 0.0001M EDTA. Carp gills were homogenized in unbuffered 0.25M sucrose. Gill and liver mitochondria, the fraction sedimenting in a centrifugal field of 600-11,000 g,* were

washed twice by resuspension and re-centrifugation at 11,000 g.

Cytochrome oxidase activity was estimated by the spectrophotometric method of Smith.⁽¹⁾ Succinate-cytochrome c reductase and NADH-cytochrome c reductase activities were measured spectrophotometrically by following the rate of reduction of cytochrome c in the presence of KCN and appropriate substrate. The concentration of cytochromes aa₃, b, c, and c₁ were determined from reduced-minus-oxidized difference spectra of deoxycholate solubilized mitochondria and computed by the procedure of Vanneste.⁽²⁾ Protein was determined with the Folin-Ciocalteu reagent using crystalline bovine serum albumen as a standard.

Cytochrome oxidase activity in brain, gill, and muscle homogenates from 10 °C acclimated goldfish was significantly higher than in preparations from 30 °C acclimated fish when determined over the temperature range of 10-40 °C (Figure 1). A similar compensation of this enzyme

* g = gravitational units

FIGURE 1. The Influence of Acclimation Temperature on Cytochrome Oxidase Activity in Goldfish Tissue Homogenates. (Specific activity is expressed as $\mu\text{moles cytochrome c oxidized/mg protein/min}$. Open circles = warm fish, 30 °C; filled circles = cold fish, 10 °C.)

was observed in goldfish liver homogenates when measured at 30 and 40 °C but was lacking at the lower assay temperatures.

In gill mitochondrial preparations from acclimated goldfish, the specific activity of cytochrome oxidase was higher in the 10 °C than in the 30 °C mitochondria when measured at subsequent periods throughout the year

(Table I). Likewise, the activities of succinate-cytochrome c reductase and NADH-cytochrome c reductase were greater in the 10 °C than in the 30 °C gill mitochondria. With the exception of September, 1966, when an unusually high activity of gill homogenate cytochrome oxidase was observed, no marked seasonal effects were noted for these enzymes.

TABLE I. Effect of Acclimation Temperature on the Activity of Fish Gill Electron Transport Enzymes^(a)

Enzyme and Source	Goldfish			Carp
	Sept. 1966	Nov. 1966	June 1967	Nov. 1967
<u>Cytochrome oxidase</u>				
Homogenate				
Cold acclimated	0.34 ± 0.02	0.26 ± 0.02	0.25 ± 0.04	0.15 ± 0.01
Warm acclimated	0.19 ± 0.01	0.18 ± 0.01	0.20 ± 0.02	0.12 ± 0.01
Mitochondria				
Cold acclimated	2.9 ± 0.2	2.5 ± 0.1	2.7 ± 0.3	0.98 ± 0.12
Warm acclimated	1.8 ± 0.2	2.0 ± 0.1	2.2 ± 0.4	1.18 ± 0.18
<u>Succinate-cytochrome c reductase</u>				
Mitochondria				
Cold acclimated	0.22 ± 0.01	0.21 ± 0.01	0.21 ± 0.03	0.10 ± 0.01
Warm acclimated	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.02	0.12 ± 0.01
<u>NADH-cytochrome c reductase</u>				
Mitochondria				
Cold acclimated	0.21 ± 0.02	0.20 ± 0.01	0.16 ± 0.03	0.07 ± 0.01
Warm acclimated	0.11 ± 0.01	0.15 ± 0.02	0.10 ± 0.01	0.11 ± 0.01

(a) Results expressed as $\mu\text{moles cytochrome c oxidized/mg protein/min}$ for cytochrome oxidase and $\mu\text{moles cytochrome c reduced/mg protein/min}$ for succinate-cytochrome c reductase and NADH-cytochrome c reductase $\pm$ standard error. Assay temperatures were 20 °C for goldfish and 25 °C for carp enzymes.

Acclimation temperatures for goldfish were: cold, 10 °C; warm, 30 °C.

Acclimation temperatures for carp were: cold, 5 °C; warm, 30 °C.

In gills from temperature-acclimated carp, the activity of homogenate cytochrome oxidase was significantly elevated in the cold fish (Table 1). However, the activity of NADH-cytochrome c reductase was lower in mitochondria from cold fish, while mitochondrial cytochrome oxidase and succinate-cytochrome c reductase were not significantly altered.

The concentration of cytochromes aa_3 , b , c , and c_1 in gill and liver

mitochondria from cold and warm acclimated goldfish were determined to see if changes in enzyme concentration paralleled the observed changes in mitochondrial electron transport rates. There were no significant differences between the two experimental groups for any of the cytochromes in gill mitochondria (Table II). However, the levels of cytochromes aa_3 and c_1 were significantly higher in the 30 °C than in the 10 °C liver mitochondria.

TABLE II. *Effect of Acclimation Temperature on the Concentration of Cytochromes in Goldfish Gill and Liver Mitochondria*^(a)

Cytochromes	Gill		Liver	
	10 °C	30 °C	10 °C	30 °C
<u>a₃</u>	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.02	0.39 ± 0.01
<u>b</u>	0.087 ± 0.007	0.084 ± 0.010	0.065 ± 0.002	0.085 ± 0.004
<u>c</u>	0.084 ± 0.009	0.094 ± 0.010	0.070 ± 0.004	0.094 ± 0.004
<u>c₁</u>	0.074 ± 0.006	0.10 ± 0.01	0.077 ± 0.005	0.12 ± 0.01

(a) Results are expressed as $\mu\text{moles/mg protein} \pm \text{standard error}$

Conclusions

In contrast to earlier suggestions that there is an increased dependence on extramitochondrial oxidations, these results suggest that increased oxygen consumption of goldfish tissues following cold acclimation is correlated with greater rates of mitochondrial electron transport. The magnitude of increase in homogenate cytochrome oxidase activity of each of the four goldfish tissues studied is similar to that reported elsewhere for tissue oxygen consumption.⁽³⁾ Increases in activity of cytochrome oxidase in goldfish gill homogenates seem partially attributable to an increase in the specific activity of this enzyme in gill mitochondria. These data support the contention that cold acclimation of goldfish results in an overall increase in the capacity for electron transport in certain

tissues. However, since there was no significant increase in gill mitochondrial cytochrome concentrations and an apparent decrease in some liver mitochondrial cytochromes with cold acclimation, it is suggested that factors other than changes in enzyme concentration are involved in the compensation of electron transport activity.

References

1. Smith, L. *Methods of Biochemical Analysis*, vol. 2, Interscience Publishers, Inc., New York. 1955. pp. 427-434.
2. Vanneste, W. H. *Biochim. et Biophys. Acta*, vol. 113, pp. 175-178. 1966.
3. Prosser, C. L. *Symposium on Arctic Biology and Medicine. Part II.*, J. P. Hannon and E. Viereck, (ed.), Arctic Aeromedical Laboratory. 1962. pp. 1-44.

TERRESTRIAL ECOLOGY STUDIES

Included in this section are reports representing the three principal areas of our terrestrial ecology effort. Two reports deal with our Alaskan studies; four reports are concerned with animal life of the Hanford Reservation; and five reports relate to plant physiology.

The continuing accumulation of seasonal data on the ^{137}Cs content of Alaskan natives from the major caribou-consumer villages, together with similar data on components of the food chain, has provided a rather complete explanation of the movement of cesium in this ecosystem. Another year's intensive sampling should complete the broad picture. Although efforts to develop a computer model of this food chain were not entirely successful, the deficiencies in the model are themselves instructive in pointing out areas where a better understanding of the processes involved is required.

Of particular interest among the animal studies was the evaluation of dose-lethality relationships for whole-body irradiation in a quite sizable population of free-ranging wild mice. There has been much speculation concerning the applicability of data obtained in highly controlled laboratory environments to the more rigorous circumstances of a native habitat. In this particular experiment there was surprisingly little difference between the laboratory and field data.

The plant physiology reports include new information regarding the uptake by plants of alpha emitters associated with ^{239}Pu contamination events. This is timely with respect to recent contaminating events. Environmental studies deal with the general desert stress factor of drought as well as with the more localized stresses from pockets of salinity. While not a new interest, our research in environmental plant physiology was stimulated by the establishment by the AEC in 1967 of the Arid Lands Ecology Reserve, a 120-square-mile area of the Hanford Reservation which will be fenced and permanently set aside for studies of desert ecology.

PUBLICATIONS

Eberhardt, L. L. Book Review: "Restoring the Quality of our Environment," Report of the Environmental Pollution Panel, President's Science Committee. The White House, 1965. J. Wildlife Management, vol. 31, p. 846. 1967.

Eberhardt, L. L. Book Review: "Future Environments of North America," edited by F. Fraser Darling and John P. Milton. Natural History Press, Garden City, New York. 1966. J. Wildlife Management, vol. 31, pp. 391-393. 1967.

Eberhardt, L. L. "Some Developments in 'Distance Sampling'," Biometrics, vol. 23, pp. 207-216. 1967.

Eberhardt, L. L. Book Review: "Review of Scientific Aspects of Pest Control," A Symposium. National Academy of Sciences, National Research Council Pub. 1402. Washington, D.C., 1966. J. Wildlife Management, vol. 31, pp. 604-605. 1967.

Hanson, W. C. "Cesium-137 in Alaskan Lichens, Caribou, and Eskimos," Health Physics, vol. 13, pp. 383-389. 1967.

Rickard, W. H. "Onset of Winter Dormancy in Lizards and Beetles," Northwest Science, vol. 41, pp. 92-95. 1967.

Rickard, W. H. "Seasonal Soil Moisture Patterns in Adjacent Greasewood and Sagebrush Stands," Ecology, vol. 48, pp. 1034-1038. 1967.

ESKIMO FOOD CHAIN STUDIES

Body burdens of ^{137}Cs in Alaskan natives were generally lower during 1967 than during the previous year. During the period 1962-1966, ^{90}Sr levels in lichens increased until 1964 and then gradually declined; those in caribou bone peaked during 1965 and plateaued, while those in wolf bone continued to gradually increase.

Investigators:

W. C. Hanson
L. L. Eberhardt

Technical Assistance:

G. W. Johnson
W. P. Neal
H. A. Sweany
Dorothy D. Wade

Measurements of ^{137}Cs body burdens of residents at Anaktuvuk Pass, Arctic Village, and Ambler in northern Alaska were continued during 1967, using the same equipment as previously described (Annual Report, 1965). Continuous sampling of lichens, caribou, and carnivores at Anaktuvuk Pass has provided a comparison of several fallout radionuclides in the three trophic levels.

Observations

Four measurements of ^{137}Cs body burdens in native residents of Anaktuvuk Pass, Arctic Village, and Ambler were made between February and October 1967 (Table I). Values at Anaktuvuk Pass during spring and fall months were about 30-40% less than

during 1966; during the annual maximum (August), the values were about 10% less than 1 year ago. It now appears that 1966 summer values were unusually high, resulting from high values in caribou killed for summer

TABLE I. *Average Adult ^{137}Cs Body Burdens of Anaktuvuk Pass, Arctic Village, and Ambler Residents During 1967 (All values expressed as nanocuries)*

Month	Location		
	Anaktuvuk Pass	Arctic Village	Ambler
February	510	190	830
May	540	450	830
August	800	350	500
October	550	290	350

meat, and similar to the unusually high values during the summer of 1964. This was perhaps due to the animals having wintered on ^{137}Cs -rich ranges not previously grazed, and/or to greater-than-normal precipitation. During 1963 and 1965, several stations in northern interior Alaska reported precipitation totals substantially greater than average. Most of the ^{137}Cs deposited on lichens is held in the upper few centimeters of green, living material and thus could be removed by grazing caribou the winter following major deposition.

During 1967, the spring caribou migration occurred later than usual and much of the landscape at Anaktuvuk Pass was bare of snow. This made transport of caribou carcasses from the killing ground to the underground caches (~12 km) too arduous for many of the people, and the caribou meat consumption by the villagers was about two-thirds normal during the summer.

Cesium-137 concentrations in the people (nCi/kg body wt) during summer maxima have usually been slightly greater than the ^{137}Cs concentration in the flesh of spring-killed caribou (nCi/g std dry wt) forming their food base; however, during 1966 and 1967, they were 7 and 9% lower, respectively. Whether this represents a decreasing dependency of the people upon caribou for their main diet or whether it is mere coincidence is still conjecture; however, the acculturation of the people is, apparently, a factor in moderating their caribou utilization.

Cesium-137 concentrations in caribou flesh obtained at Anaktuvuk Pass

followed the usual seasonal pattern, consisting of (1) a relatively stable period during late winter; (2) an abrupt increase associated with the spring caribou migration from distant winter ranges; (3) an abrupt decrease in flesh of animals returning from summer ranges; and (4) a gradual increase in fall and early winter months as the caribou change from their summer diet of green plants to the lichen diet.

Strontium-90 concentrations in various trophic levels of the lichen-herbivore-carnivore food chain at Anaktuvuk Pass during the period 1962-1966 showed (1) an increasing trend in lichens until 1964 and a subsequent gentle decrease; (2) an increasing trend in caribou bone until 1965 and then a plateau through 1966; and (3) stable values near 10 pCi/g std dry wt in wolf bone during 1960-1963, followed by an abrupt increase during 1963 to about 30 pCi/g, from whence it has continued to climb very gradually. Most of the caribou sampled were 4-9 years old, which may account for the time lag between the trends in the animals and their principal source of ^{90}Sr .

Conclusions

Seasonal differences in ^{137}Cs body burdens of native residents of Anaktuvuk Pass and Ambler reflect their contrasting caribou utilization patterns and emphasize the importance of winter range food habits of caribou in determining the Eskimo body burdens. A recurrence of unusually high ^{137}Cs concentrations in caribou flesh and

subsequently in Anaktuvuk Pass Eskimos during 1966 was again associated with the spring caribou migration. This substantiates our previous observations of the need for more basic information about caribou migration patterns and radionuclide concentrations in their winter diets. Caribou utilization at

Arctic Village resumed a normal pattern following the 1966 aberration. Continued periodic measurements of ^{137}Cs body burdens in native residents at the three villages during 1968 is expected to complete the comparison of seasonal patterns at the major caribou-consumer villages.

COMPUTER SIMULATION OF ^{137}Cs IN AN ARCTIC FOOD CHAIN

A simple deterministic computer model of the lichen-caribou-Eskimo food chain was constructed. Initial work with the model emphasizes certain gaps in the available data.

Investigators:
L. L. Eberhardt
W. C. Hanson

This report covers preliminary efforts at constructing a simple deterministic model of the food chain passage of ^{137}Cs in an Alaskan ecosystem. Efforts thus far have been devoted to an elementary model and principally devoted to attempts at estimating the necessary initial concentrations and rates. Calculations employed the "DYNAMO" program developed at Massachusetts Institute of Technology, as described by Pugh.⁽¹⁾ We are indebted to Dr. G. J. Paulik (University of Washington) for an introduction to the DYNAMO program and for arranging for initial computer runs.

Observations

The basic structural unit employed was the difference equation corresponding to the differential equation commonly used in radionuclide uptake and retention studies:

$$\frac{dy}{dt} = \lambda - \mu y$$

where:

$y = y(t)$ = concentration (or burden) of given substance at time t .

λ = intake of substance (per unit time). This is often properly $\lambda(t)$, a variable function of time.

μ = excretion or loss rate,
usually assumed to be a
constant.

In the corresponding difference equation, the time unit (Δt) was taken as 1 day throughout the study. Loss rates were considered constant, while intake rates were varied with time, usually as a function of the radionuclide concentration in a lower level in the food chain.

Major components of the model are shown in Figure 1, and include the following relationships:

- 1) Fallout inputs following the monthly pattern observed at Fairbanks, Alaska (USAEC Health and Safety Laboratory data).
- 2) Retention on lichens corresponding to a 10-year half-time.
- 3) A seasonally varying fraction of lichen in caribou diet.
- 4) An excretion rate in caribou corresponding to a 30-day effective half-time.
- 5) Two annual caribou harvests (in spring and fall migration periods) by Eskimos and varying caribou storage rates.
- 6) An excretion rate by Eskimos corresponding to a 70-day effective half-life.

Figure 2 shows the time course of an initial simulation. The time scale in Figure 2 corresponds to a period beginning June 1, 1963. Since we are still in the process of developing and refining the underlying model, the actual dates and data have not been shown.

FIGURE 1. Outline of an Alaskan Food Chain System

Conclusions

Further computer runs are needed to more clearly define the effect of the different variables. The importance of chance factors such as caribou migration dates, weather, etc., is already apparent, and are no doubt responsible for a sizable discrepancy between observations and calculations in 1964. Also apparent is the need for additional knowledge bearing on what we have called "conversion factors," i.e., from lichens to caribou flesh, and caribou flesh to Eskimo body burdens.

Reference

1. Pugh, A. L. III. *Dynamo User's Manual, Second Edition*, The M.I.T. Press, Mass. Institute of Technology, Cambridge, Mass. and London, England. 1963.

FIGURE 2. Simulated ^{137}Cs Levels in an Alaskan Food Chain

EFFECTS OF ACUTE RADIATION IN NATIVE RODENTS

The $\text{LD}_{50/30}$ values for seven species of rodents exposed to an acute dose of γ -radiation were: *Microtus oregoni*, 651 rad; *M. miurus*, 846 rad; *Perognathus parvus*, 856 rad; C57BL X BALB hybrid *Mus*, 881 rad; *Peromyscus maniculatus*, 919 rad; *Peromyscus zeucopus*, 997 rad; *Perognathus zongimembris*, 1094 rad. There was no apparent relationship between radiation resistance and either taxonomic affinities or adaptations to stresses of rigorous environments. The radiosensitivity of *Microtus oregoni* may be related to its low chromosome complement.

Investigators:

T. P. O'Farrell
G. E. Cosgrove*

Technical Assistance:

J. D. Hedlund
W. P. Neal
T. F. Winsor

Previous data (Annual Report, 1966) indicated that two species of rodents

indigenous to the Hanford Reservation had $\text{LD}_{50/30}$ values higher than those reported for laboratory mice. The present study sought to determine the resistance of several other species

* Consultant Pathologist, Biology Division, Oak Ridge National Laboratory

of native rodents to test whether radioresistance is a general phenomenon among wild species, and, if it were, to describe the possible relationships with such parameters as taxonomic affinities, karyotype, and adaptations to the stresses of rigorous environmental conditions.

Deer mice, Peromyscus maniculatus, and the Great Basin pocket mouse, Perognathus parvus, are the two most abundant rodents on the Hanford Reservation; both are adapted to a desert-steppe environment. Singing voles, Microtus miurus, were chosen as an arctic-adapted species. Oregon or creeping voles, Microtus oregoni, were tested because they have one of the lowest chromosome complements among the placental mammals ($2n = 17$ or 18). The C57BL X BALB hybrid Mus musculus was used as a comparative laboratory species. White-footed mice, Peromyscus leucopus, were included to test the similarity of our experimental conditions with those of the Oak Ridge National Laboratory, where the species is native, as a means of justifying comparisons with their data for southeastern mammals. The least pocket mouse, Perognathus longimembris, was tested to see if we could confirm the previously reported high $LD_{50/30}$ value (1520 R).⁽¹⁾

Observations

All animals were housed singly and acclimated to laboratory conditions before exposure. Exposures were effected with a 10,000-Ci ^{60}Co source at a dose rate of approximately 600 rad/min. The $LD_{50/30}$ values obtained from probit transformations

of survival curves (Figure 1) were: Microtus oregoni, 651 rad (567-717, 95% confidence interval); M. miurus, 846 rad (789-900); Perognathus parvus, 856 rad (811-897); C57BL X BALB, 881 rad (832-922); Peromyscus maniculatus, 919 rad (885-953); Peromyscus leucopus, 997 rad (946-1057); Perognathus longimembris, 1094 rad (1061-1133). Histopathological findings indicated comparable damage at similar doses for all species. There was evidence of the hematopoietic mode of death with only transient damage to the gastrointestinal tract.

Conclusions

The similarity of the responses of Perognathus parvus, Peromyscus maniculatus, and Microtus miurus to that of a laboratory hybrid of Mus was unexpected in view of previous indications of higher resistance in native species as compared with Murid mice.⁽²⁾ In our studies, animals adapted to the environmental extremes of the arctic, the desert-steppe, and the research laboratory showed no marked differences in acute radiation response.

The estimated $LD_{50/30}$ for Perognathus parvus was significantly lower than that for Perognathus longimembris,⁽¹⁾ which suggests that relatively high resistance to radiation is not a characteristic of all members of the genus. However, we were not able to confirm the previously reported $LD_{50/30}$ value of 1520 R for P. longimembris.

The unusual sensitivity of Microtus oregoni is apparently unrelated

FIGURE 1. Survivorship Curves for Seven Species of Rodents (Curves fitted to values obtained by probit transformation. Sample sizes were 10-25 animals per point. The 95% confidence intervals around the mean middlelethal doses are bracketed.)

to either taxonomic affinities or environmental conditions. The most unusual aspect of this species is its unique karyotype. Sparrow et al.,⁽³⁾ have speculated that the high correlation between sensitivities of plants to radiation and their average interphase chromosome volume might hold true for animals as well.

References

1. Gambino, J. J. and R. G. Lindberg. Radiation Res., vol. 22, pp. 586-597. 1964.
2. Golley, F. B., J. B. Gentry, E. F. Menhinick, and J. L. Carmon. Radiation Res., vol. 24, pp. 350-356. 1965.
3. Sparrow, A. H., L. A. Schairer, and R. C. Sparrow. Science, vol. 141, pp. 163-166. 1963.

RADIATION EFFECTS IN FREE-RANGING POCKET MICE

The response to radiation of free-ranging Perognathus parvus was similar to that observed for the same species in the laboratory. There was some evidence of a reduction in recapture success due to an interaction between radiation and environmental stresses at 600, 700, and 800 rad. Mice survived both depilation and subsequent replacement by white or gray hair without apparent increased mortality.

Investigator:

T. P. O'Farrell

Technical Assistance:

J. D. Hedlund

W. P. Neal

T. F. Winsor

Most studies of the effects of ionizing radiation in native mammals have been conducted either in laboratories or fenced enclosures. Such conditions are considered to be unnatural, and the results obtained might not be applicable to free-ranging mammals. Animals in their natural habitat might be more resistant to radiation because they were in a familiar environment, or they might exhibit a lowered resistance due to synergistic effects of radiation and environmental stresses. The objective of this experiment was to determine the survival of free-

ranging pocket mice exposed to acute radiation, and to compare the results with data obtained for the same species under laboratory conditions.

Observations

A group of 193 Perognathus parvus were live-trapped on a 4-hectare study area near Rattlesnake Springs. Animals were transported to the laboratory in ventilated metal cylinders containing mixed seeds. They were weighed, toe-clipped for identification purposes, assigned to an exposure level by lot, and irradiated with a

10,000-Ci ^{60}Co gamma source at a dose rate of about 600 rad/min. Absorbed doses were between 500-1100 rad, with 25 mice in each exposure group except for the 500 rad group which had 18 animals. After treatment, the mice were released at the point of capture. Because of the logistic problems involved in the irradiation, the animals were in cans for at least 15-18 hr.

Live-trapping was conducted throughout the night at 14, 21, and 28 days postirradiation. Trapping was not begun earlier because laboratory studies had indicated that 98%

of the decedents would not survive longer than 14 days and we did not want to superimpose trap stress during this critical period. Captured animals were weighed, and abnormalities were noted before they were released at their capture points shortly after sunrise.

Recapture data are shown in Figure 1. These are considered synonymous with survival data since intensive trapping of pocket mice will sample virtually the entire population. There was a sharp reduction in the number of animals recaptured 14 days postexposure at all levels including

FIGURE 1. Recapture Data for Free-Ranging *Perognathus parvus* Exposed to Acute Doses of Ionizing Radiation and the Survivorship Curve Fitted to Laboratory Data for the Same Species. (The 95% confidence interval around the mean midlethal dose is bracketed.)

the controls. Since subsequent losses in the control and 500 rad group were much smaller, it was speculated that the long confinement, disorientation during two road trips of 30 miles each, plus the stress of being released during the hottest part of the day might have been largely responsible for this initial loss. There was no large difference between the number of animals recaptured at 14 days postirradiation in the 0, 500, and 600 rad animals, but there was an inverse relationship between recapture success and increasing dose from that point. This would agree with the curve fitted to the laboratory data (Figure 1) which indicated that the threshold dose for Perognathus parvus, i.e., dose effecting 5% mortality, is about 675 rad.

From 14-28 days postirradiation, there was a similar drop in recapture success of about 4% for both controls and the mice exposed to 500 rad. The recapture success of animals exposed to 600, 700, and 800 rad dropped 12-16% during this same interval. These increased losses could be due to an interaction between radiation and environmental stresses.

Data from the 900, 1000, and 1100 rad exposure groups fell on the tail of the laboratory survival curve, indicating that most of the losses at these high doses could probably be ascribed to radiation-induced mortality. Further experimentation and

statistical analyses must be completed to test the validity of these inferences.

All irradiated mice showed depilation, including, in some instances, a total loss of hair. The replacement pelage was gray to white, rendering the animals quite conspicuous. It was speculated earlier (Annual Report, 1967) that these pathologies might constitute an important mortality factor, as the loss of hair might upset a part of the hygrothermal regulatory capabilities, and the regrowth of lighter hair might make the animals more obvious to predators. Since the animals irradiated at 500 rad exhibited both effects without influence on mortality, the original hypothesis does not appear to be valid. Several white animals are still alive 4 months postirradiation.

Conclusions

The threshold and supralethal doses for free-ranging Perognathus parvus exposed to an acute dose of ionizing radiation were similar to those obtained for the same species in the laboratory. There was some evidence of losses due to an interaction between radiation and environmental stresses at 600, 700, and 800 rad. Depilation and subsequent replacement by white to gray hair had no apparent effect on mortality.

THE RETENTION OF ^{137}Cs BY DARKLING BEETLES

Retention of ^{137}Cs following a single ingestion was measured in darkling beetles over a 28 day period. About 98% of the ^{137}Cs was lost with a half-time of 1.0 day at 90 °F, and 1.5 days at 74 °F.

Investigators:
W. H. Rickard
T. E. Hakonson

Darkling beetles are an important consumer of vegetation in the natural habitats of the desert steppe regions of southeastern Washington. One of the larger-sized and ubiquitous species is *Eleodes hispilabris*. Although there is much published information concerning the retention of ^{137}Cs in vertebrate animals, especially mammals, there is less information concerning its retention in terrestrial insects.

Observations

Beetles caught in the field were adapted to a laboratory diet of dry oatmeal flakes and to constant temperatures of 74 and 90 °F. Individual beetles from both temperature regimens were fed 0.5 μCi of ^{137}Cs on an oatmeal flake and thereafter periodically counted to record the whole-body retention of ^{137}Cs . The oatmeal diet and temperature regimens were maintained throughout the 28-day duration of the experiment.

Retention curves of ^{137}Cs are shown in Figure 1. These curves have two distinct components--a rapid loss component that accounted for about 98% of the initial burden and a slow loss or persistent component. At 74 °F the biological half-time for

FIGURE 1. Retention of ^{137}Cs in *Eleodes hispilabris* at 74 and 90 °F

the rapid loss component was 1.5 days, and for the persistent component, 7.4 days. At 90 °F the corresponding half-times were 1.0 and 24 days.

The clearly defined two component structure of the retention curves suggested that tissues with widely

different rates of mineral exchange were contributing to the total curves.

Samples of gut, muscle, and exoskeleton were taken for analysis only at the conclusion of the experiment and contained, per gram of oven-dried tissue, an average of 0.48, 1.41, and 1.50%, respectively, of the initially ingested ^{137}Cs . Assuming that 60% of the oven-dried weight of an adult beetle is comprised of exoskeleton, it was calculated that at least 70% of the persistent component was present in the exoskeleton.

During the early phases of the study, cesium was eliminated more

rapidly by beetles on the 90 °F temperature regimen, probably because of increased metabolic activity of soft tissue. On the average, the 90 °F beetles ate 40% more food than the 74 °F beetles.

Conclusions

Initial uptake of ^{137}Cs by E. hispilabris is essentially complete. Elimination of 98% of the deposited cesium is rapid, the rate being dependent on ambient temperature. Only a small amount of the initial burden is more tenaciously retained in the exoskeleton.

THE INFLUENCE OF WILDFIRE ON GROUND DWELLING BEETLES

Wildfire is not an effective factor in reducing populations of ground dwelling beetles.

Investigator:

W. H. Rickard

Technical Assistance:

T. E. Hakonson

G. W. Johnson

Fire is sometimes used in the management of desert steppe lands to economically eradicate shrubs and to stimulate the growth of livestock forage plants. The impact of fire damage on the animal components of these plant communities is seldom considered. This paper presents the results of a year-long pitfall trap survey of ground dwelling beetles in adjacent burned and unburned desert steppe vegetation on the Hanford Reservation.

Observations

Trapping was begun in the autumn of 1966. A total of 30 cans were buried to their rims at three widely separated sites. At each site five can traps were placed in a stand of burned native vegetation and five were placed in adjacent unburned vegetation.

Vegetation in the areas of the study is dominated by big sagebrush, Artemisia tridentata and bitterbrush,

Purshia tridentata. This is a widespread vegetation-type on the Hanford Reservation. The understory is comprised mostly of a sparse stand of grasses, particularly cheatgrass brome, Bromus tectorum and Sandberg bluegrass, Poe secunda. The percentage of ground covered by the shrub canopies as measured on a 20 x 50 meter plot at each unburned study site was 8.6, 18.6 and 20.5%, respectively.

Four species of adult ground dwelling beetles comprised almost all of the trap catch. These were Eleodes hispilabris, Eusattus muriaius, Pelecyporus densicollis (Tenebrionidae) and Calosoma luxatum (carabidae). Eusattus and Calosoma were most active in late winter and early spring. The trap catch declined as summer approached and these beetles became inactive in late summer and autumn (Figure 1). Eleodes hispilabris became active in early spring; the catch declined in summer and autumn but beetles remained active until November. Pelecyporus was active only for a short period in autumn.

Conclusions

The total number of beetles caught in burned and unburned areas was similar (Table I). It was concluded that populations of ground dwelling beetles typical of a desert steppe community are not adversely affected by wildfire.

In this region fires are most apt to occur in summer because of the very dry condition of aestivating plants. Such fires are not effective in

FIGURE 1. The Weekly Catch of Ground Dwelling Beetles in Burned and Unburned Vegetation - 1967

killing adult beetles because they are not active on the soil surface, and larvae are buried in the soil. Wildfire is not effective in reducing

TABLE I. Comparison of the Yearly Catch of Beetles October, 1966, to October, 1967, in Burned and Unburned Native Vegetation.

Taxa	Burned Sites				Unburned Sites			
	1	2	3	Total	1	2	3	Total
<u>Pelecyporus densicollis</u>	54	48	100	202	26	30	101	157
<u>Calosoma luxatum</u>	130	6	45	181	132	21	30	183
<u>Eleodes hispilabris</u>	108	61	110	285	139	93	118	322
<u>Eusattus muratus</u>	495	27	122	644	558	25	116	699
TOTAL	787	146	377	1312	855	168	367	1389

the long-term food supply because grass roots are not destroyed by fire. The recovery of grasses is rapid enough so that food sources are renewed as soon as autumn rains break the dormancy of perennial grasses and initiate germination of

the seeds of the annual cheatgrass brome. The food of the predaceous beetle Calosoma is not seriously affected by wildfire because they probably prey on the larvae of the other beetles.

PSYCHROMETRIC DETERMINATION OF WATER POTENTIAL OF DESERT PLANTS

The thermocouple psychrometer was used for determining leaf water potential for a few common desert plants.

Investigator:
J. J. C. Hsieh

The energy status of water in a plant-soil-atmosphere system is best described in terms of water potential, which is an expression of the capacity of this water to do work, as compared to the capacity of an equal mass of free water at the same location and temperature. The potential of plant

water in an isothermal condition is expressed as:

$$\psi_{\text{plant}} = \psi_s + \psi_m + \psi_p$$

where ψ_s is the potential due to solutes, ψ_m is the matrix potential due to adsorption, and ψ_p is the

hydrostatic pressure from such sources as the water column and friction drag in transpiring plants.

If the effects of the above forces are expressed through their influence on water vapor pressure, and assuming water vapor is an ideal gas, then

$$\Psi_{\text{plant}} = R T \ln (p/p_0)$$

where R is the ideal gas constant, T is the absolute temperature, p is the vapor pressure of water in the system at temperature T , and p_0 is vapor pressure of free water at temperature T . It is possible to evaluate p/p_0 with T known, and thus to determine the water potential in the portion of the system which is of interest, i.e., leaf, soil, etc. The units of Ψ_{plant} can be dynes/cm², or bars, or atmospheres, all of which are interconvertible with ergs/g.

Equipment for making psychrometric measurements was obtained during the summer and leaf water potentials of a few desert plants were measured in September and November, 1967.

Observations

Leaf samples from plants in the vicinity of the Rattlesnake Springs Laboratory were collected, quickly put into a sample changer⁽¹⁾ housing a microthermocouple, and the assembly placed in a water bath at 25 °C. After allowing the leaf to come to equilibrium with its environment, a current was passed through the chromel-constantan thermocouple^(2,3) and, by Peltier cooling, a film of

moisture condensed onto the thermocouple. On cutting the current, the measured thermocouple EMF, as compared with the dry reference junction, gives a measure of relative humidity of the tested sample. Standard reference curves relating water potential with EMF were previously obtained from standard KCl solutions.

Table I shows leaf water potentials obtained from leaves of four woody species taken from the same plants or from the same area in the morning and afternoon of September 21, 1967, a typically sunny day. Since ambient atmospheric conditions in the afternoon favored an increased transpiration, the internal water stresses or water potentials were

TABLE I. Comparison of Leaf Water Potentials of Desert Plants in the Morning and Afternoon

	Water Potential, atm	
	8:30 a.m.	1:15 p.m.
	66 °F RH 54%	88 °F RH 29%
Sagebrush	44	55
<u>Artemisia tridentata</u>		
Greasewood	33	38
<u>Sarcobatus vermiculatus</u>	31	35
Rabbitbrush	26	32
<u>Chrysothamnus nauseosus</u>		
Willow	20	28
<u>Salicaceae spp.</u>		
25 atm KCl control	24.5	24.5

higher in afternoon than in morning leaf samples, as shown in Table I.

Leaf water potentials measured September 25 and November 13, 1967, are compared in Table II. Leaf samples of a given species were collected from the same plant with exception of the salt grass which was collected from the same area. The decreases in water potential between these dates were probably due to late fall rains, falling temperature, and increasing relative humidity.

Conclusions

The water energy status of plants can be inferred from thermocouple psychrometer measurements. The water potentials of a few common desert plants were measured and values for diurnal as well as seasonal changes were obtained. The results are in accord with expectations and testify to the usefulness of this technique in identifying short-term physiological plant responses as well as long-term factors of phenology.

TABLE II. *Comparison of Leaf Water Potentials of Desert Plants in September and November*

	Water Potential, atm	
	Sept. 25 9:00 a.m. 73 °F RH 40%	Nov. 13 9:00 a.m. 49 °F RH 76%
Sagebrush	60	43
<u>Artemisia tridentata</u>	62	45
Greasewood	44	37
<u>Sarcobatus vermiculatus</u>		
Rabbitbrush	33	6
<u>Chrysothamnus nauseosus</u>		
Salt grass	94	40
<u>Distichlis stricta</u>		
50 atm KCl control	50.5	--

References

1. Campbell, G. S., W. D. Zollinger, and S. A. Taylor. *Agronomy*, vol. 58, pp. 315-318. 1966.
2. Spanner, D. C. *J. Exptl. Botany*, vol. 2, pp. 145-168. 1951.
3. Rawlins, S. L. *Agri. Meteorol.*, vol. 3, pp. 293-310. 1966.

THE RELATIONSHIP OF WATER POTENTIAL AND WATER CONTENT IN A LOCAL SOIL

The relationship between soil water potential and water content was determined for a local sandy loam soil.

Investigator:
J. J. C. Hsieh

Availability of soil water to plants is more closely related to water potential energy than any other single factor. With the development of thermocouple psychrometer techniques, soil water potentials can be measured. Increasing use will be made of such potential values as a parameter important to plant survival in arid areas. The present study was designed to relate previously collected water content measurements with the energy values of the soil water in question.

Observations

A composite soil sample was taken to a depth of 60 cm in the vicinity of the soil temperature station, south of the Rattlesnake Springs Laboratory. Roots of the greasewood and cheatgrass vegetative cover were removed from the sandy loam soil by passing it through a 1 mm sieve. Known amounts of water were added to soil samples in air-tight containers, and equilibrated first in a 50 °C oven for 1 day, then at a room temperature of about 20 °C for at least 4 days before water potential measurements were made. The soil samples were shaken many times during the period of equilibration.

Soil water potential measurements were made with a chromel-constantan thermocouple psychrometer in a sample changer as described in the previous report. Soil water contents were measured gravimetrically. The relationship between the two sets of data is shown in Figure 1.

Conclusions

The availability of soil water to plants is best measured as water potential energy in units of bars or atmospheres. According to the conventional concept of "permanent

FIGURE 1. *Sorption Curve Showing Relationship Between Moisture Content and Energy Potential of Water in a Composite Soil Sample from Rattlesnake Springs*

wilting point" (pwp), plants are unable to absorb sufficient water to maintain turgidity and growth when soil water potential reaches about

15 atm. The pwp for the soil tested is, thus, equivalent to a water content of about 8%.

ABSORPTION OF $^3\text{H}_2\text{O}$ AND ^{22}Na FROM SALINE SOIL LAYERS BY GREASEWOOD

*The tracers ^{22}Na and $^3\text{H}_2\text{O}$ have shown that sodium and water from saline soil layers enter into greasewood, *Sarcobatus vermiculatus*. Root stratification at boundaries between saline and nonsaline soil layers appears to be related to the availability of water at these sites.*

Investigators:
O. Van der Borgh^{*}
F. P. Hungate

Greasewood is a desert shrub characterized by high sodium content. It is considered an indicator of high soil salinity and of a high water table. Older plants are known to draw water from depths as great as 10 m below the soil surface. The question arises as to why the sodium content of leaves is about 10% of dry leaf weight. Does this accumulate from evaporation of absorbed groundwater that contains only about 12 ppm sodium, or is there uptake from the high salinity soil layers through which the roots pass? This report includes some very preliminary observations of the fate of radioactively labeled sodium and water introduced into strata of high

salinity and at locations where strong salinity gradients occur.

Observations

The distribution of rootlets was observed to be clearly related to planes of contact between saline and nonsaline layers. In upper soil levels, rootlets were located in nonsaline soil immediately overlying saline strata. A similar pattern was most frequently observed at deeper levels, though in some instances high rootlet concentrations were observed just below or lateral to the high (18-20%) moisture saline strata; but always the high rootlet concentrations occurred in soil of lower salinity. Only tap roots or very sparse rootlets were observed in the high salinity soil strata. Such a distribution pattern is consistent with the suggestion of

^{*} On leave from S.C.K., C.E.N., Mol, Belgium on a NATO Fellowship, May 5 - July 24, 1967.

Boyko⁽¹⁾ that soil moisture moves in the vapor phase in sandy soil and provides fresh water to root surfaces. Table I gives typical conductance values for soil obtained on both sides of observed root planes. Conductances were measured on a soil paste with brass electrodes.

For studies of ^{22}Na and $^3\text{H}_2\text{O}$ uptake, stainless steel needles 15 cm long, with holes in the terminal 3 cm, were used to inject 2 ml of solution into a saline soil layer at a depth of 5 cm below the mat of rootlets. This volume of solution had been previously observed to affect the moisture concentration of a soil volume approximately 3 cm in diameter. Needles were inserted 5 days prior to injection to permit healing of any damaged rootlets, though rootlet density was very low

in the saline layer and probability of damage was correspondingly slight. In the experiment to be described, 46.5 μCi ^{22}Na and 709 μCi $^3\text{H}_2\text{O}$ were slowly injected through four needles. Samples of greasewood plants growing above the injection site were collected at intervals for ^{22}Na and ^3H assay by liquid scintillation counting following sample incineration in an oxygen-filled combustion flask. Soil samples were taken at the conclusion of the experiment.

Figure 1 shows the results obtained for a single greasewood plant. While little quantitative significance

TABLE I. Conductance of Soil Samples on Both Sides of Root Planes of *Sarcobatus vermiculatus*

Conductance, $\mu\text{mhos/cm}$ (a)		Conductance Ratio
High Salinity Layer	Low Salinity Layer	
6,000	200	0.03
25,000	2,000	0.08
4,000	200	0.05
11,000	400	0.04
240	160	0.67
6,000	1,000	0.17

(a) 1000 $\mu\text{mhos/cm}$ equivalent to 7.3 meq NaCl/liter

FIGURE 1. Uptake and Retention of ^{22}Na and $^3\text{H}_2\text{O}$ by Twigs and Leaves of Greasewood Following Addition of Radionuclides to Saline Soil Stratum

can be attached to these preliminary observations, the consistent uptake of both ^{22}Na and $^3\text{H}_2\text{O}$ into the plant shows that water and sodium accumulation does occur from these soil strata between groundwater and soil surface. This finding is consistent with a rough estimate of sodium which might be derived from groundwater. Tests using cooled plastic enclosures about branches of greasewood indicate transpiration rates of about 4 g water/g dry leaves/day. Assuming this rate of transpiration to occur for 300 days and the source of water to contain 12 ppm of sodium, this would produce sodium concentrations in the vegetation amounting to less than 10% of that actually observed. Thus, another source of sodium, such as that identified by these ^{22}Na studies, would be required.

Initial higher concentrations of $^3\text{H}_2\text{O}$ in twigs (including leaves) give way to higher concentrations of ^{22}Na by the thirteenth day. Whether

this is a consequence of $^3\text{H}_2\text{O}$ loss by transpiration or to slower transfer of ^{22}Na through conducting tissue is not clear. Soil samples obtained on the fourteenth day showed the presence of ^{22}Na and $^3\text{H}_2\text{O}$ at least 7 cm above and below the injection site, the ^{22}Na concentration being higher below the injection site and the $^3\text{H}_2\text{O}$ concentration being higher above the injection site.

Conclusions

The results of these very preliminary studies can only be said to establish the feasibility of an experimental approach, and to identify some of the factors that must be controlled in future modifications of this approach.

Reference

1. Boyko, H. Scientific American, vol. 216, no. 3, pp. 89-96. 1967.

EFFECT OF SAGEBRUSH DISTILLATE ON THE GERMINATION OF CHEATGRASS SEED

Distillate from sagebrush leaves inhibited germination of cheatgrass seed. The effect was reversible and was not exhibited by a rabbitbrush distillate.

Investigator:
J. D. Davis

While collecting material for a soil-microflora relationship study, it was observed that some areas immediately surrounding well established sagebrush (Artemisia tridentata Nutt.) were devoid of plant life. Cheatgrass (Bromus tectorum L.) is the herbaceous species usually surrounding the A. tridentata stand. The essential oils of A. tridentata are known to have an effect on the life processes of some microorganisms; it was conjectured that they might affect the germination of B. tectorum seeds.

Observations

One hundred-gram samples of sagebrush and rabbitbrush (Chrysothamnus spp.) foliage were distilled in deionized water for 6 hr. Two milliliters of the approximately 200 ml of collected distillate were pipetted into a petri dish containing five seeds of B. tectorum placed between two filter papers. The dishes were incubated at 22 °C and germination scored from the fourth to the eighth day (Table I). After the eighth day, ungerminated seeds were removed from the petri dishes and returned to the

incubator in water-saturated filter paper. Within 3 days, 84% of these seeds had germinated and were developing at a normal rate.

Conclusions

The distillate obtained from A. tridentata foliage, in contrast to that obtained from Chrysothamnus spp., was clearly inhibitory to germination of B. tectorum seeds. Depending upon the results which may be obtained from microflora studies, a more thorough investigation of such inter-organism effects may be warranted.

TABLE I. *Effect of Sagebrush and Rabbitbrush Distillates on Germination of Cheatgrass Seeds (Average percent germination for five replications)*

Treat- ment (a)	Incubation Period. days				
	4	5	6	7	8
RBD	96	100	100	100	100
SBD	0	0	0	0	0
DW	96	96	100	100	100

(a) RBD - Rabbitbrush distillate
SBD - Sagebrush distillate
DW - Distilled water

UPTAKE OF ^{241}Am AND ^{239}Pu BY PLANTS

Concentrations of ^{241}Am were 20-30 times greater than those of ^{239}Pu in plant tissues grown on substrates containing equal amounts of the radioisotopes. Americium was more toxic than plutonium to growing roots. It was bound tightly to soil particles but showed some movement with irrigation water, especially in basic soils.

Investigator:
J. F. Cline

Measuring the gamma of ^{241}Am , usually present as a contaminant, has been suggested as a practical means of externally monitoring the movement of ^{239}Pu in biological systems. To realize such a tracer application, the relative uptake and distribution of the two radioisotopes by biological systems must be established. This study sought to determine something of their relative behavior in plants and soils.

Observations

Plant uptake rates were determined for beans grown in standard Hoagland's solution, and for barley grown in Cinebar (pH 4.5) and Ephrata (pH 7.5) soils, using the Neubauer technique. Plutonium was added as $^{239}\text{Pu}(\text{NO}_3)_4$ at concentrations of 1.0 $\mu\text{Ci/liter}$ of nutrient solution, or 10 $\mu\text{Ci/g}$ of soil in the Neubauer study. Americium was added as a nitrate at concentrations of 0.9 $\mu\text{Ci/liter}$ of nutrient solution, or 1.8 $\mu\text{Ci/g}$ of soil in the Neubauer study. Radionuclides were added to the substrates either as a pure solution of ^{241}Am (>99% by activity) or as a solution con-

taining, by activity, 2.9% ^{238}Pu , 95.1% ^{239}Pu , and 2.0% ^{241}Am .

Americium-241 concentrations were determined by direct counting of the gamma activity in the tissues with a NaI crystal and scintillator. Plutonium was chemically separated from the ^{241}Am and counted on a liquid scintillator. Results are shown in Table I as a ratio of the activity per gram of oven-dried tissue over the activity per gram of oven-dried soil, or milliliter of solution. Uptake of americium was 20-30 times greater than that of plutonium.

TABLE I. Plant Uptake of ^{241}Am and ^{239}Pu

Isotope	Leaf/Substrate Ratios		
	Beans in Nutrient Solution	Barley in Soil Cinebar	Ephrata
^{241}Am	0.003	0.003	0.002
$^{241}\text{Am}(\text{a})$	0.003	0.003	0.003
^{239}Pu	0.00006	0.0002	0.0001

(a) From the 2% ^{241}Am contaminant in the ^{239}Pu solution

Table II shows results of experiments in which americium placed on a soil column was leached with a 100-in. equivalent of irrigation water. There was little penetration of the acid (Cinebar) soil, but quite significant movement in the basic (Ephrata) soil. More rapid movement in basic soils has been observed previously with radionuclides, such as ^{185}W , that can function as anions.

Americium was toxic to the roots of pea seedlings grown in 0.1 mCi ^{241}Am /liter. The seedling radicles elongated approximately 1 cm following treatment, but no secondary roots were formed. No mitotic figures were observed in the root tissue grown in ^{241}Am solutions. Seedlings grown in solutions containing the same activity level of ^{239}Pu showed somewhat restricted cell division after a 24 hr treatment period, but secondary roots were formed of near normal appearance. This apparent greater toxicity of americium may reflect solubility differences occasioned by the approximately 50-fold greater mass of ^{239}Pu involved in equal-microcurie experiments. However, in view of the soil mobility data, it seems more likely that these two elements are behaving in a chemically different manner such that americium is more readily taken up and thus is able to exert a greater toxicity.

TABLE II. Penetration of ^{241}Am into Soil Columns with Irrigation Water

Depth of Soil Layer, in.	Percent of ^{241}Am in Soil Layer	
	Ephrata Soil	Cinebar Soil
0-1/2	76.5	98.2
1/2-1	2.4	1.5
1-2	2.4	T(a)
2-3	4.4	T
3-4	4.5	T
4-5	6.0	T
5-6	3.7	T
6-7	0.2	T
7-8	T	T

(a) $T = \text{less than } 0.1\%$

Conclusions

In plant systems, ^{241}Am cannot be relied upon as a tracer for ^{239}Pu , at least at the concentration levels employed in these experiments. The much greater uptake of ^{241}Am as compared with ^{239}Pu may cast doubt on previous studies of plant uptake of ^{239}Pu where the radionuclide was perhaps not fully characterized as to americium contamination. These preliminary observations suggest the need for definitive studies to clarify the comparative behavior of americium and plutonium in plant-soil systems.

AQUATIC ECOLOGY

The past year saw a great deal of public discussion of thermal pollution problems in the Columbia River. This interest in the establishment of future control limits on heat additions to the river happily coincided with the fruition of many years of study in our laboratory of temperature effects on Columbia River organisms. The first four reports in this section are concerned directly with this problem. The following two reports on the spawning of salmon near Hanford and the incidence of columnaris disease in the Columbia River are also related to the evaluation of temperature problems. While these studies, and others that have preceded them, cannot be said to completely define the problem of thermal pollution in the Columbia River, they do provide a large measure of assurance that past Hanford practices have not grossly affected the ecology of the river; they provide an indication of temperature levels that might be expected to be deleterious; and they suggest several new approaches to the study of these problems that should be pursued if realistic control limits are to be more precisely defined.

An intensive investigation of seasonal effects on radionuclide concentrations in Columbia River organisms was described in some detail in last year's Annual Report. This investigation was concluded during 1967, but the data are still being processed and evaluated. A separate report will be issued in 1968. Several reports are included on specific aspects of radionuclide cycling problems, most of them concerned with ^{65}Zn .

Important progress is being made in the interpretation of both temperature effect and radionuclide cycling data by using computer-derived mathematical models.

As in the past, we have included in this section reports on programs closely related to those of the Division of Biology and Medicine, but which receive funding from the Division of Production. These are indicated by footnotes to the titles.

PUBLICATIONS

Buhler, D. R. "Localization of Zn-65 in Highly Pigmented Tissues of Fishes," Federation Proc., vol. 26, p. 524. 1967. (Abstr.)

Cushing, C. E. "Concentration and Transport of ^{32}P and ^{65}Zn by Columbia River Plankton," Limnology and Oceanography, vol. 12, pp. 330-332. 1967.

Cushing, C. E. "Periphyton Productivity and Radionuclide Accumulation in the Columbia River, Washington, U.S.A.," Hydrobiologia, vol. 29, pp. 125-139. 1967.

Fujihara, M. P. and R. E. Nakatani. "Cold and Mild Heat Marking of Fish," Progressive Fish Culturist, vol. 29, pp. 172-174. 1967.

Pauley, G. B. "A Tumorlike Growth in the Foot of a Freshwater Mussel (*Anodonta californiensis*)," J. Fish. Res. Bd. Canada, vol. 24, pp. 679-682. 1961.

Pauley, G. B. and R. E. Nakatani. "The Uptake of the Radioisotope ^{65}Zn by Various Tissues of the Freshwater Mussel, *Anodonta californiensis* Lea," Proc. National Shellfisheries Association, vol. 57, pp. 7-8. 1967.

Pauley, G. B. and W. J. Clarke. "Preparation of Oyster and Freshwater Mussel Tissue for Histological and Histochemical Examination," The Progressive Fish Culturist, vol. 29, p. 149. 1967.

EFFECT OF ELEVATED TEMPERATURES ON MORTALITY AND GROWTH OF YOUNG
CHINOOK SALMON*

Chinook salmon spawned between October and December were reared under seven temperature regimes, each following the Columbia River's seasonal pattern, but differing by 2 °F increments. Temperatures more than 4 °F above normal river temperature resulted in excessive mortality in the October spawn, but only nominal mortality resulted from a 22 °F increase in the December spawn. All lots showed faster growth in warmer waters.

Investigators:

P. A. Olson

R. E. Nakatani

Technical Assistance:

C. O'Malley

R. E. Darrington

A local race of fall chinook salmon, Oncorhynchus tshawytscha, spawns in the Priest Rapids-Hanford reach of the Columbia River where the eggs and young may be exposed to warm reactor effluent. The temperature response of the young of this local chinook was measured in terms of mortality and growth. The data obtained should help to determine the quantity of heat which can be added to the river without significant damage to young salmon.

Observations

Four pairs of ripe chinook salmon provided by the Washington State De-

partment of Fisheries were spawned on October 10, November 14 and 23, and December 8, 1966. Approximately 5000 fertilized eggs obtained from each pair were divided into seven lots and exposed to the seven different temperature regimes illustrated in Figure 1. The reference temperature was that of the river water furnished to our hatchery; this water was heated or chilled to obtain the 2 °F intervals. The coldest temperature treatment closely approximated average river temperatures at Priest Rapids Dam located upstream from Hanford.

Table I summarizes the mortality and growth data for the seven lots from each spawn. In the lots from the October spawn, high egg mortality

* *This study supported by funds from the AEC Division of Production*

TABLE I. Mortality and Growth Summary

Date Spawned		10/30/66		11/14/66		11/23/66		12/8/66	
Date Terminated		4/24/67		5/9/67		5/18/67		6/2/67	
Lot	Temperature Condition	% Mor- tality	Mean wt, g	% Mor- tality	Mean wt, g	% Mor- tality	Mean wt, g	% Mor- tality	Mean wt, g
1	Control ^(a)	4.6	0.95	10	0.63	5.5	0.64	4.8	0.63
2	+2 °F	3.6	1.40	11	0.96	9.3	0.97	5.5	0.97
3	+4 °F	11	1.92	16	1.53	12	1.37	7.5	1.42
4	+6 °F	28	2.72	19	2.55	9.4	1.96	7.3	1.79
5	+8 °F	60	4.28	17	4.17	6.9	2.61	17	2.31
6	+10 °F	97	--	43	5.52	10	3.82	14	3.18
7	+12 °F	100	--	93	5.65	37	5.28	12	4.24

(a) Similar to temperature at Priest Rapids Dam

FIGURE 1. Experimental Trough Temperatures (Plotted at 5-day intervals)

occurred at the warmest temperatures, but even in these lots, there was considerable development of the embryo and most of the deaths occurred just prior to hatching. Lots with excessive egg mortalities also experienced greater mortality during the fry stage (from hatch to start of active feeding). In the cooler but still adverse treatment lots from the early spawn, egg or fry mortality was not obviously greater, and death occurred during the critical transition period of early active feeding. The ability to assimilate food in the new digestive tract may be the important factor for survival. Once the fish adapted to feeding, mortalities became spasmodic and inconsequential in all lots.

Increased temperature clearly accelerates growth. Fish in the warmest lots were as much as eight times

heavier than those in the coldest lots at the conclusion of the observation period (Table I).

Conclusions

Temperature increments exceeding 4 °F above the base river temperature caused excessive mortality in the October spawn of chinook salmon, but the December spawn tolerated an incremental addition of as much as 12 °F without significant mortality. A 2 °F increment in temperature resulted in increasing the body weight by approximately a factor of 1.4.

These results not only indicate that more heat can be added during winter without significant detrimental effect, but also imply that warmer water will favor the later spawners of the fall run chinook salmon population.

LETHAL TEMPERATURE RELATIONS OF JUVENILE COLUMBIA RIVER CHINOOK SALMON

The median resistance time of juvenile salmon to acute temperature stress was not affected by the developmental temperature regime, and size did not influence the times to death. There was a correlation between the time to loss of equilibrium and time to death. Eighty-five percent of the juveniles that lost equilibrium with thermal shock died, even if returned to nonlethal temperatures, and the duration of exposure to lethal temperature was a critical factor for survival.

Investigators:

J. M. Dean
C. C. Coutant

Technical Assistance:

R. G. Genoway

Fish which migrate and spawn in areas which receive heated effluent plumes can experience the high ambient

temperatures of the plumes during upstream and downstream migration and during development. We have studied

several related questions concerning lethal temperature relations of juvenile chinook salmon, a locally spawning species. These were:

- (1) How does the resistance to thermal shock of Priest Rapids stock chinook compare with stocks studied by others?
- (2) Does the developmental temperature regime affect the response of juvenile chinook to acute thermal shock?
- (3) Does fish size affect the response to acute thermal shock?
- (4) What are the relationships between the time to loss of equilibrium and times to death under thermal stress?
- (5) What is the relationship between times to death at continuous exposures to lethal temperatures and percent mortality at various durations of exposure to lethal temperatures?

Observations

Comparison of Thermal Resistance of British Columbia and Priest Rapids Juvenile Chinook Salmon

Times to death at various exposure temperatures of 50% of an exposed population of fall-run chinook salmon from Priest Rapids on the Columbia River are compared in Figure 1 with data for spring-run juveniles from British Columbia.⁽¹⁾ Data on the British Columbia fish did not extend to test temperatures above 27 °C. Somewhat greater heat tolerance was exhibited by the British Columbia fish. This apparent discrepancy is presumably due to racial differences inherent in the two stocks.

FIGURE 1. Comparison of Thermal Resistance Data on British Columbia Spring-Run Chinook and Columbia River Fall-Run Chinook (Each point represents a mean for 10 fish.)

Effect of Developmental Temperature

To determine the effect of developmental temperature on thermal sensitivity, fish reared under conditions described in the previous report in this volume were employed. These fish consisted of four spawning lots kept at five rearing temperature conditions. Control fish were held at the temperatures which approximated the 5-year average seasonal cycle for Priest Rapids Dam. The five experimental rearing groups were held at 1.12 °C (2 °F) increments above this control temperature.

Acclimation temperatures are known to effect thermal resistance time. To eliminate this effect, all fish were acclimated at 15 °C for 3 weeks following removal from experimental rearing temperature conditions. They were then placed in groups of 10 at test temperatures ranging from 26-30 °C, at 0.5 °C increments, and the

times to permanent loss of equilibrium and death were recorded. This was done for each spawning lot and each temperature rearing condition, requiring 250 tests and 2650 fish. In all experiments, one test was run as a handling control in which a group of fish was handled in the same way as the experimentals but without temperature stress. No mortalities were observed in these controls.

The geometric mean (GM) death time was calculated for each test on 10 fish. For each test temperature, differences between spawning lots and rearing temperature groups were examined by an analysis of variance. The results showed no significant difference between groups at any rearing temperature except at the highest test temperatures. In that case, the rearing temperatures influenced the time to death. However, the times involved are so short it is unlikely that they are of any ecological significance. In addition, these fish were a selected population in that they were obtained from a previously stressed experiment. From the graph (Figure 2) it is obvious that there was no shifting of the line due to rearing temperatures. Only spawning lot 3 is shown for simplicity and it represents the results obtained for all the tests.

These results can be interpreted in the following manner: the 50% resistance temperature at a given rearing temperature is a genetic adaptation, unaffected by rearing temperatures, except perhaps at high-stress temperatures. Acclimation temperature changes, not studied

FIGURE 2. Thermal Resistance Times of Juvenile Chinook Salmon Taken from Differing Developmental Temperature Regimes

here, cause an adjustment involving nongenetic changes in the response mechanism. Functional nongenetic adaptation has been visualized primarily as being fully reversible, and this is expressed in our ability to erase sensitivity differences by 3 weeks acclimation at 15 °C. The lethal temperature for these juvenile salmon is, therefore, basically a function of the animals genetic composition and immediate previous thermal history.

Fish Size and Mortality

Size has been reported elsewhere to affect thermal resistance times. The above 250 tests, conducted in determining geometric mean resistance times, were analyzed to determine if there was a correlation between size of test fish and time to death. No significant correlation was found.

In a further experiment, from 200 fish, three groups of 12 each were

selected in the size ranges 1.6-2.3, 3.5-5.2, and 7.7-10.8 g. Six fish were taken from each group and placed in a common holding tank. From this group of 18 fish, individuals were taken at random, put in individual containers at the lethal temperature, and the individual times to loss of equilibrium and death were recorded. At the end of the test, lengths and weights were recorded. The results showed no correlation between size of the fish used in this test and time to loss of equilibrium or time to death.

Duration of Exposure

A critical factor in the exposure of fish to elevated temperatures is the duration of this exposure. This can be related to the acute environmental situation of a fish encountering a thermal effluent in a flowing river. Most studies of thermal tolerance reported in the literature have involved exposing the fish until death occurs at the temperature of exposure. Such an experiment defines central lethal limits for the test populations but provides decreasing statistical confidence as one departs from the central value. To attempt to define the relationship between length of exposure and number of temperature mortalities in a population, groups of 20 fish were exposed at 27 °C for 2000-6000 sec at 500-sec increments. After the specified period of exposure, the fish were returned to the temperature of acclimation and observed for mortalities occurring within the next 48 hr. A

fitted curve of the probit analysis of the data obtained, shown in Figure 3, substantiates the relationship between length of exposure and mortality. The time of exposure at 27 °C which results in 50% mortality compares favorably with the geometric mean death time obtained for similar fish at 27 °C. The confidence limits of this experiment are very good for all levels of mortality (shown for 50% mortality in Figure 3), and such data should be useful in accurately predicting lower percentage mortalities.

FIGURE 3. Fitted Curve of Probit Analysis of Percent Mortality Versus Duration of Exposure to Lethal Temperature (27 °C) (95% confidence limits indicated for 50% mortality)

Loss of Equilibrium

A characteristic response of fish to acute thermal shock is the loss of equilibrium. Under such conditions, the animal has no control and would be subject to many types of predation. Ecologically, loss of equilibrium is probably more meaningful than physiological death. We, therefore, measured individual times to loss of equilibrium (LE) and found a good correlation between the LE_{50} and the LT_{50} .

To test whether a fish that has lost equilibrium can regain it if returned to nonlethal temperatures, three groups of 15-20 fish each were exposed to 27 °C. As each fish lost equilibrium, it was returned to the acclimation temperature of 15 °C and individual recovery times recorded. Initial recovery was 100%, however, a latent mortality resulted in 85% of the population dying within 24 hr (Table I). The time course of these

observations is illustrated in Figure 4. It is apparent from these data that most fish that have lost equilibrium under thermal stress are sufficiently harmed that death must result, even though temporary "recovery" is possible.

FIGURE 4. Time Course of Relationship Between Loss of Equilibrium and Mortality

TABLE I. Relationship Between Loss of Equilibrium and Mortality^(a)

Test	Number Tested	Percent Losing Equilibrium	Percent Mortality During Exposure	Percent Recovering Equilibrium	No. of Delayed Deaths	Percent Mortality at 24 hr
1	15	100	0	100	13	87
2	20	100	0	100	16	80
3	20	100	0	100	15	75
4	20 (Control)	0	0	0	0	0

(a) Fish were exposed to 27 °C until they lost equilibrium. At loss of equilibrium, they were returned to 15 °C and observed for recovery time, secondary loss of equilibrium, and delayed mortality. Survivors were observed for 1 week.

Conclusions

Experiments have shown that the temperature of rearing did not significantly affect the incipient lethal temperatures of juvenile chinook salmon. Columbia River fall-run chinook salmon are more sensitive to thermal stress than are British Columbia spring-run chinook reported in the literature. The lethal limit for acute temperature exposure is a function of the immediately previous thermal history of the animal. The LT_{50} s derived from the geometric mean and from a dose relationship are equivalent, but the dose relationship is better for predicting values at lower percentage mortalities. There is no relationship between size and

time to death for juvenile fish ranging in size from 50-97 mm and 1.6-10.8 g weight. Fish which have lost equilibrium due to thermal shock will recover equilibrium; however, 85% of these will die within 24 hr postexposure. These experiments show that death is of questionable value as an end point ecologically, and some criteria related to performance of the organism would be more valid in assessing the ecological impact of thermal modification of the environment.

Reference

1. Brett, J. R. *J. Fish. Res. Bd. Can.*, vol. 9, pp. 265-323. 1952.

BEHAVIOR OF ADULT SALMON AND STEELHEAD TROUT MIGRATING PAST HANFORD THERMAL DISCHARGES

Migration paths of adult summer chinook salmon and steelhead trout were determined in the section of the Columbia River near Hanford reactor outfalls. Most fish migrated close to shorelines thereby avoiding heated plumes.

Investigator:
C. C. Coutant
Technical Assistance:
T. M. Clement

This was a cooperative study with Mr. James Johnson, Fish Passage Research Program, U.S. Bureau of Commercial Fisheries (BCF). Sonic tags and tracking equipment developed by the BCF were used to follow the movement of summer chinook salmon and

steelhead trout in their upstream spawning migration. The area studied was between McNary and Priest Rapids Dams and included that reach of the river receiving thermal discharges from Hanford nuclear reactors. The purposes of the study were to observe

the paths followed by migrating salmonids in the area of a heated effluent, and to determine if there is a behavioral response to these thermal discharges.

Observations

This study concentrated on the late summer chinook run and the early peak of the summer steelhead run, both of which occur in August. Adult fish emerging from the Oregon fishway at McNary Dam were captured and tagged with the sonic device by BCF personnel. Tagged fish moving through the reach of the Columbia River between 100-H and 100-BC reactor areas were located and individuals were tracked by boat for varying lengths of time using portable monitoring units consisting of hydrophone, transistorized receiver, and earphones (Figure 1). Previous studies by BCF indicated that the presence of the boat did not alter the migrating behavior of these species. A total of over 70 individual fish were either located once or tracked for some distance.

Figure 2 illustrates the area of river studied, the approximate locations of the discharge plumes, and the areas where fish were found. Some points indicate multiple locations of the same fish.

Both chinook salmon and steelhead trout migrated almost exclusively along the shorelines at a depth of less than 10 ft (modal depths were 6-7 ft for steelhead and 4-5 ft for chinook). A definite preference was shown for the shore opposite the

Neg 0673051-10

FIGURE 1. Portable Tracking Equipment Used in Locating Sonic-Tagged Salmon and Steelhead. [(A) High Impedance Earphones, (B) Transistorized Receiver (C) Submerged Directional Hydrophone on 5-ft Handle. Gear developed by U.S. Bureau of Commercial Fisheries.]

reactors, a tendency evident several miles downstream from 100-D reactor area. Some crossing of the river to the reactor shore occurred by the midsection of this reach, although Coyote Rapids appeared to be a barrier on the reactor side. Shoreline seepages of warmed groundwater and overflows from the discharge

FIGURE 2. Observed Locations of Sonic-Tagged Steelhead Trout and Chinook Salmon, August-September, 1967

sealwells, despite being low in flow, created localized barriers to steelhead migration; fish coming in contact with these conditions crossed the river and continued migration, usually after a few hours delay. No behavioral responses were observed which could be connected directly to the main effluent discharges located in midchannel.

Conclusions

The relatively small numbers of fish (less than 10) that were tracked while passing through heated areas,

other than where discharges are mixed with river water downstream from 100-D, were not sufficient to obtain quantitatively significant data on behavioral responses to the increased heat. Further attention must be directed toward this question. The preferred migratory pathways of chinook salmon and steelhead trout do not bring them into direct contact with the discharge plumes from the reactors before nearly complete mixing of effluent with the river water has taken place. Small discharges from groundwater seepage and sealwell overflows do seem to affect migration

paths indicating the relative undesirability of even low volume shoreline discharges. Further study is needed to establish whether reactor

operations are primarily responsible for the choice of the shoreline opposite the reactors as the fishes' preferred route of migration.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT RATE OF BOTTOM ORGANISMS

Caddis flies emerged about 2 weeks earlier downstream from Hanford reactors than upstream. Temperature of emergence may be between 11-12 °C

Investigator:
C. C. Coutant
Technical Assistance:
R. M. Steele

Addition of heat to the Columbia River by Hanford nuclear reactors can possibly influence the rate of development of river organisms in downstream reaches as compared to similar organisms upstream from the thermal additions. This study explored one possible result of such increase, the time of emergence of river caddis flies (Trichoptera: Hydropsychidae) for their mating flights. Caddis larvae and adults are important food items for many fish species in the Columbia. Their time of emergence had not previously been related directly to river temperature levels. The presently reported observations were of an exploratory nature, with a detailed study planned for 1968.

Observations

Insect light traps (15 W, "black light" fluorescent) were installed at four locations along the Columbia

River: two at distances greater than 16 km upstream from the uppermost heated discharge, and two approximately 14 km downstream from the nearest outfall. The upper collecting stations were above the range of adults that could be influenced by the reactor discharges. Lamps were illuminated from late afternoon until the following morning at about twice-weekly intervals from early May until mid-June. Each lamp's overnight collection was considered a sample.

Numbers of adult caddis flies collected in each trap throughout the study period are shown in Table I. The data indicate an earlier emergence at the downstream location, with abundant collections beginning there on May 15, while abundant collections were not made above the reactors until May 31. Water temperatures were 12.5 °C at the downstream station on May 15 and 11.3 °C at the

TABLE I. *Numbers of Caddis Flies Caught in Light Traps 16 km Upstream and 14 km Downstream From Reactors*

Date	Below Reactor Catch	Above Reactor Catch
May 8	(a) 10	0 0
11	(a) 0	0 0
15	55 15	6 3
18	178 32	(a) 1
22	53 113	0 1
31	36 53	620 3
June 5	26 305	28 895
8	406 489	30 743
(a) <i>Malfunctioning light, no sample</i>		

upstream station on May 31. Following the start of emergence, numbers

of adults collected above the reactors usually exceeded those collected below.

Conclusions

Emergence of caddis flies from the Columbia River at Hanford occurs earlier at locations below the reactors. These preliminary observations would seem to substantiate the suggestion that the heated effluents influence rates of development of bottom organisms. The warming effect in the 40 km stretch of river studied is largely attributable to reactor operations during winter months when caddis larvae are developing, although solar heating is also important during the last stages of development in April and May. The relationship of emergence to river temperatures, preliminary at this time, merits further detailed investigation due to the importance of river invertebrates as food supplies for Columbia River fishes.

CHINOOK SALMON SPAWNING NEAR HANFORD - 1967*

A 21-year record number of 3270 fall chinook salmon nests were observed in 1967. The increase in numbers of fish spawning downstream from reactor outfalls was comparable to that in the spawning area immediately upstream.

Investigator:
D. G. Watson

Aerial surveys of chinook salmon spawning were conducted in the section

* *This study supported by funds from the AEC Division of Production*

of the Columbia River between Richland (river mile, 339) and Priest Rapids Dam (mile 397), continuing observations that started in 1947. These

surveys are useful in the evaluation of reactor effluent effects on natural populations in the river, and provide information on an important food and sport fish.

Observations

Seven surveys were made between September 28 and November 22. Spawning began between October 16 and October 26, was at its peak during the first week in November, and was completed by the middle of November. A total of 3270 salmon nests were estimated in the section of river from Ringold (mile 354) to Midway (mile 393). No spawning was observed between Ringold and Richland. This year's count was slightly higher than the previous record of

3100 observed in 1966. The 21 year average is 900.

Since the completion of Priest Rapids Dam in 1960, the spawning area at Midway, about four miles downstream from the dam, has had an increased proportion of the total number of nests in the survey. Before 1960, the Midway area contributed an average of 19% of the total; since 1960, it has contributed an average of 42%. This increase in spawning upstream from the reactors probably results from a partial barrier effect of the dam. Spawning areas downstream from the reactors, near 100-H Area and immediately downstream from 100-F Area, have also shown large increases relative to earlier years (Figure 1), which also may represent an influence of Priest Rapids Dam.

FIGURE 1. Comparison of Fall Chinook Salmon Run with Spawning Near Hanford

Conclusions

The population of chinook salmon spawning in the Hanford reach of the Columbia River has maintained itself over several life cycles, as well, or better than, the general run of fall

chinook as estimated from the number ascending McNary Dam (mile 292) (Figure 1). There is no evidence from these observations to indicate that the fish within the Hanford Reservation have been adversely affected by reactor operation.

COLUMNARIS EXPOSURE AND ANTIBODY PRODUCTION IN SEAWARD AND UPSTREAM MIGRANT SOCKEYE SALMON*

Columnaris exposure during seaward migration was slight until the juvenile salmonids reached the Columbia River estuary. Duration of exposure at the estuary was probably insufficient to cause mortality. Although most of the adults lost residual antibodies developed as juveniles, all were exposed upon reentering fresh water and developed antibodies. Many developed high agglutinating titers by the end of upstream migration and were probably immune to the disease. Nonimmune trout exposed to *columnaris* in Bonneville ladder water and under laboratory conditions did not die from exposure but developed antibodies against *columnaris*. Scrapfish residing in warm water areas of the Columbia River were not carriers of *columnaris* disease during winter months.

Investigator:
M. P. Fujihara
Technical Assistance:
R. L. Tramel

Earlier studies suggested that most species of Columbia River fish respond to *C. columnaris* challenge by producing specific antibacterial antibodies (Annual Reports, 1964, 1965). Ladder facilities at Columbia River dams were shown to be sites of repetitive *columnaris* exposure of upstream migrant salmon (Annual Report, 1966).

Observations

Studies were conducted to determine the severity of exposure to *columnaris*

organisms by following the disease pattern and antibody production exhibited by juvenile sockeye salmon (*Oncorhynchus nerka*) during migration from rearing areas to the ocean, and for adults during upstream migration from the ocean to spawning grounds. Gill smears were obtained and blood sera sampled during or near the peak of the run at each sampling site. Sampling sites are shown in Figure 1.

There are two main races of sockeye salmon in the Columbia River, those which spawn in the Okanogan

* This study supported by funds from the AEC Division of Production

FIGURE 1. Sampling Sites for Study of Columnaris Exposure and Antibody Development in Columbia River Sockeye Salmon

River above Lake Osoyoos and those which spawn in the Little Wenatchee and White Rivers above Lake Wenatchee. Samples of juvenile salmon were obtained after the start of seaward migration from the Okanogan River near Omak, Washington and from the Wenatchee River near Dryden, Washington. Both races were included in downstream samples obtained from

gate wells at Priest Rapids, McNary, and Bonneville Dams, and from the Columbia River estuary at Westport, Oregon.

Adult upstream migrants were sampled at Astoria, Cascade Locks, McNary, and Priest Rapids. The Okanogan race was sampled at Rocky Reach Dam and on the Okanogan River spawning grounds near Oliver,

British Columbia. The Wenatchee race was sampled at Icicle Creek, at Tum-water Dam on the Wenatchee River, and on the Little Wenatchee River spawning grounds.

Isolation of the pathogen from gill smears, with no detectable antibodies in blood sera, was taken as an indication of recent initial exposure. Presence of a low agglutinating antibody titer without direct isolation of the pathogen, was considered indicative of residual exposure from an earlier contact. The presence of high agglutinating titers, with or

without direct isolation of columnaris, was considered indicative of previous exposure and probable immunity to the disease.

The results of the studies on seaward migrants are summarized in Table I. All gill smears were negative for columnaris at the tributary sampling sites, and as far down river as McNary Dam. Positive smears were obtained from 15% of the fish sampled at Bonneville, but exposure was light with 1-2 isolates per fish. Residual agglutinating titers from columnaris exposure during the summer and fall

TABLE I. C. columnaris Exposure and Antibody Development in Sockeye Salmon During Seaward Migration

Date	Sampling Site	Temp., °F	Number of Fish Sampled	Positive Isolates, % of fish	Agglutinin Titers, % of samples(a)	Range of Positive Titers
<u>Wenatchee Race</u>						
4/24/67	Wenatchee River	45	90	0	31	1:20
<u>Okanogan Race</u>						
4/26/67	Okanogan River (Omak, Wash.)	45	100	0	32	1:20
<u>Combined Races</u>						
5/1/67	Priest Rapids	46	100	0	22	1:20-1:160
5/11/67	McNary	56	100	0	32	1:20-1:80
5/17/67	Bonneville	57	80	15	40	1:20-1:80
<u>Other Fish Sampled</u>						
6/1/67	Columbia River Estuary (Westport, Oregon)	57	60 (Silver salmon)	8	28	1:20-1:80
			40 (Scrap-fish)	30	100	1:40-1:640

(a) Pooled blood from two fish for each sample

of the previous year were observed in approximately one-third of the juveniles at all sampling sites.

An indication of greater columnaris exposure and infection of fishes was observed in the Columbia River estuary. Although no sockeye salmon were sampled at this site, 28% of 60 juvenile silver salmon (Oncorhynchus kisutch) had titers similar to the sockeyes sampled at upstream sites. Eight percent of these silver salmon migrants showed a heavier exposure of 1-24 columnaris isolates per gill plate. More significantly, 100% of 40 freshwater sculpin, including largescale suckers (Catostomus macrocheilus), squawfish (Ptychocheilus oregonensis) and Columbia River chubs (Mylocheilus caurinus), had agglutinating titers from 1:40 to 1:640, suggesting both previous and new exposure to the disease. Columnaris was isolated from 30% of the sculpin, among which 7.5% were grossly infected. These observations suggest that columnaris disease is endemic in the Columbia River estuary. Exposure to seaward migrants, however, is probably inconsequential since the disease pathogens will be destroyed when fish enter the ocean.

Results obtained from the studies of adult sockeye salmon migrating upstream are summarized in Table II. Although at least 30% of the juveniles had residual columnaris antibodies during seaward migration, only 2.5% of the returning adults had detectable titers. Antibody production and titer magnitude increased from 2.5% of the fish at Astoria with titers of 1:20, to 100% of the fish with average ag-

glutinating titers of 1:360 on the Okanogan and 1:300 on the Little Wenatchee spawning grounds. While only two columnaris organisms were isolated from 120 fish at Astoria, 34% of the spawning fish on the Little Wenatchee River and 46% of the fish on the Okanogan spawning grounds produced isolates. Approximately 16% of the salmon on the spawning grounds were heavily infected. At least 80% of the salmon on both spawning grounds were spawned out when sampled. The presence of large numbers of exposed and infected fish which survived to spawn suggests that columnaris exposure and the development of high agglutinating titers may be protecting these fish from columnaris-induced mortality during migration.

In supporting studies, yearling rainbow trout free of columnaris agglutinating titers were artificially immunized by treatment with attenuated columnaris organisms plus Freund's incomplete adjuvant over a 2-month period. Average agglutinating titers of 1:1280 were developed in these fish. Thirty immunized and thirty control trout showing no titer were exposed for 2 weeks at the Bonneville fish ladder to columnaris infection from organisms present naturally in the fish ladder water. A similar experiment was carried out under laboratory conditions, using columnaris released from infected rainbow trout as the exposure medium. The nonimmunized fish at Bonneville developed average agglutinating titers of 1:160. Titers of the immunized fish did not change. Nine columnaris per ml were isolated from

TABLE II. C. columnaris Exposure and Antibody Development in Sockeye Salmon During Upstream Migration

Date	Sampling Site	Temp., °F	Number of Fish Sampled	Positive Isolates, % of fish	Agglutinin Titers, % of fish	Range of Positive Titers
<u>Combined Races</u>						
6/26/67	Astoria	52	120	2	2.5	1:20
6/29/67	Cascade Locks	61	120	10	11	1:20
7/7/67	McNary	66	60	11	37	1:20-1:80
7/19/67	Priest Rapids	62	50	8	28	1:20-1:160
<u>Okanogan Race</u>						
7/24/67	Rocky Reach	63	40	42	40	1:20-1:160
10/10/67	Okanogan River	46	50	46	100	1:80-1:1280
<u>Wenatchee Race</u>						
8/8/67	Tumwater	58	40	65	97	1:40-1:320
9/27/67	Icicle Creek	56	28	39	100	1:20-1:640
9/28/67	Little Wenatchee River	51	50	34	100	1:20-1:640

the trough outlet indicating a thorough exposure of the fish. Since no mortality was observed, the exposure was presumably from columnaris strains of low virulence. Similar results were obtained from the laboratory experiment. This study will be repeated, exposing the fish to columnaris strains of known high virulence.

Preliminary studies were also initiated to determine whether river fishes residing in warm water areas (70-80 °F) of the Columbia River, near reactor outfalls within the Hanford Reservation, may be serving as

carriers of the columnaris disease during the winter months. General river temperatures during this period were 47-52 °F. Average titers for 40 fish from the warm water area were 1:104 as compared to average titers of 1:106 for 35 fish taken from the river proper. No columnaris organisms were isolated from any of the fish during the test period. These results suggest that fish residing in these warm water areas are not being exposed to the pathogen during the cold water months and do not serve as carriers of the disease.

Conclusions

Juvenile salmonids, which spend at least 1 year in fresh water before seaward migration, are exposed to C. columnaris and develop agglutinating antibodies against the organism. Although mortality may occur among fingerlings during initial exposure to the disease, mortality among juveniles during seaward migration is probably insignificant. Columnaris disease is a major problem for adult

salmon during upstream migration. All adults were exposed during migration and rapidly developed antibodies against the disease. The presence on the spawning grounds of many living, spawned-out salmon, with high agglutinating titers and columnaris infection, suggests that many of these fish are immune. The incidence of exposure and antibody development of the two races of sockeye salmon were similar.

RETENTION OF RADIONUCLIDES IN COLUMBIA RIVER BOTTOM ORGANISMS

The Limpet snail and rock-surface periphyton in their natural environment show seasonal changes in ^{65}Zn retention times, progressing from shorter to longer retention between October and April.

Investigator:
C. C. Coutant

The purpose of this study has been to estimate rates of elimination of radionuclides from river bottom organisms in their natural environment. Preliminary estimates of effective half-lives for the limpet snail and periphyton algae were presented last year (Annual Report, 1966), with the conclusion that there were seasonal differences in the elimination rates. Studies during 1967 have more precisely defined these seasonal variations with emphasis on ^{65}Zn .

Observations

Limpets, Fisherola nuttalli Halde-
man, were collected downstream from reactors (hence, radioactively labeled) and transported to a site upstream from the reactors where they were placed on rocks held in polyethylene buckets. Rock-surface periphyton communities were similarly transported and maintained on steel trays. The rock-surface periphyton community is presently considered as

a single developing community starting with a relatively clean stand of *Gomphonema parvulum* (Kutz) Grun after the late spring freshet, and developing into the stable winter community of *Gomphonema*, detritus, and other algal species.

Serial samplings from such snail collections and periphyton communities, displaced at different seasons of the year, led to retention curves for ^{65}Zn as displayed in Figures 1 and 2. In both instances, there is a progressively slower turnover of ^{65}Zn from October through April.

Conclusions

The limpet snail in the Columbia River has a 1-year life cycle and the lengthening retention times may be related to increasing body weight. Seasonal water temperature changes and other factors probably also contribute.

Changes in retention times in the rock-surface periphyton may be related to the proportion of the total mass represented by actively metabolizing algal cells as opposed to that represented by organic or inorganic detritus. Although quantitative data on detrital components of the community have not yet been obtained, there is an obvious progressive accumulation of detritus throughout the period October through April.

FIGURE 1. Retention of ^{65}Zn by Bodies of the Limpet Snail at Different Times of Year

FIGURE 2. Retention of ^{65}Zn by Rock-Surface Periphyton, Principally *Gomphonema parvulum*, at Different Times of Year

UPTAKE AND RETENTION OF ^{65}Zn BY PERIPHYTON

Investigations of ^{65}Zn uptake and retention rates by periphyton communities of different age and composition show similar times to reach equilibrium (16-24 hr) and similar accumulations of ^{65}Zn per unit weight. The total ^{65}Zn accumulated is related to the age and type of community

Investigator:

C. E. Cushing

Technical Assistance:

R. M. Steele
G. W. Johnson

In a continuation of previously reported studies (Annual Reports, 1965, 1966), information was sought on the uptake and retention of ^{65}Zn by periphyton, to aid in interpretation of data collected in the Columbia River Ecology program. The system employed was slightly modified from that described previously (Annual Report, 1965). An open 6 liter reservoir was incorporated to provide both aeration and sufficient water to buffer physicochemical changes in the media.

Observations

Six experiments, including four uptake and two uptake and retention studies, were performed. Experimental conditions for all experiments included continuous lighting at about 1500 ft-c, water exchange of about 640 ml/min, and an initial ^{65}Zn concentration of 3 nCi/ml. Table I describes the periphyton communities studied and summarizes the data obtained. Figure 1 illustrates the uptake and retention curves.

The time required to attain an approximate equilibrium level of ^{65}Zn

was about 20 hr, irrespective of the type of community studied, and the activity accumulated on a weight basis was about 0.02 nCi/mg dry weight. The greater range of total ^{65}Zn accumulated is probably related to the greater biomass of the "young" spring communities with their mass of filamentous Ulothrix. This difference is also apparent in the curves in Figure 1. In the young communities, initial uptake rates were about 40 counts/min for the first 7-8 hr and then lessened to about half of that until equilibrium was attained. Initial uptake rates for the mature communities approximated 20 counts each minute falling after the first few hours to rates of only about 2 counts/min.

Retention measurements were made in the last two experiments and the responses were similar in both cases. Initial loss rates were of the order of 30 counts/min, and the half-retention time was about 15 hr.

Conclusions

These results emphasize the importance of studying cycling phenomena

TABLE I. Selected Data on Six Periphyton ^{65}Zn Cycling Experiments^(a)

Experiment Number ^(b)	Date	Approximate Time to Equilibrium, hr	^{65}Zn Accumulated at End of Experiment		Retention Half-Time, hr	Community Description
			Total nCi	nCi/mg Dry Wt		
1	Dec 1-2, 1966	16	2.59	0.026	--	104 days old; diatoms, detritus
2	Dec 21-22, 1966	18	2.05	0.023	--	124 days old; diatoms, detritus
3	Jan 19-20, 1967	16	2.00	0.022	--	153 days old; diatoms, detritus
4	Mar 9-11, 1967	20	3.95	0.021	--	52 days old; <u>Ulothrix</u> , diatoms
5	Mar 22-24, 1967	24	3.30	0.022	15	34 days old; <u>Ulothrix</u> , diatoms
6	Apr 4-6, 1967	20	2.66	0.014	14.5	47 days old; <u>Ulothrix</u> , diatoms

(a) See text for experimental conditions

(b) See corresponding curves in Figure 1

FIGURE 1. Uptake and Retention Curves for Six Periphyton ^{65}Zn Cycling Experiments. (Arrows denote change to unspiked river water. See text and Table I for experimental details)

at different times of the year so that information on the effect of community structure can be more readily applied to field situations. We have just begun to obtain meaningful data from this system and much work is needed to define the complexities of community mineral cycling as influenced by environmental factors.

Since completion of the work described in this report, the electronic portion of the system has been refined by replacement of the decade

scalers with a completely automated programmer, timer, and printing scalers. The GM detector tubes have been replaced by water- and light-

proof calcium fluoride crystals and photomultiplier tubes to provide a more sensitive detector system.

BEHAVIOR OF CERTAIN RADIONUCLIDES IN A MARINE BENTHIC AMPHIPOD

The following is the abstract of a thesis presented by Ford A. Cross to Oregon State University in partial fulfillment of the requirements for a degree of Doctor of Philosophy with a major in oceanography. A substantial portion of the radionuclide studies were conducted by Dr. Cross while assigned to our Zaboratory as an AEC Richland Graduate Fellow.

Investigator:
F. A. Cross^x

A new species of gammarid amphipod (Anonyx sp.) was captured in 80 meters of water off the Oregon coast. These abundant scavengers are probably an important food for bottom-feeding fish. Laboratory studies were conducted to determine the effects of temperature, feeding, and sediments on the ⁶⁵Zn metabolism of Anonyx sp. In addition to ⁶⁵Zn, other radionuclides used in the feeding experiments included ¹⁴⁴Ce, ⁴⁶Sc, and ⁵¹Cr.

Zinc-65 accumulation and elimination rates were temperature dependent although the effect appeared minimal within the seasonal temperature range of the organism. Individual dif-

ferences in ⁶⁵Zn accumulation rates for similar sized amphipods had mean standard deviations ranging from 14-19%. Accumulation of ⁶⁵Zn from labeled sediments was not significant; whereas, elimination rates were increased when sediment was present.

Adult brine shrimp (Artemia salina), labeled simultaneously with ⁶⁵Zn, ¹⁴⁴Ce, ⁴⁶Sc, and ⁵¹Cr, were fed to individual amphipods. Radionuclide transfer from Artemia to Anonyx averaged 55% for ⁶⁵Zn and less than 10% for ¹⁴⁴Ce and ⁴⁶Sc. Transfer of ⁵¹Cr was not measurable by using this technique. Zinc-65 elimination rates in feeding amphipods were significantly greater than in nonfeeding ones.

The percentage of ⁶⁵Zn lost at molt was dependent upon the mode of accumulation. When uptake was from

* U.S. Department of Interior, Fish and Wildlife Service, Bureau of Commercial Fisheries, Radiobiological Laboratory, Beaufort, North Carolina

sea water, approximately 20% of the ^{65}Zn body burden remained with the case exoskeleton while only 2.0% was lost if ^{65}Zn accumulation had been from feeding.

Autoradiographs of amphipods that accumulated ^{65}Zn from sea water showed radiozinc localization in the exoskeleton and interstitial spaces of muscle tissue. Labeling also occurred in the gut and hepatopancreas.

SOME SITES FOR RADIONUCLIDE BINDING IN FISH

The highest concentrations of ^{65}Zn and stable zinc in the eyes of Hanford Reservation animals were found in fish from the Columbia River. Zinc-65 and other radionuclides were identified in the melanin-rich tissues of the choroid, iris, and peritoneal membrane. The distribution in subcellular fractions was investigated.

Investigator:

D. R. Buhler

Technical Assistance:

Mary E. Rasmussen

Certain of the reactor-produced radionuclides released into the Columbia River at Hanford ultimately are concentrated in aquatic or terrestrial animals. Differences in tissue radionuclide concentration exist but little is known about the parameters which control these differences. The present investigation was, therefore, aimed at establishing the biochemical factors which influence radionuclide binding in tissues. Initial phases of the study were concerned with identifying the loci for ^{65}Zn binding in Columbia River fishes.

Observations

The highest tissue concentration of ^{65}Zn in a given species from the Hanford Reservation was found in the

eye (Figure 1). Previous studies (Annual Report, 1966) have shown that the greatest amount of ^{65}Zn in the eye was localized in melanin-rich tissues (argentea, iris, and choroid). The low specific activities of these tissues further indicated that the turnover of radiozinc was quite slow. Other tissues which contained appreciable melanin, such as the peritoneal lining from certain fish, also contained a high ^{65}Zn concentration. Highly melanized tissues of this type also accumulated appreciable ^{59}Fe and $^{140}\text{Ba-La}$.

To establish the subcellular site of ^{65}Zn binding, pooled choroid, iris, and peritoneal membrane fractions from a group of Columbia River large-scale suckers (Catostomus macrocheilus) were homogenized in 0.25M sucrose and the cellular debris and

FIGURE 1. Zinc-65 Concentrations in the Eye of Various Aquatic and Terrestrial Species of the Hanford Reservation

nuclear fractions removed by low-speed centrifugation. Crude melanin granules were separated and purified by sedimentation techniques. Figure 2 shows an electron micrograph of a purified melanin granule preparation.

The ^{65}Zn in choroid and iris was associated primarily with the purified melanin granules; very little radioactivity was recovered in other subfractions (Figure 3). Specific activities of the various centrifugation fractions were quite comparable and ranged from 2.4-2.8 pCi $^{65}\text{Zn}/\mu\text{g}$ Zn for choroid and 2.9-3.0 for iris. The distribution of ^{59}Fe within the choroid and iris paralleled that of ^{65}Zn .

20,000X

FIGURE 2. Purified Melanin Granules

FIGURE 3. Subcellular Distribution of ⁶⁵Zn in Choroid and Iris of Largescale Suckers

A similar pattern emerged with the melanin-rich peritoneal membrane (Figure 4). In this case, however, two melanin fractions were isolated: a grayish light melanin fraction that failed to sediment in 2M sucrose and a normal heavy black melanin fraction that did sediment. The distribution of ⁶⁵Zn (and ⁵⁹Fe) was directly related to melanin concentration with the greatest accumulation of radiozinc being observed in purified heavy melanin granules. Fraction specific activi-

ties ranged from 2.9-3.3 pCi ⁶⁵Zn/ μ g Zn. The light melanin granules are presumably still attached to fragments of the peritoneal membrane. Subsequent studies also have shown that sonication increases the number of heavy melanin particles while the light fraction decreases.

Conclusions

Tissues which contain appreciable melanin are known to accumulate heavy metals. Melanin apparently

FIGURE 4. Subcellular Distribution of ^{65}Zn in Peritonea2 Membrane of Largescale Suckers

behaves as a weak cationic exchange resin and is capable of binding many heavy metals and radionuclides by simple ion exchange processes. It is possible, therefore, that

significant radionuclide binding can occur in melanin-rich areas of an animal which can cause highly localized radiation damage to certain tissues.

MODELING OF AQUATIC SYSTEMS

Preliminary models have been developed for two aquatic systems: fish death in fluctuating lethal temperatures and maintenance of ^{65}Zn concentrations in the limpet snail.

Investigators:
C. C. Coutant
C. R. Cole*

Attempts were made to develop models of predictive value for two aquatic systems that have been under study in our laboratory: a) the accumulation by freshwater fishes of lethal doses of high temperature under fluctuating thermal conditions, and b) the maintenance of ^{65}Zn body concentrations in the field by the limpet snail, Fisherola nuttalli. The first system is relevant to thermal modifications of the aquatic environment at Hanford and elsewhere; the second to ^{65}Zn cycling in the Columbia River and to limpet physiology.

Observations

A preliminary model for fish death in fluctuating temperatures was programmed, based upon a graphical model presented by Fry et al.⁽¹⁾ Input data for this model include experimentally determined times to death of 50% of a test population at constant lethal test temperatures for a series of acclimation temperatures, and the temperature-time curve being experienced by the population in question. The model assumes that from the time-to-death data, a dying or "mortifica-

tion" rate can be calculated for each temperature--indicating the percentage of the lethal dose accumulated at that temperature per unit time. The model as presently programmed yields the dose that has been accumulated at each half-minute interval during the exposure to the fluctuating temperature condition. From this, one can determine the time when 100% mortification, or a dose sufficient to kill 50% of a test population, has been accumulated.

Presently work is underway to test the predictions of this model against actual death times of young rainbow trout. The same population of fish has been used to establish the times to death at constant lethal temperatures, and to provide death times under continuously recorded fluctuating temperature conditions. Preliminary results suggest that while the model is basically satisfactory, some modification may be necessary to improve the accuracy of prediction.

A considerable amount of data has been accumulated in recent years on the spatial and temporal distribution of ^{65}Zn in the Columbia River ecosystem, including data for the limpet snail and its presumed algal food. Attempts were made to develop an analog model simulating the mechanisms

* *Applied Physics and Electronics
Department*

by which ^{65}Zn is transferred to the limpet in this natural system. By comparing the goodness-of-fit of simulated and actual annual patterns of ^{65}Zn concentration in the limpet, tests were made both of gaps in environmental data and of the validity

of presently conceived transport mechanisms and assumptions.

Figure 1 illustrates the basic block diagram presently conceived for the uptake system for the limpet. A variety of alternative assumptions regarding mechanisms (e.g., source of

FIGURE 1. Flow Diagram Indicating the Relationships Considered in Computing the Concentration of ^{65}Zn in the Limpet

^{65}Zn , whether from river water or from algae, or some combination) and rates (e.g., feeding rate of limpet on algae) have been tested in attempts to "match" observed concentration values of ^{65}Zn in the limpet. One of our better simulations is presented in Figure 2, where river water is assumed to be the only source of the isotope and the consumption rates of both algae and water are proportional to river water temperature. Poor fits were obtained when the dominant uptake role was assigned to the algal food chain, which suggests that food chain uptake may be of minor significance in the river.

During the course of this simulation, preliminary models were also developed for the mechanisms controlling ^{65}Zn in river water and in the bottom algae community, both of which are subsystems contributing to the limpet. Sufficient data were obtained, or identified as being available, on the distribution of ^{65}Zn in the physical components of the river system (reactor input, transport by sediment, water, etc.) to suggest that a more detailed simulation of the physical aspects of ^{65}Zn dynamics in the river, especially between the reactors and McNary Dam, might be undertaken profitably. Too few data are available on the algal uptake system in the river to allow prepara-

FIGURE 2. Computer-Drawn Graphs Showing the Observed Seasonal Burden of ^{65}Zn in a Representative Limpet Snail and the Burden Generated from Assumptions Described in the Text

tion of a useful model, although initial attempts have served a useful purpose by emphasizing the deficiencies.

Conclusions

Sufficient data have been accumulated for certain aquatic systems to allow development of computer models. These models enhance the predictive utility of available data and pinpoint areas for further study.

Reference

1. Fry, F. E. J., J. S. Hart and K. F. Walker. *Lethal Temperature Relations for a Sample of Young Speckled Trout (*Salvelinus fontinalis*)*. *Univ. of Toronto Stud. Biol., Ser. No. 54; Publ. Ont. Fish. Res. Lab., vol. 66, pp. 5-35, 1946.*

TUMOR INCIDENCE AMONG FRESHWATER MUSSEL POPULATIONS

Several populations of freshwater mussels were examined for the presence of neoplasms. The lowest incidence observed was 4.2% and the highest was 15.7%. The majority of the growths were found on the foot of the animals.

Investigator:
G. B. Pauley
Technical Assistance:
L. H. Heaton

Tumors among mollusks have been considered rare. Last year (Annual Report, 1966) on the basis of limited sampling, we reported what appeared to be an unusually high incidence of benign tumors in freshwater mussels from the Columbia River. We have confirmed this observation in more extensive sampling of the Columbia River, and have observed a similarly high tumor incidence in mussels collected from other locations.

Observations

Table I summarizes tumor incidence data for three species of freshwater

mussels collected from four sites. The lowest incidence observed was 4.2% and the highest was 15.7%. Tumors ranged in size from 0.5-10 mm in diameter. Almost all of the tumors were observed on the foot. However, among the Anodonta population from the Columbia River, two mussels were observed with small lesions on the mantle and two animals were observed with large tumors on the gills (Figure 1). Tumors arising from the gills have not previously been recorded for mollusks. Multiple tumors were observed on 12 of the Anodonta examined from the Columbia

TABLE I. Incidence of Tumors in Several Freshwater Mussel Populations

Collection Site	Species	Number of Mollusks Examined	Number of Tumors Observed	Number of Animals with Tumors	Percent of Animals with Tumors
Ozette River, Washington	<u>Margaritifera margaritifera</u>	123	8	8	6.5
Chehalis River, Washington	<u>Margaritifera margaritifera</u>	71	4	4	5.6
Devil's Lake, Oregon	<u>Anodonta oregonensis</u>	388	17	17	4.2
Columbia River, Washington	<u>Anodonta californiensis</u>	299	78	47	15.7

FIGURE 1. Tumors Located on Both the Gill (T) and the Foot (t) of a Freshwater Mussel (*Anodonta californiensis*)

River, but were not noticed on mussels from the other areas. The

histological appearance of these tumors was previously described (Annual Report, 1966).

Conclusions

The cause of these lesions and the reasons for the varying incidence at different collection sites are unknown, but may be due to differing environmental conditions or genetic differences among the various genera and species. It seems probable that neoplasms in mollusks are more common than the past literature would indicate. Most tumors recorded from mollusks have been large, plainly visible neoplasms. The small size of many of the tumors observed in the present study suggests that they may have been overlooked in previous investigations.

THE PATHOLOGY OF "SPONGY DISEASE" IN FRESHWATER MUSSELS

An account is given of a previously undescribed disease of freshwater mussels, apparently of amoeboid origin.

Investigator:
G. B. Pauley

Technical Assistance:
L. H. Heaton

While examining freshwater mussels for tumor incidence, it was noted that a large proportion of *Margaritifera margaritifera* possessed large watery lesions on the foot. There are no previous reports of diseases among freshwater bivalves, even though

these animals are ubiquitous in many areas of the world.

Observations

Of 123 animals examined, 75 (61%) possessed large watery lesions of a

resilient character similar to a sponge (Figure 1); hence the tentative name, "spongy disease." The disease was confined entirely to tissues in the foot of M. margaritifera. As much as 90% of the foot was sometimes involved. Several stages in the pathogenesis were often present in a single lesion.

The first noticeable feature of the disease was the destruction of the normal architecture of the foot by edema. Subsequent to this destruction, progressive reparative changes took place that resulted in the eventual filling of the damaged area by collagen in the form of a dense scar.

In most of these reparative stages, cells were present that had large basophilic nuclei and one or more inclusions of various sizes in the cytoplasm (Figure 2). These cells appeared histologically to resemble an amoeba similar to Vahlkampfia found in oysters. However, positive taxonomic identification of this parasite and the elucidation of its life cycle will require fresh living material.

Conclusions

While evidence from the later stages of this disease in M. margaritifera suggest that the mollusks repair the damage by formation of a dense collagenous scar, it seems evident that this scar tissue impairs normal function. Many of the

FIGURE 1. Gross Appearance of Watery Lesion on the Foot of Freshwater Mussel

FIGURE 2. Inclusion-Containing Cells Thought to Be the Cause of "Spongy Disease"

mussels when collected seemed unable to dig and orient themselves properly in the sand. The cause of this disease is thought to be the inclusion-containing cells that superficially resemble Vahlkampfia, an amoeba found in oysters.

BIOLOGY DEPARTMENT STAFF

H. A. Kornberg, Ph.D. - Manager
Evelyn G. Swezea - Clerical

V. G. Horstman, B.S. - Specialist, Administration

R. C. Thompson, Ph.D. - Senior Research Associate
Sally A. Schuster - Clerical⁽¹⁾
Paulette Teal - Clerical

Elizabeth H. Groff - Librarian

D. R. Yost, B.S. - Financial Representative, Finance Department

P. B. Pearson, Ph.D., Port Chester, N.Y. - Consultant

BIOLOGY ADMINISTRATION SECTION⁽²⁾

P. M. Potter, B.A. - Manager
Corinne Breazeale - Clerical
Ruth E. Gates - Clerical
Ruby B. Shelton - Clerical

(1) *Transferred to another department*

(2) *Section formed on July 1, 1967 from previous Technical and Administrative Support Section*

CELL BIOLOGY SECTION

W. H. Matchett, Ph.D. - Manager
Marguerite S. Stack - Clerical

Professional Staff

R. R. Adee, B.S.
J. D. Berlin, Ph.D.
J. R. Turner, Ph.D.
W. R. Wiley, Ph.D.
A. L. Allenspach, Ph.D. (1)
R. C. Kelly, B.S. (2)

Technical Staff

L. C. Cronk (3)
Carla J. Farrell (4)
Barbara Hughes (5)
Josephine A. Mars
Mary M. Quentin, B.S.
Laura S. Winn

Consultant

J. A. DeMoss, Ph.D.
University of California
San Diego, California

COMPARATIVE TOXICOLOGY SECTION

J. L. Palotay, D.V.M. - Manager
Dev Felton - Clerical
R. D. Burdett - Clerical
M. Betty Soper - Clerical (6)

Professional Staff

R. L. Amster, D.V.M., Capt. (USAF-VC)
J. L. Beamer, B.S.
M. F. Gillis, D.V.M.
M. P. Hamlet, B.S. (2)
R. W. Hilwig, B.S. (2)
M. T. Karaglanes, D.V.M.
M. E. Kerr
Beatrice J. McClanahan, Ph.D. (3)
H. A. Ragan, D.V.M. (7)
V. H. Smith, Ph.D. (8)
C. R. Watson, M.S. (3)

Technical Staff

B. L. Anderson
A. Jacqueline Clary
W. L. Daniels
B. T. Didway
L. C. Hall
R. F. Howard
O. L. Jackson
R. C. Joyce
W. B. Peterson
K. L. Scherbarth
Linda G. Smith, M.S.
V. D. Tyler
F. L. Wallace
W. A. Zimmerman

Consultants

D. C. England, Ph.D.
Oregon State University
Corvallis, Oregon

J. P. Flanagan, M.D.
Kadlec Methodist Hospital
Richland, Washington

Dean Franklin
Scripps Institute
La Jolla, California

R. L. Van Citters, M.D.
University of Washington
Seattle, Washington

-
- (1) *Richland Faculty appointee*
(2) *Summer employee*
(3) *Transferred to Pathology Section*
(4) *Terminated*
(5) *AEC summer trainee*
(6) *Transferred to another department*
(7) *On educational leave of absence
from 6/16/67 to May 1969*
(8) *Transferred to Physiology Section*

ECOLOGY SECTION

R. E. Nakatani, Ph.D. - Manager
 Laura M. Benedickt - Clerical
 Sharon French - Clerical⁽¹⁾
 Nancy W. Gray - Clerical⁽²⁾
 Lorraine Murray - Clerical

Aquatic Biology Unit

Professional Staff

W. L. Templeton, B.S.
 C. D. Becker, Ph.D.
 D. R. Buhler, Ph.D.⁽²⁾
 C. C. Coutant, Ph.D.
 J. M. Dean, Ph.D.
 D. M. DesVoigne, M.S.
 S. W. Fowler, M.S.⁽³⁾
 G. B. Pauley, M.S.
 R. M. Stokes, Ph.D.⁽⁴⁾
 D. G. Watson, B.S.
 T. F. Winsor, B.S.⁽⁵⁾

Technical Staff

T. M. Clement⁽⁵⁾
R. E. Darrington⁽²⁾
R. G. Genoway
L. H. Heaton, M.S.
G. W. Johnson, B.S.⁽²⁾
W. P. Neal, B.A.⁽²⁾
C. O'Malley
J. D. Maulsby⁽⁶⁾
E. F. Prentice, B.S.
Mary E. Rasmusson, B.S.
Alice M. Russell, M.S.
R. M. Steele, B.S.
E. G. Tangen
R. L. Tramel

Terrestrial Ecology Unit

Professional Staff

L. L. Eberhardt, Ph.D.
 W. C. Hanson, B.S.
 T. P. O'Farrell, Ph.D.
 W. H. Rickard, Ph.D.

Technical Staff

T. E. Hakonson, M.S.
J. D. Hedlund, B.S.
H. A. Sweany
Dorothy D. Wade

Consultant

G. J. Paulik, Ph.D.
 University of Washington
 Seattle, Washington

(1) Transferred to Pathology Section
(2) Terminated
(3) AEC Graduate Fellow
(4) Richland Faculty appointee
(5) Summer employee
(6) On military leave

INHALATION TOXICOLOGY SECTION

W. J. Bair, Ph.D. - Manager
Dorothea N. Dixon - Clerical

Professional Staff

M. G. Brown
J. V. Dilley, Ph.D.
N. Matsusaka, D.V.M., Ph.D. (1)
J. F. Park, D.V.M.
B. O. Stuart, Ph.D.
C. L. Sanders, Ph.D. (2)
E. G. Tombropoulos, Ph.D.
A. P. Wehner, Dr. Med. Dent.

Technical Staff

Sandra T. Baker (3)
P. E. Bergam
J. C. Gaven
T. C. Kinnas
K. E. McDonald
W. G. Morrow
I. L. Newcomb
L. R. Richardson
W. W. Roberts
W. Skinner
M. D. Snyder
K. C. Upton
E. L. Wierman

PATHOLOGY SECTION

W. J. Clarke, D.V.M., Ph.D. - Manager
Gertrude G. Haggard - Clerical
Sharon French - Clerical

Professional Staff

P. H. Cooper, Ph.D. (2)
M. E. Frazer, M.S. (4)
Patricia L. Hackett, Ph.D.
E. B. Howard, D.V.M., Ph.D.
Beatrice J. McClanahan, Ph.D.
R. F. Palmer, M.S.
C. L. Sanders, Ph.D. (5)
L. A. Temple, B.S.
Glenda S. Vogt, B.A.
C. R. Watson, M.S. (6)
E. A. Wellman, B.S. (4)

Consultants

J. E. Alexander, D.V.M.
Washington State University
Pullman, Washington

D. K. Merkeley, M.D.
W. A. Ricker, M.D.
Laboratory of Clinical Medicine
Seattle, Washington

E. E. Osgood, M.D.
University of Oregon Medical School
Portland, Oregon

R. N. Ushijima, Ph.D.
University of Montana
Missoula, Montana

H. A. Wenner, M.D.
University of Kansas Medical School
Kansas City, Kansas

Technical Staff

T. K. Andrews, B.A. (7)
Marilyn J. Bottorff
L. C. Cronk (7)
Eugenia T. Edmerson
V. T. Faubert
Daphne S. Harrah
Darlene H. Hunter
C. C. Jannke, B.S.
Margaret L. Lawson
Judi K. Lund (4)
Mary M. Morrow, B.A. (3)
Monique E. Quanstrom (3)
Jean D. Stearns
R. L. Tramel (8)
Starleen M. White, B.S.

-
- (1) Completed one year assignment - returned to National Institute of Radiological Sciences, Chiba-shi, Japan
(2) AEC Postdoctoral Fellow
(3) Terminated
(4) Summer employee
(5) AEC Postdoctoral Fellow - transferred to Inhalation Toxicology Section
(6) Transferred to Technical Services Section following 3-month educational leave
(7) Transferred to another department
(8) Transferred to Ecology Section

PHYSIOLOGY SECTION

M. F. Sullivan, Ph.D. - Manager
N. Kay Killingstad - Clerical

Professional Staff

D. D. Mahlum, Ph.D.
J. R. McKenney, Ph.D.⁽¹⁾
M. R. Sikov, Ph.D.
V. H. Smith, Ph.D.
B. W. Wachholz, Ph.D.

Technical Staff

D. L. Catt
Alison T. Clark
Alma L. Crosby
Joan O. Hess
S. A. Hughes, B.S.
Nancy L. Lukens⁽¹⁾
C. L. Mason, M.S.

Consultant

T. D. Mahony, M.D.
Medical Arts Building
Richland, Washington

PLANT PHYSIOLOGY AND AGRICULTURE SECTION

F. P. Hungate, Ph.D. - Manager
Marguerite S. Stack - Clerical

Professional Staff

J. F. Cline, B.S.
J. D. Davis, M.S.
J. J. C. Hsieh, Ph.D.
D. B. Menzel, Ph.D.
R. L. Uhler, Ph.D.
M. S. Rupp, M.S.⁽²⁾
O. Van der Borcht, Ph.D.⁽³⁾

Technical Staff

Frances A. DeMers
R. H. Kirkpatrick⁽¹⁾
R. M. Oliver, M.S.
C. W. Wigger⁽¹⁾

TECHNICAL SERVICES SECTION⁽⁴⁾

L. J. Kirby, Ph.D. - Manager
N. Kay Killingstad - Clerical

Professional Staff

A. C. Case, B.A.
J. P. Herring
R. F. Keough, M.S.
C. R. Watson, M.S.

Technical Staff

Juanita S. Barnett
F. Dean, B.S.⁽⁵⁾
Yvonne W. Hendrickson⁽⁶⁾
Kathleen S. Longwell
Margaret B. Patrick
G. J. Powers
Sara K. Richards
Dorothy A. Ricketts
Eddie L. Skinner
C. H. St. John

(1) *Terminated*

(2) *AEC Richland Graduate Fellow*

(3) *Assigned to Biology for a 3-month training assignment on a NATO Fellowship. Returned to the Radiobiology Department, S.C.K., Mol, Belgium*

(4) *Section formed on July 1, 1967 from previous Technical and Administrative Support Section*

(5) *Summer employee*

(6) *Transferred to another department*

ATTACHED PERSONNEL

R. J. Olson, M.S. - Statistics Section, Mathematics Department
H. W. Slater, M.B.A. - Planning and Technical Development Section,
Planning, Programming, and Engineering Department
J. M. Thomas, Ph.D. - Statistics Section, Mathematics Department

GENERAL SERVICES
ENGINEERING SERVICES DEPARTMENT

Glassblower - W. D. Leach

Janitors - C. E. Gant (Foreman)
W. D. Brewer
A. W. Funk(1)
P. R. Garza
O. L. Jackson(2)
Ruby Maupin
Alma Moore

MAINTENANCE
ENGINEERING SERVICES DEPARTMENT

K. E. Harding - Foreman

Carpenters - E. C. Bolin
A. J. Uhlman

Electrical - J. T. Caraway
J. B. Collins

Instruments - R. D. Tucker
S. A. Wilson

Material Coordinator - W. Leichty

Millwright - F. W. Reynolds

Pipefitters - R. R. Brodaczynski
D. L. Dudley

RADIATION MONITORING
ENVIRONMENTAL HEALTH AND ENGINEERING DEPARTMENT

Specialist - G. M. Rolph

Radiation Monitors - H. E. Preston
A. L. Stinnett

(1) *Retired*

(2) *Transferred to Biology Department*

PUBLICATIONS AND PRESENTATIONS

DOCUMENTS

Eberhardt, L. L. "An Outline of Some Aspects of Animal Population Control," TAB-R-6. April 1967.

Eberhardt, L. L. "Some Ecological Aspects of Nuclear War," TAB-R-7. April 1967.

Staff of Biology Department. 1967. Pacific Northwest Laboratory Annual Report for 1966 in the Biological Sciences. BNWL-480, Pacific Northwest Laboratory, Richland, Washington.

OPEN LITERATURE PUBLICATIONS

Bair, W. J. and J. V. Dilley. "Pulmonary Clearance of $^{59}\text{Fe}_2\text{O}_3$ and $^{51}\text{Cr}_2\text{O}_3$ in Rats and Dogs Exposed to Cigarette Smoke," In: C. N. Davies (ed.) Inhaled Particles and Vapours II, pp. 251-271. Pergamon Press. 1967.

Bair, W. J., J. F. Park, and W. J. Clarke. "The Radiosensitivity of Alligators," Radiation Res., vol. 31, p. 532. 1967. (Abstr.)

Ballou, J. E., R. C. Thompson, W. J. Clarke, and J. L. Palotay. "Comparative Toxicity of Plutonium-238 and Plutonium-239 in the Rat," Health Physics, vol. 13, pp. 1087-1092. 1967.

Berlin, J. D. "Early Irradiation Effects on the Localization of Acid Mucopolysaccharide in Intestinal Absorptive Cells," J. Cell Biol., vol. 35, p. 12A. 1967. (Abstr.)

Berlin, J. D. "The Localization of Acid Mucopolysaccharides in the Golgi Complex of Intestinal Goblet Cells," J. Cell Biol., vol. 32, pp. 760-766. 1967.

Berlin, J. D. and J. M. Dean. "Temperature-Induced Alterations in Hepatocyte Structure of Rainbow Trout (*Salmo gairdneri*)," J. Exptl. Zoo., vol. 164, pp. 117-132. 1967.

Buhler, D. R. "Localization of Zn-65 in Highly Pigmented Tissues of Fishes," Federation Proc., vol. 26, p. 524. 1967. (Abstr.)

Cushing, C. E. "Concentration and Transport of ^{32}P and ^{65}Zn by Columbia River Plankton," Limnology and Oceanography, vol. 12, pp. 330-332. 1967.

Cushing, C. E. "Periphyton Productivity and Radionuclide Accumulation in the Columbia River, Washington, U.S.A.," Hydrobiologia, vol. 29, pp. 125-139. 1967.

Eberhardt, L. L. Book Review: "Restoring the Quality of Our Environment," Report of the Environmental Pollution Panel, President's Science Committee, The White House, 1965. J. Wildlife Management, vol. 31, p. 846. 1967.

Eberhardt, L. L. Book Review: "Future Environments of North America," Ed. by F. Fraser Darling and John P. Milton. Natural History Press, Garden City, New York. 1966. J. Wildlife Management, vol. 31, pp. 391-393. 1967.

Eberhardt, L. L. "Relationship of Cesium-137 Half-Life in Humans to Body Weight," Health Physics, vol. 13, pp. 88-90. 1967.

Eberhardt, L. L. "Some Developments in 'Distance Sampling'," Biometrics, vol. 23, pp. 207-216. 1967.

Eberhardt, L. L. Book Review: "Scientific Aspects of Pest Control," A Symposium. National Academy of Sciences, National Research Council Pub. 1402. Washington, D.C. 1966. J. Wildlife Management, vol. 31, pp. 604-605. 1967.

Fujihara, M. P. and R. E. Nakatani. "Cold and Mild Heat Marking of Fish," Progressive Fish Culturist, vol. 29, pp. 172-174. 1967.

Hanson, W. C. "Cesium-137 in Alaskan Lichens, Caribou, and Eskimos," Health Physics, vol. 13, pp. 383-389. 1967.

Mahlum, D. D. "Effect of Hepatocarcinogens on the Metabolism of Some Radionuclides by Rats," Toxicol. Appl. Pharmacol., vol. 11, pp. 264-271. 1967.

- Mahlum, D. D. and M. R. Sikov. "Effect of Age on Cesium Metabolism in the Rat," Federation Proc., vol. 26, p. 307. 1967. (Abstr.)
- Matchett, W. H. "Methionine and the Regulation of Ribonucleic Acid Synthesis in Escherichia coli," J. Bacteriol., vol. 92, pp. 90-97. 1967.
- Matchett, W. H. "Ribosomal Precursors in Steady State Cells of Escherichia coli," Bacteriological Proc., p. 119. 1967. (Abstr.)
- McClellan, R. O., J. L. Beamer, and P. L. Sheldon. "Some Aspects of Strontium-90 Metabolism in Miniature Swine Ingesting Strontium-90 Daily," pp. 213-222. Ed. by J. M. A. Lenihan, J. F. Loutit, and J. H. Martin. Strontium Metabolism, Academic Press, Inc. 1967.
- McKenney, J. R. "Chloride Transport by Isolated Rat Intestine," Federation Proc., vol. 26, p. 260. 1967. (Abstr.)
- Muth, O. H., H. W. Pendell, C. R. Watson, J. E. Oldfield, and P. H. Weswig. "Uptake and Retention of Parenterally Administered Se in Ewes on Different Selenium Regimens," Am. J. Vet. Res., vol. 28, pp. 1397-1406. 1967.
- Park, J. F., W. J. Bair, W. J. Clarke, and E. B. Howard. "Chronic Effects of Inhaled Plutonium Dioxide in Dogs," Radiation Res., vol. 31, p. 553. 1967. (Abstr.)
- Pauley, G. B. "A Tumorlike Growth in the Foot of a Freshwater Mussel (Anodonta Californiensis)," J. Fish. Res. Bd. Canada, vol. 24, pp. 679-682. 1967.
- Pauley, G. B. "Four Freshwater Mussels (Adenomas Californiensis) With Pedunculated Adenomas Arising From the Foot," J. Invertebrate Pathol., vol. 9, pp. 459-466. 1967.
- Pauley, G. B. and W. J. Clarke. "Preparation of Oyster and Freshwater Mussel Tissue for Histological and Histochemical Examination," Progressive Fish Culturist, vol. 29, pp. 149. 1967.
- Pauley, G. B. and R. E. Nakatani. "The Uptake of the Radioisotope ^{65}Zn by Various Tissues of the Freshwater Mussel, Anodonta Californiensis Lea," Proc. National Shellfisheries Association, vol. 57, pp. 7-9. 1967.
- Rickard, W. H. "Onset of Winter Dormancy in Lizards and Beetles," Northwest Science, vol. 41, pp. 92-95. 1967.
- Rickard, W. H. "Seasonal Soil Moisture Patterns in Adjacent Greasewood and Sagebrush Stands," Ecology, vol. 48, pp. 1034-1038. 1967.
- Sanders, C. L. "Phagocytosis and Translocation of $^{239}\text{PuO}_2$ Particles Deposited in the Peritoneal Cavity of Rats," J. Reticuloendothelial Soc., vol. 4, p. 432. 1967. (Abstr.)
- Sikov, M. R. "Effect of Age on the Avidity for Radioiodine and the Radiation Sensitivity of the Rat Thyroid," Radiation Res. J., vol. 31, p. 589. 1968. (Abstr.)
- Sikov, M. R., J. M. Thomas, and D. D. Mahlum. "Mathematical Analysis of Passage Through the Gastrointestinal Tract," Physics in Medicine and Biology, vol. 12, p. 15. 1967. (Abstr.)
- Smith, V. H., H. A. Ragan, and J. L. Beamer. "Absorption of ^{238}Pu from Ceramic, Plutonium Oxide Particles Fed to Swine," Health Physics, vol. 13, p. 938. 1967. (Abstr.)
- Stuart, B. O. "Inhalation of Promethium Oxide by Beagle Dogs," Health Physics, vol. 13, p. 929. 1967. (Abstr.)
- Stuart, B. O. and T. M. Beasley. "Non-Equilibrium Tissue Distributions of Uranium and Thorium Following Inhalation of Uranium Ore by Rats," In: C. N. Davies. (ed.) Inhaled Particles and Vapours II, pp. 291-298, Pergamon Press. 1967.
- Sullivan, M. F. "The Effect of Bile on Intestinal Function of Irradiated Rats," Radiation Res., vol. 31, pp. 592-593. 1967. (Abstr.)

- Sullivan, M. F. "Intestinal and Gonadal Injury After Exposure to Fission Neutrons," Proceedings of the First International Symposium on the Biological Interpretations of Dose From Accelerator Produced Radiation, Berkeley, California, March 13-16, 1967. USAEC Report TID-4500, pp. 180-191. 1967.
- Swinth, K. L. "Dependence of Plutonium X-Ray Counting on Isotopic Composition," Health Physics, vol. 13, p. 930. 1967. (Abstr.)
- Swinth, K. L., B. I. Griffin, and J. F. Park. "Whole Body Counting of Inhaled Plutonium in Dogs," Health Physics, vol. 13, pp. 1223-1231. 1967.
- Thompson, R. C. "Biological Factors," In: O. J. Wick (ed) Plutonium Handbook; A Guide to the Technology, vol. 2 Chapter 23, Gordon and Breach, New York. 1967.
- Thompson, R. C. and P. L. Hackett. "Urinary/Fecal Excretion Ratios of Strontium and Calcium in the Rat," pp. 250-260. Strontium Metabolism, Academic Press, Inc. 1967.
- Tombropoulos, E. G. "Biochemical Changes in Lung Tissues After Thoracic X-Irradiation," In: Proceedings of Seventh International Congress of Biochemistry, Tokyo. p. 1039. Pergamon and Oxford, 1967. (Abstr.)
- Tombropoulos, E. G. "Fatty Acid Synthesis by Subcellular Fractions of Lung Tissue," In: C. N. Davies (ed.) Inhaled Particles and Vapours II, pp. 17-23. Pergamon Press. 1967.
- Tombropoulos, E. G. "Synthesis of Long-Chain Fatty Acids by the Mitochondrial Fraction of Lung Tissue," Progr. in Biochem. Pharmacol., vol. 3, pp. 32-39. 1967.
- Turner, J. R. and W. H. Matchett. "Derepression of Tryptophan Synthetase in *Neurospora crassa*," Bacteriol. Proc., p. 115. 1967. (Abstr.)
- Watson, C. R. and R. O. McClellan. "In Vivo Thermoluminescence Dosimetry of Gamma Rays from Ingested Cesium-137 in Sheep," pp. 393-401. In: Frank H. Attix (ed) Proceedings of International Conference Held at Stanford University, June 21-23, 1965 to USAEC Symposium Series No. 8. 1967.
- Wiley, W. R. and W. H. Matchett. "Metabolic Control of Tryptophan Transport in *Neurospora*," Bacteriol. Proc., p. 106. 1967. (Abstr.)
- Wiley, W. R. and W. H. Matchett. "Tryptophan Transport in *Neurospora crassa*. I. Specificity and Kinetics," J. Bacteriology, vol. 92, pp. 1698-1705. 1967.
- Willard, D. H. "Inhalation and Retention of ^{238}Pu Microspheres in Beagle Dogs," Health Physics, vol. 13, p. 929. 1967. (Abstr.)
- PRESENTATIONS AT SOCIETY MEETINGS AND SYMPOSIA
- Adee, R. R. and C. L. Sanders. "The Ultrastructure of Peritoneal Mononuclear Phagocytes Exposed in Vivo to $^{239}\text{PuO}_2$ Particles." Society for Experimental Biology and Medicine, Northwest Section, Vancouver, B.C., Canada, October 21, 1967.
- Bair, W. J., B. O. Stuart, and J. F. Park. "Interpretation of Excretion Data From Beagle Dogs After ^{239}Pu Inhalation." Symposium on Diagnosis and Treatment of Deposited Radionuclides, Richland, Washington, May 15-17, 1967.
- Bair, W. J., J. F. Park, and W. J. Clarke. "The Radiosensitivity of Alligators," Fifteenth Annual Meeting of the Radiation Research Society, San Juan, Puerto Rico, May 7-11, 1967.
- Bair, W. J., J. F. Park, and W. J. Clarke. "Long-Term Study of Inhaled $^{239}\text{PuO}_2$ in Dogs." Twelfth Annual Meeting of the Health Physics Society, Washington, D.C., June 18-22, 1967.

Becker, C. D. and G. B. Pauley. "A Parasite From the Ova of the Pacific Oyster *Crassostrea gigas*." Annual Conference of the Pacific Coast Section of the National Shellfisheries Association, Olympia, Washington, August 23-25, 1967.

Berlin, J. D. "Early Irradiation Effects on the Localization of Acid Mucopolysaccharide in Intestinal Absorptive Cells." Society for Experimental Biology and Medicine, Northwest Section Meeting, Vancouver, B.C., Canada, October 21, 1967.

Berlin, J. D. "Early Irradiation Effects on the Localization of Acid Mucopolysaccharide in Intestinal Absorptive Cells." Seventh Annual Meeting of the American Society for Cell Biology, Denver, Colorado, November 13-15, 1967.

Brown, M. G. and J. F. Park. "Control of Dental Calculus in Experimental Beagles." Eighteenth Annual Meeting of the American Association for Laboratory Animal Science, Washington, D.C., October 1-7, 1967.

Buhler, D. R. "Localization of ^{65}Zn in Highly Pigmented Tissues of Fish." Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Illinois, April 16-21, 1967.

Clarke, W. J., E. B. Howard, and P. L. Hackett. Strontium-90 Induced Neoplasia in Swine. Delayed Effects of Bone-Seeking Radionuclides Symposium, Sun Valley, Idaho, September 12-14, 1967.

Cline, J. F. "The Effects of Substrate Conditions on the Uptake Rate of ^{137}Cs by Plant." Second National Symposium on Radioecology at the University of Michigan, Ann Arbor, Michigan, May 15-17, 1967.

Cooper, P. H. "Marrow Hematopoiesis in Normal Miniature Swine." Meeting of the Society for Experimental Biology and Medicine, Northwest Section, Vancouver, B.C., Canada, October 21, 1967.

Coutant, C. C. and J. M. Dean. "Temperature Tolerances of Young Chinook Salmon with Differing Developmental Temperatures." Annual Meeting, Pacific Northwest Pollution Control Association, Yakima, Washington, October 27, 1967.

Cross, F. A., J. M. Dean, and C. L. Osterberg. "The Effect of Temperature, Sediment, and Feeding Upon the Behavior of Four Radionuclides in a Marine Benthic Amphipod." Second National Symposium on Radioecology, Ann Arbor, Michigan, May 15-17, 1967.

Cushing, C. E. and N. S. Porter. "Radionuclide Cycling by Periphyton: An Apparatus for Continuous, In Situ Measurements and Initial Data on ^{65}Zn Cycling." Second National Symposium on Radioecology, Ann Arbor, Michigan, May 15-17, 1967.

Dean, J. M., F. A. Cross, and S. W. Fowler. "Zinc in the Tissues of Marine Crustaceans." American Society of Zoologists, New York, New York, December 29, 1967.

Eberhardt, L. L. "Abundance of Fallout Radionuclides in Forest and Range Environments." Thirty-Second North American Wildlife and Natural Resources Conference, San Francisco, California, March 13-15, 1967.

Eberhardt, L. L. and R. E. Nakatani. "Modeling the Behavior of Radionuclides in Some Natural Systems." Second National Symposium on Radioecology, Ann Arbor, Michigan, May 15-17, 1967.

Eberhardt, L. L. and W. C. Hanson. "Problems in Modeling the Behavior of Radionuclides in Arctic Food Chains." Fourth Symposium on Radioactivity in the Arctic, Oslo, Norway, October 19-20, 1967.

Fowler, S. W., L. F. Small, and J. M. Dean. "Metabolism of Zinc-65 in Euphausiids." Second National Radioecology Symposium, Ann Arbor, Michigan, May 15-17, 1967.

Fujihara, M. P. "Columnaris Exposure and Antibody Production in Seaward and Upstream Migrant Sockeye Salmon." Eighteenth Annual Northwest Fish Culture Conference, University of Washington, Seattle, Washington, December 14-15, 1967.

Gillis, M. F. "Measurement of Blood Flow in the Miniature Swine Using the Ultrasonic (Doppler) Technique." Conference on Engineering in Medicine and Biology, Twentieth Annual Meeting, Boston, Mass., November 13-16, 1967.

Hanson, W. C. and L. L. Eberhardt. "Biological Half-Times of Cesium and Strontium on Radioecology." Second National Symposium on Radioecology, Ann Arbor, Michigan, May 15-17, 1967.

Hanson, W. C. and L. L. Eberhardt. "Effective Half-Times of Radionuclides in Alaskan Lichens and Eskimos." Second National Symposium on Radioecology, Ann Arbor, Michigan, May 15-17, 1967.

Hanson, W. C. "Fallout Radionuclides in Northern Alaskan Ecosystems." 1967 Symposium on Circumpolar Health Related Problems, Fairbanks, Alaska, July 23-28, 1967.

Howard, E. B. and W. J. Clarke. "Radiation-Induced ^{90}Sr Lymphoproliferative Disease in a Miniature Pig Resembling Hodgkin's Disease of Man." Annual Meeting of Pacific Northwest Society of Pathologists, Seattle, Washington, April 28-29, 1967.

Howard, E. B., W. J. Clarke, and P. L. Hackett. "Experimental Myeloproliferative and Lymphoproliferative Diseases of Swine." Third International Symposium on Comparative Leukemia Research, Paris, France, July 11-13, 1967.

Hsieh, J. J. C. "Mass-Flow of Ions in Soil Under Irrigation." Western Society of Soil Science, Los Angeles, California, June 19-24, 1967.

Kornberg, H. A. "Introductory Talk in Panel Discussion on Biological Effects of Fallout." Symposium on Radioactive Fallout (Part of the Fifth World Congress on Gynecology and Obstetrics) Sydney, Australia, September 25-30, 1967.

Mahlum, D. D. "Effect of Hepatocarcinogens on the Metabolism of Some Radionuclides by Rats." Annual Meeting of the Society of Toxicology, Atlanta, Georgia, March 24-26, 1967.

Mahlum, D. D. and M. R. Sikov. "Effect of Age on Cesium Metabolism in the Rat." Federation of American Societies for Experimental Biology, Chicago, Illinois, April 16-21, 1967.

Matchett, W. H. "Ribosomal Precursors in Steady State Cells of *Escherichia coli*." Annual Meeting of the American Society for Microbiology, New York, New York, May 1967.

Matchett, W. H. "Ribosomal Precursors and Control of RNA Synthesis." Meeting of American Society for Microbiology, Seattle, Washington, September 8-9, 1967.

McClanahan, B. J. and H. A. Kornberg. "Treatment of Plutonium-Contaminated Wounds with Diethylenetriaminepentaacetic Acid in Rats." Symposium on Diagnosis and Treatment of Deposited Radionuclides, Richland, Washington, May 15-17, 1967.

McKenney, J. R. "Chloride Transport by Isolated Rat Intestine." Federation of American Societies for Experimental Biology, Chicago, Illinois, April 16-21, 1967.

O'Farrell, T. P. "Effects of Acute Ionizing Radiation on Selected Pacific Northwest Rodents." Second National Symposium on Radioecology, Ann Arbor, Michigan, May 15-17, 1967.

Olson, P. A. "Effect of Elevated Temperature on Mortality of Chinook Salmon." Annual Meeting Pacific Northwest Pollution Control Association, Yakima, Washington, October 27, 1967.

Park, J. F., W. J. Bair, W. J. Clarke, and E. B. Howard. "Chronic Effects of Inhaled Plutonium Dioxide in Dogs." Fifteenth Annual Meeting of the Radiation Research Society, San Juan, Puerto Rico, May 7-11, 1967.

Pauley, G. B. "The Pathology of 'Spongy' Disease in Freshwater Mussels (*Margaritana margaritifera*).\" Annual **Conference** of the Pacific Coast Section of the National Shellfisheries Association, Olympia, Washington, August 23-25, 1967.

Rickard, W. H. "Cesium-137 in Cascade Mountain Vegetation - 1966.\" Second National Radioecology Symposium, Ann Arbor, Michigan, April 3, 1967.

Rickard, W. H. "Cesium-137 in Cascade Mountain Vegetation - 1966.\" American Association for the Advancement of Science Meeting, Los Angeles, California, June 17-19, 1967.

Rickard, W. H. "The Effects of Wild-fire on the Ecological Distribution of Ground Dwelling Beetles.\" Society for Experimental Biology and Medicine, Northwest Section Meeting, Vancouver, B.C., Canada, October 21, 1967.

Sanders, C. L. "Phagocytosis and Translocation of $^{239}\text{PuO}_2$ Particles Deposited in the Peritoneal Cavity of Rats.\" Fourth National Meeting of the Reticuloendothelial Society, Winston-Salem, North Carolina, December 3-6, 1967.

Sanders, C. L. "Phagocytosis and Translocation of $^{239}\text{PuO}_2$ Particles by Peritoneal Phagocytes of the Rat.\" Symposium on Diagnosis and Treatment of Deposited Radionuclides, Richland, Washington, May 15-17, 1967.

Sanders, C. L. and R. R. Adee. "The Ultrastructure of Peritoneal Mononuclear Phagocytes Exposed in Vivo to $^{239}\text{PuO}_2$ Particles.\" Fourth National Meeting of the Reticuloendothelial Society, Winston-Salem, North Carolina, December 3-6, 1967.

Sikov, M. R. "Effect of Age on the Avidity for Radioiodine and the Radiation Sensitivity of the Rat Thyroid.\" Fifteenth Annual Meeting of the Radiation Research Society, San Juan, Puerto Rico, May 7-11, 1967.

Smith, V. W., H. A. Ragan, and J. L. Beamer. "Absorption of Pu-238 from Ceramic, Plutonium Oxide Particles Fed to Swine.\" Health Physics Society Annual Meeting, Washington, D.C., June 18-22, 1967.

Stuart, B. O. "Studies on Inhalation Hazards in Nuclear Industries.\" Columbia Chapter of Health Physics Society, Richland, Washington, May 9, 1967.

Stuart, B. O., J. F. Park, and W. J. Bair. "Interpretation of Excretion Data from Beagle Dogs After ^{239}Pu Inhalation.\" Symposium on Diagnosis and Treatment of Deposited Radionuclides, Richland, Washington, May 15-17, 1967.

Stuart, B. O. "A Model for the Long-Term Retention and Distribution of $^{239}\text{PuO}_2$.\" Panel on "Evaluation of the Risks of Inhalation of Air-Borne Contaminants\" in Vienna, Austria, June 26-30, 1967.

Stuart, B. O. "Inhalation of Promethium Oxide in Beagle Dogs.\" Twelfth Annual Meeting of the Health Physics Society, Washington, D.C., June 18-22, 1967.

Sullivan, M. F. "Intestinal and Gonadal Injury After Exposure to Fission Neutrons.\" Symposium on the Biological Interpretation of Dose from Accelerator Produced Radiation, Berkeley, California, March 13-16, 1967.

Sullivan, M. F. "The Effect of Bile on Intestinal Function of Irradiated Rats.\" Fifteenth Annual Meeting of the Radiation Research Society, San Juan, Puerto Rico, May 7-11, 1967.

Swinth, K. L. "Dependence of Plutonium X-Ray Counting on Isotopic Composition.\" Twelfth Annual Meeting of the Health Physics Society, Washington, D.C., June 18-22, 1967.

Thompson, R. C. "Properties of Radionuclides in Tissues - A New Look at Some Old Problems.\" Symposium on Diagnosis and Treatment of Deposited Radionuclides, Richland, Washington, May 15-17, 1967.

Tombropoulos, E. G. "Biochemical Changes in Lung Tissue After Thoracic X-Irradiation." Seventh International Congress of Biochemistry Science Council, Ueno Park, Tokyo, Japan, August 21-25, 1967.

Tombropoulos, E. G. "Lipid Synthesis by Lung Mitochondria." Annual Meeting of the Northwest Section of the Society for Experimental Biology and Medicine, Vancouver, British Columbia, Canada, October 21, 1967.

Turner, J. R. "Derepression of Tryptophan Synthetase in Neurospora crassa." Annual Meeting of the American Society for Microbiology, New York, New York, May 1967.

Watson, C. R. "A Method of Quantitative Thermoluminescence." Multidisciplinary Conference on Solid State Optical Dosimetry Development, Seattle, Washington, November 20, 1967.

Watson, D. G., C. E. Cushing, C. C. Coutant, and W. L. Templeton. "Effect of Hanford Reactor Shutdown on Columbia River Biota." Second National Symposium on Radioecology, Ann Arbor, Michigan, May 15-17, 1967.

Wehner, A. P. "Technical, Biological and Clinical Aspects of Electro-Aerosols." Sixth Annual Scientific Program of the West Coast Allergy Society, Phoenix, Arizona, November 30-December 2, 1967.

Willard, D. H. "Inhalation and Retention of ^{238}Pu Microspheres in Beagle Dogs." Twelfth Annual Meeting of the Health Physics Society, Washington, D.C., June 18-22, 1967.

INDEX OF INVESTIGATORS

Adee, R. R.	6.20	Haverfield, A. J.	5.24
Amster, R. L.	5.20	Howard, E. B.	1.10, 1.12, 2.3, 2.8, 2.14, 2.18, 2.19, 3.22
Bair, W. J.	1.18, 3.3, 3.8, 3.12, 3.20, 3.22, 4.11, 6.17	Hsieh, J. J. C.	4.20, 4.21, 4.23, 8.16, 8.19
Beamer, J. L.	1.12, 5.3	Hungate, F. P.	4.23, 8.20
Berlin, J. D.	7.14, 7.18	Jackson, P. O.	3.11
Buhler, D. R.	9.26	Jannke, C. C.	2.14, 2.18
Caldwell, R. S.	7.23	Jones, Y. M.	3.11, 3.32
Clarke, W. J.	1.18, 2.3, 2.8, 2.19, 3.3	Karagianes, M. T.	1.10, 1.14, 2.3, 2.8, 4.12, 4.16, 5.3
Cline, J. F.	8.24	Mahlum, D. D.	5.5, 5.8, 5.15, 5.18
Cole, C. R.	9.30	Matsusaka, N.	3.20
Cooper, P. H.	2.16	McClanahan, Beatrice J.	1.12, 2.21, 6.3
Cosgrove, G. E.	8.7	McDonald, K. E.	6.9, 6.11
Coutant, C. C.	9.5, 9.10, 9.13, 9.21, 9.30	Nakatani, R. E.	9.3
Cross, F. A.	9.25	Nelson, I. C.	5.24
Cushing, C. E.	9.23	O'Farrell, T. P.	8.7, 8.10
Davis, J. D.	8.23	Olson, P. A.	9.3
Dean, J. M.	7.21, 9.5	Olson, R. J.	3.37
Dilley, J. V.	3.17, 6.9, 6.11, 6.12	Palmer, R. F.	2.21
Dionne, P. J.	3.5	Palotay, J. L.	1.10, 5.3
Eberhardt, L. L.	8.3, 8.5	Park, J. F.	1.18, 3.3, 3.22, 3.37
Frazier, M. E.	2.19	Pauley, G. B.	1.16, 9.33, 9.34
Fujihara, M. P.	9.16	Perkins, R. W.	3.12
Gillis, M. F.	1.14, 4.16, 5.3	Ragan, H. A.	1.12, 1.14
Hackett, Patricia, L.	2.10, 2.21	Richardson, L. R.	3.37
Hakonson, T. E.	8.13	Rickard, W. H.	8.13, 8.14
Hanson, W. C.	8.3, 8.5		

Roesch, W. C.	4.23	Thomas, J. M.	1.14, 4.21
Sanders, C. L.	6.13, 6.17, 6.20	Tombropoulos, E. G.	3.34, 3.35
Schultz, W. W.	5.24	Turner, J. R.	7.10
Sikov, M. R.	5.5, 5.8, 5.15, 5.18	Ushijima, R. N.	2.19
Smith, V. H.	4.12, 4.16, 4.18, 5.20, 6.7	Van der Borght, O.	8.20
Snyder, M. D.	4.7	Vogt, Glenda S.	2.10
Stuart, B. O.	3.5, 3.27, 3.29, 3.32, 4.3, 4.7, 4.11	Wachholz, B. W.	1.7, 1.9
Sullivan, M. F.	1.3	Watson, C. R.	2.23, 3.15, 4.12, 4.16, 5.20
Swinth, K. L.	3.8	Watson, D. G.	1.16, 9.14
		Willard, D. H.	4.3, 4.9
		Wiley, W. R.	7.3, 7.6

DISTRIBUTIONNo. of
Copies

1	<u>Atlantic Richfield Hanford Company</u> W. P. McCue	<u>Battelle-Northwest (contd)</u> W. L. Fisher R. F. Foster J. J. Fuquay E. P. Galbraith F. P. Hungate R. L. Junkins D. R. Kalkwarf A. R. Keene L. J. Kirby H. A. Kornberg (401) W. H. Matchett R. E. Nakatani C. E. Newton R. L. Newton J. M. Nielsen J. L. Palotay H. M. Parker R. S. Paul D. W. Pearce P. M. Potter W. C. Roesch G. M. Rolph H. W. Slater A. J. Stevens D. H. Stewart M. F. Sullivan W. L. Templeton R. C. Thompson C. R. Tipton, Jr. C. M. Unruh F. W. Van Wormer E. E. Voiland D. C. Worlton D. R. Yost Biology Library (2) Technical Information Files (5) Technical Publications (2)
269	<u>Division of Technical Information Extension</u>	
1	<u>Douglas United Nuclear, Inc.</u> DUN File	
2	<u>Hanford Environmental Health Foundation</u> P. A. Fuqua G. H. Crook	
4	<u>Richland Operations Office</u> C. L. Robinson (2) R. K. Sharp Technical Information Library	
1	<u>U.S. Testing</u> 2800 George Washington Way Richland, Washington 99352 D. B. Wilcox	
454	<u>Battelle-Northwest</u> F. W. Albaugh W. J. Bair C. A. Bennett L. M. Bonnefond W. A. Burns S. A. Bush W. J. Clarke G. M. Dalen F. G. Dawson D. R. de Halas L. L. Eberhardt	