1966
BIOLOGY

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AEC
RESEARCH
and
DEVELOPMENT
REPORT

PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1966
TO THE
USAEC DIVISION OF BIOLOGY AND MEDICINE

VOLUME 1 BIOLOGICAL SCIENCES

JULY, 1967

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

3 3679 00047 1427

BNWL-480

UC-48, Biology and Medicine

PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1966
TO THE
USAEC DIVISION OF BIOLOGY AND MEDICINE

VOLUME I BIOLOGICAL SCIENCES

by

The Staff of the
Biology Department

Edited by

R. C. Thompson and Evelyn G. Swezea

July, 1967

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PACIFIC NORTHWEST LABORATORY
RICHLAND, WASHINGTON

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-481 1; (2) Radiological Sciences, BNWL-481 2; (3) Earth Sciences, BNWL-481 3; (4) Instrumentation, BNWL-481 4.

This is the sixteenth 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), and BNWL-280 (1965).

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 Annual Report follows the general format established last year. The number of individual reports has increased quite significantly, as compared with the previous year, but we have endeavored to keep them brief. We continue to rely primarily on publication in the open literature for presentation of our detailed findings.

Individual reports are grouped to correspond approximately with the USAEC's Division of Biology and Medicine budget categories. Each of these groupings is introduced by a brief general discussion of our program in that area. Major research accomplishments are highlighted in these introductions.

In the foreword to last year's Annual Report, we listed six "priority areas" in which we hoped to expand our activities, if necessary, at the expense of less critical or less productive areas. Our progress toward these objectives is summarized below:

- Inhalation studies have been somewhat expanded and we expect to continue this trend with additional dog facilities becoming available in 1967.
- Chronic ⁹⁰Sr toxicity studies in swine were thoroughly re-evaluated in late 1966, and while overall expansion is slight, there will be a major intensification of effort in certain selected areas.
- Columbia River ecology studies were somewhat expanded, but more significant, here also, was the focusing of effort on an integrated river sampling program, the results of which should be apparent in the next year's report.

- Early in the year, terrestrial ecology studies were subordinated to the possibly more urgent river program, but at year end prospects appeared excellent for the establishment of a large segment of the Hanford Reservation as an ecology study area. This is known as Project ALE, signifying Arid Land Ecology.
- Internal emitter metabolism and toxicity studies, particularly in small animals, were substantially expanded, as evidenced by reports in this volume.
- Gastrointestinal radiation damage studies were greatly stimulated by the Symposium on Gastrointestinal Radiation Injury held in Richland, September 25-28, 1966, under the general chairmanship of Dr. M. F. Sullivan.

There were two major organizational changes during 1966. The former Aquatic Biology and Ecology Sections were combined under the direction of Dr. R. E. Nakatani in order to foster a more unified approach to environmental problems. Primary responsibility for our major program on radiostrontium toxicity studies in miniature swine was transferred from the Comparative Toxicology Section to Dr. W. J. Clarke in the Pathology Section.

An exciting event of the past year was the final approval of funding for the construction of a new Biology laboratory. Planning for this structure has occupied a considerable portion of many people's time. Progress has been rapid and we are presently scheduled to occupy the new building by the summer of 1969. We are, indeed, appreciative of the confidence expressed by the USAEC's Division of Biology and Medicine in making this development possible.

H. A. Kornberg, Manager
Biology Department

TABLE OF CONTENTS

FOREWORD	iii
RADIATION EFFECTS - GENERAL	1
Effect of Radiation, Bile, Radiomimetics, and Radioprotective Agents on Sodium and Water Transport in the Rat Intestine - M. F. Sullivan	3
Irradiation and Bile Salt Effects on Electrolyte Transport and Voltage Measurements in Rat Ileum - J. R. McKenney	7
Vitamin B ₁₂ Absorption from the Intestine of the Irradiated Rat - M. F. Sullivan	11
Localization of Acid Mucopolysaccharides in the Golgi Complex of Intestinal Goblet Cells - J. D. Berlin	12
Ontogeny and X-Radiation Sensitivity of the Flour Beetle, <u>Tribolium</u> - H. E. Erdman	16
Effects on Flour Beetles Irradiated as Pupae - H. E. Erdman	17
Dimethylsulfoxide Toxicity for Seeds - H. E. Erdman	18
TOXICITY OF RADIOELEMENTS	21
Effects of Strontium-90 in Miniature Swine - Seventh Progress Report - H. A. Ragan, C. R. Watson, M. F. Gillis, J. L. Beamer, W. J. Clarke, E. B. Howard, Patricia L. Hackett, Glenda S. Vogt, and J. L. Palotay	23
Radiostrontium Studies in Pygmy Goats - C. R. Watson and H. A. Ragan	28
Hematopoiesis in Swine Chronically Ingesting Strontium-90 - P. H. Cooper	29
Chromosome Studies in Miniature Swine and Goats - Patricia L. Hackett and H. A. Ragan	32
Metabolism of ¹⁴ C-Choline in Marrow and Blood Cell Phospholipids of Strontium-90 Burdened Rats - J. C. Pekas and Linda G. Smith	35
Effects of Radioiodine in Sheep and Swine - H. A. Ragan, J. L. Palotay, and W. J. Clarke	38
Effect of Age on Absorption of Plutonium from the Gastrointestinal Tract of Rats - D. D. Mahlum and M. R. Sikov	40
Cross-Placental Passage of Actinides in the Rat - M. R. Sikov and D. D. Mahlum	42
Neptunium-Induced Fatty Livers in Rats: Electron Microscopic and Biochemical Studies - J. D. Berlin and D. D. Mahlum	43
Distribution of Cerium-144 in the Fetal and Newborn Rat - D. D. Mahlum and M. R. Sikov	47
Age-Related Differences in the Metabolism of Cesium-137 by the Rat - D. D. Mahlum and M. R. Sikov	48
Interrelationships Between Environmental Temperature and Diet in the Distribution and Retention of Cesium-137 in the Rat - D. D. Mahlum and M. R. Sikov	50
Age Dependence of Radioiodine Metabolism and Effect in Rats - M. R. Sikov	52

INHALATION STUDIES	55
Chronic Effects of Inhaled Plutonium Dioxide in Dogs - J. F. Park, W. J. Bair, W. J. Clarke, and E. B. Howard	57
Plutonium-Americium Ratios in Dogs After Inhalation of $^{239}\text{PuO}_2$ - W. J. Bair and T. M. Beasley	61
Comparative Disposition of Four Types of Plutonium Dioxides Inhaled by Dogs - W. J. Bair and J. F. Park	63
Acute Lethality of Inhaled $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ in Rats - B. O. Stuart	66
Distribution and Excretion of $^{106}\text{Ru-RhO}_2$ Inhaled by Beagle Dogs - B. O. Stuart	67
Retention of Inhaled $^{144}\text{Ce-PrO}_2$ in Beagle Dogs - B. O. Stuart	68
Retention and Translocation of Inhaled Calcined Promethium Oxide in Beagle Dogs - B. O. Stuart	70
Taurine Excretion in Beagle Dogs After Inhalation of Plutonium Oxide - J. V. Dilley	73
Taurine Excretion in Extracorporeally Irradiated Lymphocytic Leukemia Patients - J. V. Dilley	74
Taurine Excretion in Rats Treated with Lymphopenic Agents - J. V. Dilley	76
Effects of X-Irradiation on Lung Biochemistry - E. G. Tombropoulos	77
Effect of $^{239}\text{PuO}_2$ Inhalation on Lung Tissue - E. G. Tombropoulos and B. O. Stuart	80
Effect of Chronic Cyanide Ingestion on Lung Collagen - J. V. Dilley and J. D. Berlin	81
SPACE NUCLEAR SYSTEMS STUDIES	85
Retention of Plutonium Oxide Microspheres in Beagle Dog Lungs - D. H. Willard	87
Passage Time and Pathology Following Feeding of $^{238}\text{PuO}_2$ Microspheres to Swine - V. H. Smith, H. A. Ragan and J. L. Beamer	88
Absorption of $^{238}\text{PuO}_2$ from the Gastrointestinal Tract of Swine - V. H. Smith and J. L. Beamer	91
Effect of Age and Particle Size on Passage Time Through the Gastrointestinal Tract of the Rat - M. R. Sikov and D. D. Mahlum	93
Resuspension of Particulates from a Surface - J. J. C. Hsieh	96
COMBATING DETRIMENTAL EFFECTS OF RADIATION	99
Plutonium-Contaminated Wound Studies in Swine - Beatrice J. McClanahan and H. A. Ragan	101
Phagocytosis of $^{239}\text{PuO}_2$ Particles - C. L. Sanders	103
Chelates as Contrast Media: Uranium DTPA - V. H. Smith and H. A. Ragan	105
MOLECULAR AND CELLULAR LEVEL STUDIES	107
Ribosomal Precursors in Steady State Cells - W. H. Matchett	109
Turnover of Chloramphenicol RNA During Recovery - W. H. Matchett	113
Tryptophan Transport in <u>Neurospora</u> : Attachment Sites - W. R. Wiley	115

Tryptophan Transport in <u>Neurospora</u> : Metabolic Control - W. R. Wiley	118
Regulation of Enzyme Synthesis in <u>Neurospora Crassa</u> - J. R. Turner and W. H. Matchett	121
Control of Sterol Synthesis in Yeast - J. R. Turner	125
ENVIRONMENTAL RADIATION STUDIES - TERRESTRIAL	127
Cesium-137 in the Food Chain of Alaskan Natives - W. C. Hanson and L. L. Eberhardt	129
Population Studies of Hanford Geese and Gulls - W. C. Hanson	130
Effects of Radiation on Native Rodents - T. P. O'Farrell and G. E. Cosgrove	132
Elevational Distribution of <u>Eleodes Hispilabris</u> on the Hanford Reservation - W. H. Rickard	135
Effects of Moisture and Temperature on the Physiology of Cheatgrass - R. L. Uhler and W. H. Rickard	137
Cesium-137 in the Vegetation of a Mountain Bog - W. H. Rickard	138
Cesium-137 Movement in a Root-Soil Profile - J. F. Cline	140
Availability to Plants of Cesium-137 Absorbed to Clay - J. F. Cline	141
Toxic Effect of Cesium in the Cellular Development of Plants - J. F. Cline and Mary W. Magula	143
Hevesy Revisited: The Absorption and Translocation of Lead by Plants - R. L. Uhler	145
Cuticular Foliar Sorption of Iodine - J. J. C. Hsieh	147
Effect of Irradiation on Growth and Dormancy of Potato Tubers - R. L. Uhler	150
ENVIRONMENTAL RADIATION STUDIES - AQUATIC	153
Metabolism of Acetate and Palmitate by Tissues of Warm and Cold Adapted Trout - J. M. Dean	155
Temperature-Induced Differences in Acid Phosphatase Levels of Rainbow Trout Livers - J. D. Berlin	157
The Effects of Temperature and X-Irradiation on the Intestinal Epithelial Cells of Juvenile Silver Salmon - T. S. Johnson, F. P. Conte, and R. E. Nakatani	159
Temperature of Tissues of Freshwater Fishes - J. M. Dean	162
Radionuclides in Columbia River Organisms - D. G. Watson, C. E. Cushing, C. C. Coutant, and W. L. Templeton	164
Retention of Radionuclides in Columbia River Bottom Organisms - C. C. Coutant	170
Tissue Binding of Zinc-65 in Fish and Other Vertebrates - D. R. Buhler	172
Zinc-65 Metabolism in Freshwater Mussels - G. B. Pauley and R. E. Nakatani	174
Apparatus for Measuring Voluntary Swimming Activity: Effect of Zinc-65 in Trout - R. C. Erickson, A. H. Seymour, and R. E. Nakatani	176
Radionuclide Cycling by Periphyton - C. E. Cushing	178
Reactor Effluent Monitoring: Comparison of Puget Sound and Priest Rapids Chinook Salmon - P. A. Olson	180

Chinook Salmon Spawning Near Hanford - 1966 - D. G. Watson . . .	181
Immune Response of Salmonids and Exposure of River Fishes to <u>Chondrococcus Columnaris</u> - M. P. Fujihara . . .	183
Upstream Dispersion of Adult Caddis Flies - C. C. Coutant . . .	186
Fairy Shrimp in Gravel Pit Ponds - C. C. Coutant . . .	188
Observations of the Life History of the Limpet Snail <u>Fisherola</u> <u>Nutalli</u> Haldeman - C. C. Coutant, D. G. Watson, C. E. Cushing, and W. L. Templeton . . .	190
Freshwater Mussels with Pedunculated Adenomas - G. B. Pauley . .	191
Interoceanic Canal Study - Freshwater Ecology - W. L. Templeton and D. G. Watson . . .	193
BIOLOGY DEPARTMENT STAFF . . .	195
PUBLICATIONS AND PRESENTATIONS . . .	201
Documents . . .	201
Open Literature Publications . . .	201
Presentations at Society Meetings and Symposia . . .	203
INDEX OF INVESTIGATORS . . .	206

RADIATION EFFECTS - GENERAL

This section of our Annual Report includes four reports concerned with the mechanism and treatment of gastrointestinal radiation effects, two reports of irradiation studies with flour beetles, and a report on dimethylsulfoxide toxicity, which may seem misplaced, but was initially conceived as part of a synergistic effects study involving radiation.

The past year has seen a continuing expansion of our approaches to the study of gastrointestinal radiation effects. These approaches are well illustrated by this year's reports, one concerned with rather straight-forward in vivo studies of sodium and water transport as influenced by radiation and auxiliary factors, one involving quite sophisticated in vitro studies of electrolyte transport, one concerned with effects on the absorption of a very large molecule, and one involving ultrastructural studies of the intestinal goblet cell.

This area of research interest was greatly stimulated last year by the Symposium on Gastrointestinal Radiation Injury co-sponsored by the U.S. Atomic Energy Commission and Battelle Memorial Institute, held September 25-28 in Richland. Over 40 papers were presented and there were more than 80 off-site participants, including 17 from England, Belgium, France, Germany, Italy, and Japan. The proceedings of this symposium will be published by Excerpta Medica in the fall of 1967.

Several years' studies of radiation effects on flour beetles were brought to completion during 1966. In addition to the two reports included here, a considerable body of data on the competitive survival of several flour beetle strains under conditions of chronic irradiation is yet to be interpreted.

EFFECT OF RADIATION, BILE, RADIOMIMETICS, AND RADIOPROTECTIVE
AGENTS ON SODIUM AND WATER TRANSPORT IN THE RAT INTESTINE

Movement of tritiated water and ^{22}Na from intestinal lumen to blood was depressed in irradiated rats. Bile diversion did not affect this depression when function was measured by an in vivo perfusion technique, but did eliminate a major portion of the effect when labeled buffer was placed in an isolated segment of the small intestine. The effect of X-irradiation on intestinal function was mimicked by nitrogen mustard and was largely prevented by prophylaxis with cysteine or AET.

Investigator:
M. F. Sullivan

Technical Assistance:
Alma L. Crosby

Cellular damage in the intestinal mucosa resulting from exposure to radiation is considered sufficient to explain the sequence of events that is termed the intestinal radiation syndrome. We have shown, however, that a prominent feature of this sequence, diarrhea, can be prevented by diverting the bile from the lumen of the irradiated intestine. We have demonstrated decreases in intestinal function that seemed sufficient to explain the excessive losses of fluid and electrolyte which characterize the radiation-induced diarrhea (Annual Report, 1965).

This report describes studies which were designed to demonstrate the effect of bile on intestinal function.

Information is also presented on the influence of radiomimetic and radioprotective drugs on gastrointestinal function.

Observations

The common bile ducts and duodenae of rats were intubated with polyethylene cannulae before exposure of either the abdomen or the lower body to X rays. Salt and water were injected daily through the duodenal cannula to replace that fraction lost with the bile. Intestinal function was measured in the anesthetized animal at 3 days after radiation or drug treatment by an intestinal perfusion technique as previously described (Annual Report,

1965). Intestinal function was also tested by another technique which involved the injection of 10 ml of labeled buffer into a segment of the intestine ligated at the pylorus and cecal junctions. Measurement of sodium or tritium loss was made over a 2-hr period by the perfusion method, but for only 30 min with the isolated segment technique. The radiomimetic drug methyl bis 2 (chloroethyl) amine hydrochloride (HN2) was injected intraperitoneally 3 days before function was measured. Both cysteine, 1.0 g/kg, and 2-aminoethylisothiuronium bromide hydrobromide (AET), 150 mg/kg, were injected intraperitoneally 10 min before exposure to X rays.

The effects of radiation and/or bile diversion on water and sodium transport, as measured by the perfusion technique, are shown in Figure 1. Both the absence of bile from the lumen of the intestine for several days and X ray depress insorption (lumen to blood), but bile has little effect on movement in the opposite direction. Since these results did not help in explaining the observed effect of bile diversion in preventing diarrhea in the irradiated animal, another radiation dose, 1250 R, and the isolated segment technique, which should have less effect on intestine motility, were employed in further studies. The preliminary results obtained using this

FIGURE 1. Sodium and Water Transport in the in situ Perfused Small Intestine of the Bile Duct Cannulated and/or Irradiated Rat (Number of animals shown in parentheses)

FIGURE 2. Sodium Insorption in the Ligated Small Intestine of the Bile Duct Cannulated and/or Irradiated Rat (Number of animals shown in parentheses)

isolated segment method are shown in Figure 2. They differ significantly from those obtained with the perfusion method, and are compatible with the observed effect of bile diversion in preventing diarrhea.

The influence of X ray, HN2, cysteine, or AET on the insorption of water and sodium from the perfused buffer is shown in Figure 3. The damage to in-

testinal function caused by X rays is mimicked by HN2 and largely eliminated by prior treatment with the protective drugs.

Conclusions

Judging from the results obtained using the perfusion technique, the major effect of bile diversion on water

FIGURE 3. Sodium and Water Transport in the *in situ* Perfused Small Intestine of the Rat After Irradiation and/or Treatment with Various Drugs (Number of animals shown in parentheses)

and sodium transport is to reduce movement from lumen to blood, even in the unirradiated bowel. Such an effect is hardly compatible with the preservation of fluid and electrolyte necessarily associated with the prevention of diarrhea. In contrast, the depression of absorption in irradiated animals was markedly alleviated by bile diversion

as measured by the isolated segment technique. These contradictory results might be due to the different radiation dose, 1250 R vs. 1000 R, but are more likely explained by complicating effects of the techniques on intestinal function and motility. Further studies are clearly required to resolve these discrepancies.

IRRADIATION AND BILE SALT EFFECTS ON ELECTROLYTE TRANSPORT
AND VOLTAGE MEASUREMENTS IN RAT ILEUM

Three days after 1500 R of abdominal X-irradiation, passive ^{22}Na , ^{36}Cl , and ^3HOH fluxes across isolated rat ileum were relatively normal, but net electrolyte transport was severely depressed. Taurocholate caused changes in the bioelectric and transport properties of nonirradiated segments that were similar to the radiation effects.

Investigator:

J. R. McKenney

Technical Assistance:

W. P. Neal

The sequence of events that leads to the depression of fluid and electrolyte absorption by irradiated intestine, and subsequent diarrhea, has not been established. This is in part due to lack of knowledge of the detailed mechanisms, or even the predominant pathways, of fluid and electrolyte transport across the mucosa. To study this function, intestinal segments from rats were everted and mounted in apparatus for potential difference (PD) and radioactive flux measurements. The experimental procedures and media used have been described previously.⁽¹⁾

Observations

The media were labeled with ^{22}Na , ^{36}Cl , and ^3HOH . The two kinds of transport measurements described are the apparent absorption permeability, K_{ms} , and steady state ratio of the serosal and mucosal concentrations, S/M. The K_{ms} values are the flux rates of the radioactive labels from the mucosal to the serosal media, divided by their concentrations in the mucosal media.

During exposure to X rays, either lead shields were used which permitted complete exposure of the abdomen

but protected the rest of the rat, or the intestine was exteriorized and the remainder of the body shielded. Portions of the ileum were protected by lead shields during the latter exposure for comparison of the characteristics of contiguous irradiated and shielded portions of the ileum.

The results of transport measurements with contiguous irradiated and shielded segments, 3 days following irradiation of the exteriorized intestine, are shown in Figure 1. The K_{ms} values for sodium and chloride were divided by the respective K_{ms} values for ^3HOH and are recorded as K'_{ms} . Included in Figure 1 are the averages of the PD's (serosal surface - mucosal surface) and the K_{ms} measurements for ^3HOH .

The segments were initially placed into normal bicarbonate-buffered media. Under this condition the PD's across the irradiated and shielded segments were similar and there was a slight depression in the K_{ms} values for tritiated water by the irradiated segments. The apparent absorption permeabilities of both the irradiated and shielded segments, for either sodium or

FIGURE 1. Effects of 1500 R X-Irradiation of the Exteriorized Intestine on ^{22}Na , ^{36}Cl , and 3HOH Transport and on Bioelectric Potential Measurements with Isolated Rat Ileum (Values shown are the average of data from four to six rats. The segments were first placed into normal bicarbonate buffer for PD and flux measurements and then were transferred into mannitol bicarbonate buffer ($[Na]$ 50 mM, $[Cl]$ 20 mM) for PD, flux, and S/M measurements.)

chloride, were about one-half the corresponding values for 3HOH . The effects of mannitol media on the PD and transport measurements for the shielded segments were similar to those that we have obtained for nonirradiated ileum. The K_{ms} values for chloride approached or exceeded those for 3HOH . Chloride absorption occurred against both opposing PD and concentration differences as indicated by the corresponding S/M values. The K_{ms} values for sodium approached and sometimes exceeded those for 3HOH . These results support previous observations of active

chloride absorption in the rat ileum. In contrast to the shielded segments, and in conjunction with the effects of the mannitol media on the ability of the irradiated segments to develop negative PD's, the K_{ms} values for the irradiated segments did not respond to the sodium and chloride concentrations in the mannitol media, and the S/M values for the irradiated segments were depressed.

Measurements of PD and K_{ms} were also made for ileal segments 3 days following 1500 R abdominal X-irradiation. Both irradiated and control rats were fasted during the interval from 24 hr prior to

irradiation until the rats were sacrificed. The body weights of both the irradiated and control rats dropped about 12% during this period. The results of this study are shown in Figure 2. In the first experiment, the effects of the mannitol media on ileal segments from the irradiated and fasted rats were tested; in the second, effects of lowering only the chloride concentration were tested by partial substitution of Na_2SO_4 for NaCl , reducing the chloride concentration to 20 mM. The effects of the mannitol media on the control and irradiated rat ileum were like those shown for the contiguous shielded and irradiated segments. In the sulfate media, K_{ms} values for chloride by the irradiated segments were

also depressed in comparison to K_{ms} values for chloride by the nonirradiated ileal segments.

During preliminary experiments, taurocholate concentrations as low as 0.2 mM were found to markedly alter the bioelectric potentials across isolated ileum. Since it has been demonstrated that a 6 mM taurocholate concentration will depress fluid transport by isolated rat intestine, this concentration was used in our initial transport studies. Ileal segments from three rats were exposed to the mannitol media, and segments from a second group of three rats were exposed to the sulfate media for PD and K_{ms} measurements. As shown in Figure 3, 6 mM taurocholate in the mucosal media had

FIGURE 2. Effects of 1500 R Abdominal X-Irradiation on ^{22}Na , ^{36}Cl , and ^3HOH Transport and Bioelectric Potential Measurements with Isolated Rat Ileum (Values shown are the average of data from six rats.)

FIGURE 3. Effects of 6 mM Taurocholate on ^{22}Na , ^{36}Cl , and ^3HOH Transport and Bioelectric Potential Measurements with Isolated Rat Ileum (Values shown are the average of data from six segments, two from each of three rats.)

essentially the same effect as irradiation on PD and K_{ms} measurements.

Conclusions

The effects of radiation on electrolyte absorption became more obvious when the sodium or chloride concentrations in the bathing media were reduced sufficiently to demonstrate changes in active ion transport relative to passive ion movement. Effects on the bioelectric potential differences were also more apparent when the intestine was placed into media with the sodium concentration reduced.

The similarity in effects caused by irradiation and the bile salt, sodium taurocholate, suggests that the depression of fluid and electrolyte absorption following X-irradiation may be, in part, secondary to a deleterious effect of bile salt on irradiated intestinal mucosa.

Reference

1. McKenney, J. R. "Electrolyte Transport and Voltage Measurements in a Preparation of the Irradiated Rat Ileum," *Proceedings of Symposium on Gastrointestinal Radiation Injury*, 1966. *Excerpta Medica Foundation*. (In Press)

VITAMIN B₁₂ ABSORPTION FROM THE INTESTINE OF THE IRRADIATED RAT

Vitamin B₁₂ absorption from the X-irradiated (1250 R) intestine was reduced to half of control value by a mechanism that was not dependent upon the availability of gastric intrinsic factor.

Investigator:
M. F. Sullivan

Technical Assistance:
Alma L. Crosby

We have previously shown that absorption from the intestine by active, passive, and facilitated transport mechanisms is decreased after exposure to radiation, although not all transport mechanisms are equally affected (Annual Report, 1965). Although the mechanism of B₁₂ absorption is not clearly understood, the effect of radiation on the transport of such a large molecule is of interest both for the light it may shed on the nature of the injury process and from a clinical standpoint when enteric administration of B₁₂ is considered after abdominal irradiation.

Observations

Rats were installed with an indwelling duodenal cannula through which test materials were administered, thus circumventing the loss of normal gastric function that occurs after irradiation. The abdominal region, including all of the intestine proximal to a terminal few centimeters of colon, was exposed to 1250 R. At 3 days after X ray, when function is known to be most severely impaired and when diarrhea was clearly evident, either ⁵⁷Co-labeled B₁₂ (50 µg) or labeled B₁₂ combined with rat gastric intrinsic factor (derived from half a rat stomach) was injected through the duodenal cannula.

The radioactivity not absorbed was measured in cumulative 5-day fecal collections. The radioactivity which was absorbed and retained was determined by whole-body counting at 5 and 10 days. Total recovery in the retained and excreted fractions was better than 90% in all experiments. Absorption data are shown in Figure 1.

FIGURE 1. Effect of Radiation and Intrinsic Factor (IF) on Gastrointestinal Absorption of Vitamin B₁₂ (Average of total-body counts on fifth day with standard deviation and number of animals shown in parentheses)

Conclusions

The results clearly indicate that:

- (1) absorption of vitamin B₁₂ is depressed at 3 days after 1250 R,
- (2) that absorption is stimulated

by the addition of exogenous intrinsic factor in both control and irradiated rats, and (3) that the functional defect caused by irradiation was not lessened by the addition of intrinsic factor.

LOCALIZATION OF ACID MUCOPOLYSACCHARIDES IN THE GOLGI COMPLEX OF INTESTINAL GOBLET CELLS

Localization of acid mucopolysaccharides in the intracisternal spaces at the mature face of the Golgi complex, in Golgi vacuoles, and in mucous droplets suggests that this complex is involved in synthesizing the final secretory product of the goblet cell. Such localization is further evidence for the existence of a functional as well as a morphological polarity in the Golgi complex.

Investigator:
J. D. Berlin

Technical Assistance:
R. R. Adee

The Golgi region of the intestinal goblet cell has long been considered the probable site of formation of the mucin droplets secreted by these cells. Recently, the Golgi complex was shown to be the site of sulfation of the mucopolysaccharides secreted by the intestinal goblet cell.⁽¹⁾ The present study utilized an acid mucopolysaccharide stain at the electron microscope level to further determine the extent to which the Golgi complex is involved in mucin droplet formation.

Observations

Tissue samples were obtained from the terminal ileum of Charles River strain rats and Hanford-Labco miniature swine. Representative electron

micrographs are shown in Figure 1 (rat) and Figure 2 (swine). The Golgi complex is a prominent structure in a mature goblet cell. It is composed of a stack of three or more parallel cisternae and usually exhibits a curved array. The terms "forming face" and "mature face" will be used to describe the convex and concave sides of the Golgi apparatus, respectively.

The Golgi cisternae at the forming face are dilated and their intracisternal spaces appear electron-transparent by the methods of preservation used in this study. Thorium particles, which are extremely electron-dense, are rarely observed within the cisternae at the forming face. The cisternae comprising the region between the forming face and the mature

FIGURE 1. This electron micrograph of rat ileum shows the localization of acid mucopolysaccharide in the Golgi region of a mature goblet cell. Thorium particles are found concentrated only in the cisternae at the mature face of the Golgi complex (MF), in Golgi vacuoles (V), and in mucin droplets (D). The possible coalescence of Golgi vacuoles to form the mucin droplets is indicated by unlabeled arrows. Osmium-fixed, methacrylate-embedded, and stained with thorium for acid mucopolysaccharides and with uranyl acetate and lead citrate to enhance contrast.

FIGURE 2. This micrograph shows acid mucopolysaccharide localization in the Golgi region of a mature goblet cell from swine ileum. The thorium particles are localized in the intracisternal spaces at the mature face (MF) of the Golgi complex, but not at the forming face (FF). Preparation similar to that of Figure 1.

face are similar in appearance to the cisternae at the forming face except that they are rarely dilated. The cisternae at the mature face are not dilated, but are frequently observed to contain a substance of moderate electron density and are stained intensely with thorium. The thorium

is found in the intracisternal spaces and does not appear to be attached to the limiting membrane. The cisternum at the extreme mature face is frequently dilated and appears to be forming Golgi vacuoles by a process of fragmentation. This cisternum and the Golgi vacuoles are also intensely

stained with thorium. That the Golgi vacuoles appear to coalesce with one another to form the larger mucin droplets with uniform concentrations of thorium is shown in Figure 1.

The staining mechanism of acid mucopolysaccharides by colloidal thorium is thought to result from the interaction of positively charged colloidal micelles with strong acidic groups, such as uronic carboxyl and/or sulfate carried by acid mucopolysaccharides. Although it is presently impossible to equate the sulfation which is known to occur in the Golgi complexes of these cells with thorium staining, it seems likely that these two phenomena may be related. If so, then sulfation of acid mucopolysaccharides is localized in the cisternae at the mature face of the Golgi complex.

Conclusions

The present investigation substantiates and extends our knowledge of the Golgi complex. It would appear that the intestinal goblet cell Golgi complex is composed of at least three, and possibly four, distinct morphological regions and that each region has a separate function. These regions are: (1) The forming face characterized by dilated cisternae containing no visible electron-dense materials.

These cisternae are generally thought to function as collection points for various materials, some of which are probably synthesized by the rough endoplasmic reticulum. (2) The mid-region zone, located between the forming face and the mature face, where the cisternae are not dilated and intracisternal material, as recognized by its electron density, is not present. The function of this region is not clear, but it may involve condensation of various materials collected at the forming face. (3) The mature face with nondilated cisternae which frequently contain a substance of moderate electron density. The localization of thorium within these cisternae is regarded as evidence that the materials may be sulfated at this location; certainly the materials are chemically modified in such a way as to give a reaction product with low pH thorium staining. (4) There may be a region immediately adjacent to the mature face composed of fragmenting cisternae which are in the process of forming Golgi vacuoles containing the secretory product.

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ONTOGENY AND X-RADIATION SENSITIVITY OF THE FLOUR BEETLE, *TRIBOLIUM*

Early egg stages were most sensitive to irradiation. Mature larvae of *T. castaneum* were more sensitive than younger larvae or more advanced stages. Lethal doses for adults of two strains of this species were 14-15 kR and sterilizing doses were 5 kR. *T. confusum* was more sensitive with adult lethality at 8 kR and sterility at 4-5 kR.

Investigator:
H. E. Erdman

Technical Assistance:
Ethel H. Jaschek

Information on relative radiation sensitivities of different stages in development is useful both for its practical application to pest control problems and for its value in identifying the nature of radiation damage in a differentiating organism. This report defines the radiation sensitivities of *Tribolium castaneum* and *T. confusum* following exposure at various stages in the life cycle, as measured by sterility and mortality.

Observations

Approximately 100 individuals were X-rayed at each of nine different stages during the life cycle. X rays were delivered at 1 kR/min by a generator operated at 30 ma, 250 kvp, 0.25 mm copper + 1.0 mm aluminum filtration, and a 2.5-in. target-subject distance. Culture conditions were 30 ± 1 °C and $70 \pm 10\%$ relative humidity. Virgin irradiated adults were crossed following exposure. An exposure was considered as sterilizing if no progeny developed within 2-3 months and as lethal if individuals failed to mature or died within 3-4 weeks posteclosion. Minimum doses resulting in sterility or lethality are shown in Figure 1.

FIGURE 1. X-ray Sensitivity During the Life Cycle of Species and Strains of Flour Beetles, *Tribolium*

Conclusions

The most susceptible link in the life cycle was the 1-3 hr old egg in which nuclei are rapidly dividing and undifferentiated. The radiosensitivity of somatic cells and gonad anlagen

diverged markedly during postlarval stages, presumably because: (1) germ cells remained at a radiosensitive stage in cell division, and (2) the increased differentiation of somatic tissue required greater exposures for lethality.

Increased sensitivity of older larvae of both the Brazil CI and sooty strains of T. castaneum suggests that

some selection of undamaged cells or repair of radiation damage may be occurring in the larval stages. This decline was not noted in T. confusum. This species was notably more sensitive to radiation and showed a progressive rise in minimum sterilizing and lethal exposures throughout the life cycle.

EFFECTS ON FLOUR BEETLES IRRADIATED AS PUPAE

Incomplete metamorphosis and reduced adult life span were observed when pupae of the flour beetle Tribolium confusum were X-irradiated.

Investigator:

H. E. Erdman

Technical Assistance:

Ethel H. Jaschek

During metamorphosis there is an extensive reorganization of insect tissue such that radiation damage at the cellular level might show minimal expression in the resulting adult. This investigation was undertaken to determine whether metamorphosis and length of life could be altered when flour beetle pupae were X-irradiated.

Observations

T. confusum Duval ('Chicago Standard' stock) pupae, age 25-26 days, were sexed and irradiated. The X-ray unit operated at 250 kV, 0.86 mm copper half value layer, 0.25 mm copper and 1.0 mm aluminum filtration. The anode was 2.5 in. from the subject

and the apparatus delivered approximately 1 kR/min. Doses were 0, 1, 2.5, 5, 7.5, 10, 20, 30, 40, 50, 60, and 70 kR. Each of the five replicates at each dose contained ten pupae of each sex in 10 g food. Deaths were noted twice weekly. Culture conditions were $29 \pm 1^\circ\text{C}$ and 60 to 75% relative humidity. Lethality data for males and females were combined since no difference in effect was found between sexes. Results are shown in Figure 1. Dose-response curves omitted fall in the expected intermediate positions.

Emergence of adults from pupae given 1 or 2.5 kR was comparable to that of controls. Incomplete metamorphosis increased progressively

FIGURE 1. Percent Lethality During Adult Life of *Tribolium Confusum* X-rayed as 25 to 26-Day-Old Pupae (Numbers on the curves refer to the dose in kR)

for doses from 5 to 70 kR, but in no case was metamorphosis delayed. Partial metamorphosis occurred in some individuals given 5 kR and greater doses; i.e., thorax and head

were adult but the elytra, although pigmented, was pupal in size and position and the abdomen retained pupal characteristics. Some individuals given 30 kR and greater were unable to cast off their meconium. Death probably resulted from intestinal obstruction and not from starvation since beetles irradiated as adults continue to ingest food even following heavy exposures.

Conclusions

Reduction in length of life of adults irradiated as pupae was a sensitive indicator of radiation damage. Mortality increased progressively with increased exposure. We can only speculate on causes of early death and presume a relationship to somatic mutation.

DIMETHYLSULFOXIDE TOXICITY FOR SEEDS

Toxicity of DMSO was more clearly indicated by growth than by germination data. Data suggest that toxic effects would severely limit the usefulness of DMSO as a synergist for fertilizers, insecticides, etc.

The exceptional miscibility of dimethylsulfoxide (DMSO) with organic and inorganic chemicals suggests its possible usefulness as a synergist for fertilizers, insecticides, etc. This report is concerned with two studies of DMSO toxicity on seeds

Investigator:
H. E. Erdman

Technical Assistance:
Ethel H. Jaschek

and seedlings of four economically important crops.

Observations

Seeds of kidney beans (*Phaseolus vulgaris*), peas (*Pisum sativa*),

barley (*Hordeum vulgare*), and rye (*Secale cereale*) were placed in petri dishes containing a series of concentrations of DMSO in distilled water. Incubation was in the dark at 20 °C. Measurements were made on the sixth day. Data of Table I show that no germination occurred on 10% or higher concentrations of DMSO. Plumule growth was the most sensitive to DMSO with retardation evident at all concentrations tested.

Kidney bean seedlings grown in standard nutrient solution wilted within one-half hour following addition to the solution of DMSO at concentrations of 2% or more. After 3 days, wilting was evident at DMSO concentrations as low as 1%, the degree of effect increasing with greater concentrations of DMSO. There was no evidence of recovery during the 3 days of the test.

The uptake of calcium, magnesium, potassium, and phosphorus appeared to have been stimulated by DMSO when measured in terms of wet weight of

plant tissue. However, when measured in terms of dry weight, no significant changes occurred except for potassium which showed a slight increase after 48- and 96-hr exposures. This difference between wet and dry weight analyses is a reflection of the wilting and suggests that the plant either lost capacity to absorb water or to retain water, or both.

Conclusions

Growth rather than seed germination was the most sensitive indicator of DMSO toxicity in these plant species. Although the mechanism(s) of toxicity remains uncertain, alteration of physiological processes such as poisoned metabolic pathways and modified cell permeability seem more likely than chromosomal changes associated with nuclear effects. For agricultural use DMSO concentrations greater than 0.1% are contraindicated.

TABLE I. Germination and Growth Characteristics of Seeds Subjected to DMSO for 6 Days

	% DMSO			
	0	1	5	10
% Seed germination				
Beans	100	84	90	0
Peas	98	100	94	0
Rye	84	94	88	0
Barley	94	96	94	0
Root length (cm)				
Kidney beans	3.7 ± 0.3*	4.0 ± 0.3	2.6 ± 0.3	
Peas	4.1 ± 0.2	4.2 ± 0.2	2.0 ± 0.01	
Plumule height (cm)				
Rye	6.5 ± 0.6	4.5 ± 0.4	2.1 ± 0.2	
Barley	7.0 ± 0.3	3.4 ± 0.2	1.8 ± 0.1	

* Standard error.

TOXICITY OF RADIOELEMENTS

Most of the research conducted in the Biology Department is concerned in one way or another with the toxicity of radioelements. This section of our Annual Report does not include the major effort on inhalation studies, nor the group of studies related to problems of radionuclides in space programs; these are included in separate sections which follow. Several reports on the toxicity and metabolism of radionuclides in aquatic organisms are also separately grouped with related environmental studies.

The first five reports in this section are concerned directly or indirectly with our major continuing study of ^{90}Sr toxicity in miniature swine. A year ago, we were impressed with the high incidence of leukemia and the total absence of bone tumors in certain chronic exposure groups. During the past year, a number of bone tumors have been diagnosed. The complete picture will, of course, not be available for several more years. While awaiting these developments we are making detailed hematopoietic studies, searching for chromosomal aberrations, comparing ^{90}Sr metabolism in pygmy goats, and studying marrow and blood cell phospholipid turnover in ^{90}Sr -burdened rats. Although not represented in these reports, progress is also being made in developing tissue culture and electron microscopy techniques for application to the basic problem of leukemogenesis.

A single report summarizes the current status of the 16-year program of studies of radioiodine in sheep and swine. Although now reduced largely to a "holding" operation, some additional work was initiated to permit better comparison of the results from sheep and swine.

The remaining seven reports in this section describe studies on the deposition, retention, and biological effect of a variety of radionuclides in rats. This represents something of a resurgence of activity in a field we had somewhat neglected for several years past. Five of these reports deal, in one way or another, with effects of age on radionuclide metabolism or toxicity.

EFFECTS OF STRONTIUM-90 IN MINIATURE SWINE - SEVENTH PROGRESS REPORT

Hematopoietic and lympho-reticular tissue neoplasms continue to occur in miniature swine exposed to ^{90}Sr by daily ingestion, particularly at the 125 $\mu\text{Ci/day}$ level. Of special interest has been the development of bone tumors in this same group after times approximately the same as those required for development of the hematopoietic tissue neoplasms. The distribution of ^{90}Sr among various skeletal parts appears to be independent of body burden, age, or generation.

Investigators:
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Patricia L. Hackett
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J. L. Palotay

Previous progress reports have described the experimental design and the biological effects of ^{90}Sr fed daily to miniature swine at levels of 0, 1, 5, 25, 125, 625, or 3100 $\mu\text{Ci/day}$ (Annual Reports, 1959 et seq.). At levels of 25 $\mu\text{Ci/day}$ and less there have been no definitive changes observed in the formed elements of the blood. At levels greater than 25 $\mu\text{Ci/day}$, there has been a progressive decline in leukocytes and platelets, and a terminal precipitous drop in the red blood cells. In addition, there have been numerous cases of leukoproliferative disorders, particularly in the 125- $\mu\text{Ci/day}$ group of swine at 2-4 years of age, and in the offspring of dams receiving 625 $\mu\text{Ci/day}$. These disorders have ranged from frank leukemias, lymphosarcomas, and reticulum

cell sarcomas to localized myeloproliferative lesions (Annual Report, 1965).

Observations

During the past year, 14 miniature swine on the ^{90}Sr study died or were killed because of a deteriorating clinical condition. Table I lists pertinent details on each of these animals. Three additional cases of leukoproliferative disorders were diagnosed this year in the 125- $\mu\text{Ci/day}$ group, making a total of 21 confirmed cases of leukemia and related diseases from a total of 45 animals over 2 years of age (16 in this group are still living). There were 2 cases of benign uterine neoplasms; one in a control and another in a 1- $\mu\text{Ci/day}$ swine. Another uterine

TABLE I. Deaths Among ^{90}Sr Swine During 1966^(a)

Pig, number	Age, years	Feeding level, $\mu\text{Ci/day}$	Est. dose to skeleton, rads	Comments
299	7.0	0	None	Clinical - observed down, died within hour Necropsy - uterine tumor, 14 kg Histology - leiomyoma
471	5.9	1	300	Clinical - anorexia 6 days; obstipation Necropsy - pneumonia and gastritis Histology - no significant lesions
480	6.0	1	300	Clinical - anorexia 1 day; moribund Necropsy - uterine tumor Histology - leiomyoma
470	6.2	1	300	Clinical - flacid posterior paralysis, 1 day Necropsy - subdural cerebral congestion Histology - no significant lesions
325	6.9	1	350	Clinical - lymphocytosis; anorexia 4 days; died Necropsy - abdominal neoplasm Histology - lymphocytic leukemia
28	8.0	1 ^(b)	350	Clinical - large palpable abdominal mass Necropsy - abdominal tumor, 8.5 kg Histology - metastasizing adenocarcinoma (possibly from uterus)
407	6.7	25	8,500	Clinical - anorexia 1 day; died Necropsy - chronic enteritis Histology - not complete
1678	1.9	125	12,000	Clinical - anorexia 5 days; necrotic stomatitis Necropsy - necrotic periodontitis and stomatitis Histology - osteosarcoma
1675	2.0	125	12,000	Clinical - sporadic copious vaginal hemorrhage; died Necropsy - uterine hemorrhage Histology - lymphogranulomatous disease resembling Hodgkin's disease
1255	3.2	125	20,000	Clinical - leukocytosis; anorexia Necropsy - soft tissue cellular infiltration Histology - granulocytic leukemia
1228	3.8	125	22,000	Clinical - anorexia and vomiting; died Necropsy - icterus; peritonitis Histology - hematopoietic crisis; no neoplasia observed

(a) Five additional control swine were killed for other studies.

(b) Started at age 9 months, all others exposed in utero.

TABLE I. (Contd)

Pig, number	Age, years	Feeding level, $\mu\text{Ci/day}$	Est. dose to skeleton, rads	Comments
1258	3.9	125	23,000	Clinical - weight loss last 2 months; died Necropsy - pericarditis; mild general hemorrhage Histology - vegetative endocarditis and lymphocytic leukemia
288	7.3	125 ^(b)	42,000	Clinical - progressive emaciation; no anorexia Necropsy - serous atrophy of fat; pulmonary consolidation Histology - not complete
1061	4.5	(c)	(c)	Clinical - progressive anorexia 2 weeks; paralysis Necropsy - neoplasm associated with dura Histology - meningioma; osteosarcoma

(b) Started at age 9 months, all others exposed in utero.

(c) See text.

neoplasm in a 1- $\mu\text{Ci/day}$ animal was diagnosed as an adenocarcinoma with metastases. These three tumors were in animals 6 to 8 years of age and were, in all probability, not radiation-induced.

Bone tumors, diagnosed for the first time in this study this year, are of particular interest. Table II summarizes these cases of osteosarcomas and giant cell tumors. A representative radiogram of the lesion found in one skull is shown in Figure 1. With the exception of animal 1061, these bone tumors have all occurred in the 125- $\mu\text{Ci/day}$ group, between 2-3 1/2 years of age. Exposure to ^{90}Sr started in utero. Average skeletal radiation dose at death was estimated to be 12,000-20,000 rads. In five cases, the neoplastic lesion occurred in the skull. In animals 1063 and 1068, there were tumors in other bones also, but

no evidence of metastases to soft tissue. Animal 1061 was born to a dam ingesting 625 $\mu\text{Ci } ^{90}\text{Sr/day}$. Consequently, it was exposed in utero and to ^{90}Sr from the dam's milk. After weaning, at 6 weeks of age, it was fed 156 $\mu\text{Ci/day}$ for one month, and then was removed from ^{90}Sr feeding. At this time, the estimated average skeletal dose was 5,000 rads. This animal lived for an additional 4 years before developing an osteogenic sarcoma in the ulna.

At necropsy, each skeleton is separated into individual bones, cleaned of flesh, ashed, and radioanalyzed to determine terminal body burden of ^{90}Sr . The percent distribution of ^{90}Sr in the bones of swine, representing both original dams and their F_1 and F_2 offspring, is shown in Table III. Original females were started on daily ingestion of ^{90}Sr at 9 months of age; offspring F_1 and F_2 received ^{90}Sr in

TABLE II. Resume of Miniature Swine That Have Developed Bone Tumors While Ingesting ^{90}Sr

Pig, number	Age, years	Feeding level, $\mu\text{Ci/day}$	Dose to skeleton, krads	Description of neoplasia (a)
1061	4.5	625	(b)	OS - ulna
1063	2.8	125	15	OS - skull - rib GCT - mandible Gran. leuk.
1065	3.5	125	20	OS - skull Gran. leuk.
1068	3.2	125	20	OS - skull GCT - mandible Gran. leuk.
1254	2.8	125	15	OS - skull Gran. leuk.
1678	1.9	125	12	OS - skull

(a) OS = osteogenic sarcoma, GCT = giant cell tumor, Gran. leuk. = granulocytic leukemia.

(b) Exposed in utero, from dam's milk, fed 156 $\mu\text{Ci } ^{90}\text{Sr/day}$ for one month and then discontinued. No estimate of dose.

FIGURE 1. Representative Radiogram of Lesion in the Skull

utero, via the milk, and finally were sequentially brought to the same daily feeding level as their dam. From these data, it appears that within the limits of the sample preparation and radioanalysis methods employed, distribution

of ^{90}Sr among the various bones is relatively constant regardless of age, body burden, or generation. This observation, however, awaits verification by statistical analysis. Detailed analysis of ^{90}Sr concentration in teeth, compared to skeletal concentration, is underway and will appear in a future progress report. Radioanalysis of individual teeth is complicated by the tartar accumulation which tends to have a high ^{90}Sr concentration.

The distribution of skeletal ^{90}Sr in a hypothetical "average" miniature swine is shown in Table IV. This is a compilation of data from 27 animals. Note that over 30% of the body burden is in the head.

Conclusions

It can now be stated that chronic feeding of ^{90}Sr at the 125- $\mu\text{Ci/day}$

TABLE III. *Skeletal Distribution of Chronically Ingested ^{90}Sr in Representative Miniature Swine*

Pig number	Original sows				F ₁ offspring			F ₂ offspring			
	125	488	291	1345	453	804	795	802	699	1063	1073
μCi daily fed	5	25	125	625	25	125	125	5	25	125	125
Years on feed	3.0	3.8	1.8	0.5	2.0	1.0	3.0	2.0	3.0	2.6	2.2
μCi in body	31	200	820	2590	124	750	1350	45	265	1170	1500
% in ribs	10	11	10	10	9.9	13	8.3	12	8.8	12	10
% in scapula	4.3	3.4	4.2	3.8	3.6	3.4	4.2	4.3	4.2	4.6	4.4
% in pelvis	5.3	5.7	5.0	5.8	4.7	4.4	5.6	5.2		6.2	5.5
% in sternum and tail		0.7		0.9			0.9	1.6	0.8	1.0	0.5
% in lumbar vertebrae	5.8	5.0	6.0	5.6	3.4	4.8	5.8	5.6	4.2	5.2	5.2
% in thoracic vertebrae	6.1	5.2	9.1	9.1	9.8	6.1	5.2	7.8	6.5	6.5	6.1
% in hind limbs	11	10	8.0	11	9.5	12	12	12	9.6	13	14
% in fore limbs	11	10	7.9	11	11	10	11	12	10	11	10
% in mandible and teeth	13	11	14	10	15	12	13	12	12	10	11
% in skull and teeth	17	19	22	18	20	19	20	18	19	17	18
% remainder	17	19	14	17	14	16	15	10	25	14	14

TABLE IV. *Skeletal Distribution of Chronically Ingested ^{90}Sr in the "Average" Miniature Swine (Data from 27 animals)*

Ribs-----	10%
Scapula-----	4%
Pelvis, sternum and tail-----	6%
Vertebrae-----	12%
Legs-----	22%
Head-----	31%
Other (neck, feet, etc.)-----	15%

level will produce bone tumors in miniature swine, as well as hematopoietic tissue tumors. While diagnosis of the bone tumors came later than diagnosis of the hematopoietic neoplasms, the "latency" period for both tumors appears to be approximately 2-4 years, after an accumulated average dose to the bone of 12,000-20,000 rads. As the animals that are receiving less than 125 $\mu\text{Ci}/\text{day}$ accumulate doses approximating 20,000 rads, it is quite possible that they, too, will show an increased tumor incidence.

RADIOSTRONTIUM STUDIES IN PYGMY GOATS

Pygmy goats have ingested 125 μCi of ^{90}Sr daily for 13 months without clinical evidence of radiation damage except for a 50% reduction in leucocytes. The goats are still accumulating ^{90}Sr in contrast to swine which reach equilibrium in 6-9 months.

Investigators:
C. R. Watson
H. A. Ragan

A group of pygmy goats is being fed ^{90}Sr daily for comparison with our radiostrontium studies in miniature swine. The daily dose of 125 μCi was chosen as possibly comparable to a 625 $\mu\text{Ci/day}$ dose in miniature swine, which results in death of the swine in about one year with a characteristic hemorrhage syndrome. Since the goats are alive and apparently healthy after 13 months, it is possible that the 125 $\mu\text{Ci/day}$ dose may lie in the leukemogenic range, which would also be most interesting, since, if leukemia could be induced in ruminants, studies of possible viral transmission via milk would be facilitated.

Observations

Five of seven male pygmy goats, aged 1 to 2 years at the start of the experiment, are still alive after 13 months of daily ingestion of 125 μCi ^{90}Sr . The death of one goat 9 months into the experiment was apparently not due to radiation damage. Another goat was sacrificed at 1 year. Tissues of these animals were grossly normal. Histopathologic examination is not complete, nor is skeleton radioanalysis. Estimates of body burdens from whole-body counts are 500

and 800 μCi , respectively. The clinical blood picture (Figure 1) has not changed markedly, except for a steady decline in leucocytes, perhaps indicative of radiation damage to bone marrow. After a year, whole-body counts of the goats are still increasing (Figure 2). This is in contrast to the situation in miniature swine which attain an equilibrium ^{90}Sr body burden of about ten times the daily intake within 6-9 months. The goats appear to be in good physical condition and their average weight has increased from 20.8-22.8 kg.

FIGURE 1. Clinical Blood Picture of Pygmy Goats Ingesting 125 μCi ^{90}Sr Per Day

FIGURE 2. Strontium-90 Burden of Pygmy Goats Ingesting 125 μCi ^{90}Sr Daily (Based on approximate calibration of total body counts)

Conclusions

From these very preliminary results, one can conclude little more than that goats are not pigs. It does seem clear that goats either absorb a smaller percentage of strontium from their diet or retain it less tenaciously than miniature swine.

HEMATOPOIESIS IN SWINE CHRONICALLY INGESTING STRONTIUM-90

Hemograms of swine ingesting high levels of ^{90}Sr daily for prolonged periods of time indicate that granulocytes are damaged more than erythrocytes. Hematopoietic studies suggest that granulocyte precursors may spend more time in the radiation sensitive phases of the generation cycle than do erythrocyte precursors. This could result in subnormal functional integrity of mature neutrophils or the inability of their early forms to increase proliferation rates as do erythroblasts.

Investigator:
P. H. Cooper

The numbers of circulating erythrocytes in swine ingesting high levels of ^{90}Sr daily for prolonged periods of time are maintained within normal limits, while blood neutrophil counts steadily decline (Annual Report, 1962). Histopathologic evidence indicates depletion of hematopoietic marrow, with the residual marrow restricted primarily to the sternum, ribs, and vertebrae. In the absence of extensive extramedullary hematopoiesis, maintenance of the normal red blood

cell count is difficult to explain. Hematopoietic studies to examine this inconsistency have been partially completed on a limited number of swine in the control group and in those animals chronically ingesting 125 and 625 μCi ^{90}Sr per day.

Observations

One thousand marrow cells, obtained by sternal puncture, were classified as to cell type on at least three

Wright's-Giemsa stained smears, while 5000 cells were examined for the presence of mitotic figures. Shortly after aspiration, marrow was incubated at 37 °C for 1 hr with 1 μ Ci/ml of tritiated thymidine. Determination of cell class and presence of label over the nucleus was noted on 1000 cells of the developed and stained autoradiograms.

Table I shows the proportion of immature erythrocytes and granulocytes expressed as percent of the total number of cells, the frequency of division, and uptake of tritiated thymidine (i.e., DNA synthesis) in these immature cells. Assuming that the proliferating cells are randomly distributed throughout the generation cycle, the fraction of time spent in any one stage is equal to the fraction of cells in that stage. Table I shows that erythroblast mitoses are more frequent than granuloblast mitoses in marrow from all animals. If immature erythroblast and granuloblast generation times are approximately equal in the pig, as they are in humans and dogs, then mitosis in

the erythrocyte series lasts longer than mitosis in the granulocyte series, and, using the same reasoning, their DNA synthesis times are approximately equal. The remainder of the cell cycle ($G_1 + G_2$) must last longer in the granulocyte series than it does in the erythrocyte series (Table II). Since both G_1 and G_2 are more sensitive to ionizing radiation than are DNA synthesis or mitosis, immature granulocytes might be damaged more than immature erythrocytes.

Selective damage to granulocyte precursors, resulting in a greater than normal percentage of erythrocyte precursors in the marrow, was not seen. Damage and destruction of myelocytes, resulting in a subnormal percentage of maturing and mature granulocytes, was also not seen. Thus, selective damage to granulocytes must be realized after exit from the marrow, perhaps by a decreased functional integrity leading to a decreased life span.

Studies are in progress to determine the time taken to complete various stages of the generation cycle in both erythrocyte and granulocyte precursors.

TABLE I. Immature Marrow Cells and Their Frequency in Mitosis and DNA Synthesis

Treatment	Number of animals	Granulocytes			Erythrocytes		
		Immature cells, as % of total cells	Percent of immature cells		Immature cells as % of total cells	Percent of immature cells	
			Dividing	Labeled		Dividing	Labeled
Control average	3	8.4	8.5	36	10	1.2	32
125 μ Ci 90 Sr/day	Alive	12	7.0	33	12	1.4	30
		7.2	11	20	12	2.4	32
	Dead	7.0	14	30	13	2.0	20
		11	6.8	16	14	1.4	19
		22	6.5	33	17	2.5	23
	625 μ Ci 90 Sr/day Dead	12	11	31	5	2.5	36

TABLE II. Generation Time of Immature Marrow Cells

	<u>Erythrocyte series</u>	<u>Granulocyte series</u>
Total generation time	100%	100%
Mitotic time	8%	1%
DNA synthesis time	36%	34%
(G ₁ + G ₂) time	56%	65%

Information concerning neutrophil blood transit time will also be obtained.

Increased rate of erythropoiesis without an apparent increase in marrow elements could result from an increased proliferation rate, i.e., a decreased generation cycle. A decreased generation cycle would not cause any differences in the normal differential classification, mitotic, or labeling indices if all stages of the generation cycle were equally decreased.

Studies are being planned to determine the generation cycle of hematopoietic cells in ⁹⁰Sr-fed swine. Other hematopoietic studies indicated to support and extend available information are: (1) volume and cellularity of remaining hematopoietic and extramedullary sites, (2) life span of

mature neutrophil and erythrocytes, and (3) marrow cell transit times.

Conclusions

These preliminary observations indicate that immature granulocytes spend more time in the radiation sensitive stages of the generation cycle than do erythroblasts. This implies that ⁹⁰Sr marrow exposure may damage cells in the granulocyte series more than those in the erythrocyte series. This could explain the subnormal neutrophil counts and normal erythrocyte counts seen in these swine that are ingesting ⁹⁰Sr daily. Studies in progress will supply information concerning generation time of erythrocyte and granulocyte precursors and the blood transit times of neutrophils.

CHROMOSOME STUDIES IN MINIATURE SWINE AND GOATS

Leukocytes of peripheral blood and marrow were cultured and spreads prepared for study of chromosomal aberrations following daily ingestion of ^{90}Sr by Pitman-Moore swine and African Pygmy goats.

Investigators:
Patricia L. Hackett
H. A. Ragan

Technical Assistance:
T. K. Andrews

The induction of chromosomal aberrations in peripheral blood and marrow leukocytes by ionizing radiations and the appearance of chromosome abnormalities in certain human blood dyscrasias have stimulated interest in comparable studies in miniature swine and goats on a regimen of daily ^{90}Sr feeding. A description of hematopoietic tissue neoplasms and myeloproliferative lesions in the miniature swine appeared in an earlier report (Annual Report, 1965).

Observations

The culture of peripheral blood leukocytes and preparation of chromosome spreads from blood and marrow were performed by conventional methods. Medium containing NCTC 109 and fetal calf serum was found to support good cellular growth of both swine and goat blood.

The karyotype of a male ^{90}Sr -exposed, Pitman-Moore swine is shown in Figure 1. The somatic chromosomes of these swine were found to be very similar to those breeds of domestic pigs classified by McConnell et al.⁽¹⁾ The long constriction of both members of pair 13 appear to be constant in Pitman-Moore swine while the long constriction in pair 8, reported in other breeds of swine, was found in only one of our animals.

Gross chromosomal aberrations were observed in spreads obtained from sam-

ples of blood or marrow from swine with evident peripheral blood dyscrasias at the time of death. Decreased erythrocyte values were a constant observation in these animals as was the presence of immature cell forms. Leukocyte and platelet levels were variable. All of the animals in which gross abnormalities were detected were either exposed in utero with a subsequent feeding level of 125 μCi ^{90}Sr /day for 39 to 44 months or had been fed the same level of ^{90}Sr for 72 months.

Figure 1 shows a weak gap in one member of pair 17 while Figure 2 shows a strong gap in the same pair as well as a gap in one chromosome of pair 5. The gaps in pair 17 have been observed only in animals receiving ^{90}Sr but sufficient control animals have not been studied to attribute this effect to radiation. A very large size discrepancy is also evident in the largest metacentric pair of Figure 2.

The classification of chromosomes from Pygmy goats (Figure 3) is based on size alone since all chromosomes are morphologically similar. Evans⁽²⁾ studies on the determination of the sex chromosomes by tritium-labeling the late-labeling X chromosome suggest that at least one of the members in the goat is in the third longest pair. These experiments have not been performed with the Pygmy goat. Only a few observations have been made on the Pygmy goats, but

FIGURE 1. Chromosomes from a Male Pitman-Moore Swine on a ^{90}Sr -Feeding Regimen Showing Elongation in One Member of Pair 8 and Gap in One Member of Pair 17 ($n = 38$)

FIGURE 2. Chromosomes from a Male Pitman-Moore Swine on a ^{90}Sr -Feeding Regimen Showing Gaps in Both Members of Pair 17 and One Member of Pair 5

FIGURE 3. Somatic Chromosomes from a Normal Cell of a Male African Pygmy Goat ($n = 60$)

no chromosomal aberrations were detected in the marrow of one animal after 12 months on the $125\text{-}\mu\text{Ci } ^{90}\text{Sr/day}$ feeding regimen.

Conclusions

The observations reported here are of a preliminary nature and indicate that definitive correlations of ^{90}Sr level and associated blood dyscrasias, with aberration frequencies, should be attempted. Observations of pre-exposure karyotypes from direct marrow preparations are needed to evaluate the persistence of abnormal clones. Hopefully,

the appearance of an abnormal spread may be an early indicator of the presence of myeloproliferative lesions. Future experiments will include in vivo and in vitro studies of the kinetics of tritium labeling of chromosomes in control and ^{90}Sr -fed animals for detection of alteration in DNA replication.

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METABOLISM OF ^{14}C -CHOLINE IN MARROW AND BLOOD CELL PHOSPHOLIPIDS OF STRONTIUM-90 BURDENED RATS

Rats burdened with various levels of ^{90}Sr by repeated injection were given a single injection of ^{14}C -labeled choline. Subsequent appearance of ^{14}C in three phospholipid fractions from marrow and blood cells showed no effects attributable to irradiation of marrow by ^{90}Sr . Some conclusions are drawn relative to phospholipid kinetics in blood and marrow cells.

Investigators:
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Linda G. Smith

The voluminous studies of radiosensitivity of the hematopoietic system have usually employed large doses of radiation which result in acute reactions. Lower doses over longer periods may affect hematology only slightly, but eventually lead to such long-term effects as leukemias and lymphosarcomas, as in our chronic ^{90}Sr studies in miniature swine. The present study sought to determine whether ^{90}Sr body burdens, which do not induce measurable hematopenia, may induce some subtly measurable hyperactivity in the hematopoietic tissues which compensates for blood cell destruction.

Observations

Four groups of young rats were injected intraperitoneally with 0.00, 0.02, 0.10, and 0.50 μCi ^{90}Sr per 100 g body weight, twice weekly, for 14 weeks. Ten days after the last ^{90}Sr injection, each rat received 50 μCi methyl- ^{14}C -labeled choline. Two rats from each group were sacrificed at 1.5, 13, 24, 48, and 100 hr, and at 6, 13,

20, and 27 days after ^{14}C -choline injection. Urine and feces collections from three rats of each group were made throughout the experimental period.

The ^{90}Sr body burden changes are shown in Figure 1. There was no effect of ^{90}Sr burdens on growth. Table I summarizes the hematologic data. Total white cells and lymphocytes were depressed in the ^{90}Sr rats as compared with controls, but there were no statistically significant differences among the irradiated groups. No extramedullary hematopoiesis in the ^{90}Sr rats was indicated by the available histological data.

The behavior of ^{14}C -choline was not significantly affected by the differing

FIGURE 1. Plan of Experiment and ^{90}Sr Body Burdens Attained

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TABLE I. Average Blood Values for All Rats at Sacrifice

	Semiweekly ^{90}Sr injection rate ($\mu\text{Ci}/100 \text{ g body weight}$)			
	0	0.02	0.10	0.50
No. of rats	16	17	16	18
Hematocrit (%)	35	35	35	34
Hemoglobin (g %)	13.9	13.7	13.5	13.4
RBC ($\times 10^6 \text{ cells/mm}^3$)	6.63	6.56	6.44	6.43
WBC ($\times 10^3 \text{ cells/mm}^3$)	5.85	4.46(a)	4.37(a)	3.63(a)
Differential leukocyte counts ($\times 10^3 \text{ cells/mm}^3$)				
Neutrophils	1.78	1.42	1.53	1.03
Lymphocytes	3.98	2.95(a)	2.75(a)	2.59(a)
Mononucleocytes	0.03	0.02	0.04	0.02
Eosinophils	0.05	0.04	0.06	0.04

(a) Strontium-90 groups statistically different from controls ($P < 0.01$).

body burdens of ^{90}Sr present in the different groups of rats; thus, the principal objective of the experiment was not realized. Pooled data from all groups on ^{14}C activity in plasma, blood cells, and marrow cells are shown in Figure 2. Partition of this activity between aqueous and organic phases according to the method of Folch et al.⁽¹⁾ showed a shift of ^{14}C with time to the organic phase. Within 48 hr, 80% of the plasma ^{14}C was in the organic phase. Blood cell ^{14}C activity was better than 90% in the organic extract within 24 hr. Movement of ^{14}C to the organic phase was much slower in marrow cell extracts, reaching 75% only after 6 days, suggesting a marrow pool of water-soluble intermediates.

Aliquots of the chloroform-methanol (Folch) extracts of marrow and blood cells were further separated into phospholipid fractions by thin-layer chromatography, utilizing Silica Gel G and a chloroform-methanol-water (5:5:1 -

FIGURE 2. Incorporation and Retention of ^{14}C in Plasma, Marrow, and Blood Cells of Rats Following Injection of ^{14}C -Choline

V:V:V) solvent system. Fractions were obtained which consisted of lysophosphatidyl choline (LPC), sphingomyelin (SM), and phosphatidyl choline (PC). Phosphatidyl choline was the predominant phospholipid in both marrow and blood. Peak specific activity of PC ($\text{c/min } ^{14}\text{C}/\mu\text{g P}$) occurred at 40 and

73 hr after ^{14}C -choline injection for marrow and blood cells, respectively. This difference suggests that release of marrow cells to the blood may be delayed for about 33 hr following maximal PC synthesis. Sphingomyelin behaved similarly. Maximum specific activity of marrow and blood SM occurred at 60 and 80 hr, respectively, suggesting a 20-hr delay in release of marrow cells following maximum SM synthesis. Lyso-phosphatidyl choline behaved similarly in marrow but showed a markedly different picture in blood. Specific activity peaked in blood 33 hr before it peaked in marrow, and showed, in blood, a more rapid turnover rate than was observed for the other phospholipid fractions. This would suggest extramedullary synthesis and cellular uptake of LPC.

Conclusions

The levels of ^{90}Sr employed in this study were too low to elicit any measured effects on phospholipid metabolism,

although total leukocytes and lymphocytes were depressed. With regard to the normal kinetics of marrow and blood cell phospholipids, the data obtained are compatible with the concept that PC and SM of blood cells are derived predominantly from synthesis within the marrow. This is not true for LPC which one may presume is also synthesized in the liver and incorporated by the blood cells from blood plasma.

The turnover kinetics indicate that all three of the choline-containing phospholipids in marrow are synthesized at about the same rate, have about the same probability of incorporating ^{14}C -choline, and show similar turnover behavior. This would suggest that these phospholipids are derived from the same metabolic pool and are carried from the marrow by the same population of cells.

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EFFECTS OF RADIOIODINE IN SHEEP AND SWINE

There was a 36% incidence of thyroid adenomas in 125 sheep thyroids examined following various ^{131}I dosing regimens. Only 10% of 38 control thyroids contained adenomas. A partial thyroid ablative dose of ^{131}I was given a group of young miniature swine for a comparative study of the long-term effects.

Investigators:

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Technical Assistance:

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Bustad et al. have reported on acute and chronic effects of ^{131}I in sheep observed over the past 16 years at this laboratory (Annual Reports, 1951 et seq.).⁽¹⁾ A primary objective of these continuing studies has been to ascertain if any increase in thyroid neoplasms occurred in the sheep on long-term studies. Included in this report is a summary of observations, to date, in this area of our investigations. Also included is the description of a newly initiated study in miniature swine which should provide data for comparison of long-term effects in sheep and swine.

Observations

Table I summarizes thyroid and pituitary weights and tumor incidence in sheep on various ^{131}I treatment regimens. Although there was considerable individual variation in thyroid weights, even in the control group, there was a marked decrease in thyroid weight at all ^{131}I treatment levels above 0.15 $\mu\text{Ci/day}$. Much of the weight variation in treatment groups was due to large cysts and/or adenomas. In the 3000- μCi single-dose group, many of the thyroids were reduced to small foci of glandular tissue interspersed in fat and fibrous tissue impossible to weigh meaningfully.

Histologically, thyroid damage consisted of fibrosis, atrophy, areas of necrosis, calcification, hemorrhage, and edema, and the formation of tumors. Thyroid damage was slight, if evident at all, at levels below 5 $\mu\text{Ci } ^{131}\text{I/day}$, slight to moderate in the 5- μCi group, and moderate to severe at 45 and 135 $\mu\text{Ci/day}$. Most thyroids were severely damaged in the animals that received 3000 μCi of ^{131}I in a single dose.

The tumors were classified as follicular, trabecular, or papillary. Follicular patterns were most common and were subdivided as micro and macro. In some glands there was a mixture of all adenoma types. These benign tumors were usually well encapsulated; there were only four cases of capsular and vascular invasion with pre-cancerous cells present. The one case of malignant fibrosarcoma has been previously reported by Bustad et al.⁽²⁾ Tumors were not observed in animals younger than 5 years. At doses below 1.5 $\mu\text{Ci/day}$, the apparent latent period was 10 years.

Pituitary glands were not consistently submitted for histologic examination, thus, inter-group comparison is difficult. Damage, when present, usually consisted of focal areas of

TABLE I. *Thyroid and Pituitary Weights and Incidence of Neoplasia in Sheep on Various ^{131}I Feeding Regimens*

Feeding level, $\mu\text{Ci/day}$	Age at initial feeding	Duration of feeding, months	Estimated dose to thyroid, krads	Thyroid weight ^(a) ($\pm\text{SD}$)	Pituitary adenoma incidence ^(b)	Pituitary weight ^(a) ($\pm\text{SD}$)	Pituitary adenoma incidence
0	--	--	0	12 $\pm$ 5.0	4/38	2.2 $\pm$ 0.8	2/9
0.15	Fetal or young adults	36-120	0.4-2	14 $\pm$ 6.6	1/31	2.2 $\pm$ 0.8	0/1
0.5	Fetal or young adults	36-120	2-3	5 $\pm$ 2.5	6/13	2.0 $\pm$ 0.5	1/6
1.5	Fetal or young adults	36-120	7-10	9 $\pm$ 6.8	8/13 ^(c)	1.7 $\pm$ 0.5	2/6
5	Fetal or young adults	24-96	20-35	6 $\pm$ 4.6	12/31 ^(d)	2.2 $\pm$ 0.8	3/8
45	15 months	12	60-70	0.8 $\pm$ 0.8	2/5	2.4 $\pm$ 0.8	0/0
135	15 months	9	100	1 $\pm$ 0.6	2/4	1.6 $\pm$ 0.4	0/0
<hr/>							
3000	5-7 months	Single feeding	30-50	2 $\pm$ 2.3	14/28	1.9 $\pm$ 0.7	7/15

(a) At 3-8 years of age.

(b) At 5-12 years of age.

(c) Includes three with premalignant cells present.

(d) Includes one with premalignant cells present, a metastasizing fibrosarcoma was also observed in one thyroid.

necrosis, calcification, cyst formation, and/or tumors. In all cases the neoplasms were chromophobe adenomas in the pars distalis.

In an experiment initiated this year, ten 5-month-old female miniature swine were fed a single dose of 2000 μCi ^{131}I . Thyroid deposition of the ^{131}I was monitored externally, with a peak of approximately 25% of the fed dose occurring near 45 hr. This should have resulted in a thyroid dose of 17,000-25,000 rads to a 3-4 g thyroid. Serum protein-bound iodine values obtained prior to and 7 days following the 2-mCi ^{131}I dose were not greatly different.

There has been one death in this group, probably not related to ^{131}I treatment. The thyroid in this animal

was small (2 g) but otherwise grossly normal. The remainder of the animals show no visible signs of hypothyroidism 4 months postadministration. Thyroid function will be assayed annually in both the treated animals and in a group of five control animals. When the animals are 4 years old, studies will be performed to determine the effects of hypothyroidism on reproductive ability.

Conclusions

There was an increased incidence of benign thyroid adenomas in sheep ingesting ^{131}I at all levels resulting in a dose of 3000 or more rads to the thyroid. In general, the severity and

extent of thyroid damage was dependent on the dose received by the gland and was most severe in the 3000- μ Ci single-dose group. The incidence of pituitary adenomas appears to be increased in animals exposed to ^{131}I . The studies initiated this year in swine will complement the studies in sheep.

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EFFECT OF AGE ON ABSORPTION OF PLUTONIUM FROM THE GASTROINTESTINAL TRACT OF RATS

The absorption of plutonium(IV) citrate was substantially greater in day-old rats than in rats of 20, 21, or 35 days of age. Differences in retention and redistribution were also noted.

Investigators:
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Technical Assistance:
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Joan O. Hess

It was reported by Ballou⁽¹⁾ that very young rats retained a substantially greater fraction of orally administered plutonium(IV) nitrate than older rats at 21 days postfeeding. It was concluded that absorption from the gastrointestinal tract of neonatal rats was greater than from that of adults, although possible differences in retention were not ruled out. The present studies were undertaken to examine this conclusion more directly.

Observations

A preliminary study was performed, the results of which essentially verified Ballou's findings. In further

experiments, nonpolymeric plutonium(IV) citrate (14-fold citrate excess) was administered by gavage to groups of rats of 1, 20 (1 day before weaning), 21 (immediately postweaning), or 35 days of age. Animals from these groups were sacrificed at serial times ranging from 1 hr to 20 days postadministration.

The initial absorption is depicted by the plutonium content of the total rat (minus gastrointestinal tract) measured at 4 hr postadministration (Figure 1). The one-day-old rats absorbed a greater fraction of the administered plutonium than did the older animals. No significant differences in absorption by the rats of 20,

21, or 35 days of age were noted. There was a pronounced tendency for increased deposition of absorbed plutonium in the liver of the day-old rats as compared with the older animals. The absolute values obtained are somewhat higher than those reported by Ballou,⁽¹⁾ likely due to the form in which the material was administered (citrate vs. nitrate).

The retention of the absorbed plutonium at 6 days postadministration is shown in Figure 2. Retention was higher in the preweanling (1- and 20-day) rats than in the older animals. There was an evident tendency for translocation of plutonium to bone in

the preweanling rats which was less evident with increasing age.

Conclusions

These studies extend the earlier results of Ballou, indicating an enhanced gastrointestinal absorption of plutonium(IV) citrate in the very young. It further appears that there are age-related differences in the retention and redistribution of plutonium.

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FIGURE 1. Effect of Age on Gastrointestinal Absorption of Plutonium (Retention at 4 hr postfeeding--average of three animals of each age)

FIGURE 2. Effect of Age at Injection on Redistribution of Deposited Plutonium (Retention at 6 days postfeeding--average of three animals of each age)

CROSS-PLACENTAL PASSAGE OF ACTINIDES IN THE RAT

Only small amounts of activity were found in the fetus following the administration of ^{239}Pu , ^{237}Np , or ^{233}U to pregnant rats although substantial concentrations of actinide were measured in the placenta and fetal membranes. The partition of these materials among the fetal tissues was often dissimilar to that found in the corresponding maternal tissues.

Investigators:
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Technical Assistance:
Joan O. Hess

This study was undertaken to compare the cross-placental passage of ionic and colloidal solutions of plutonium and to determine their distribution in the fetus. The two actinides immediately preceding plutonium in the periodic table, neptunium and uranium, were also studied.

Observations

Ionic solutions were prepared by adjusting the pH of an acid solution of the nuclides to 4.5 in the presence of excess sodium citrate which results in solutions containing little or no polymerized nuclide. The colloidal or polymeric form was obtained by adjusting the pH without added citrate. Pregnant rats were intravenously injected with doses of approximately 50 μCi of ^{239}Pu (ionic or colloidal), 3 μCi of ^{237}Np (ionic), or 4 μCi of ^{233}U (ionic). Injection of each solution was made after 15 or 19 days of gestation and the rats killed 24 hr later.

Table I indicates the extent of cross-placental transfer of these materials and summarizes their distribution in the various maternal and fetal tissues. In all cases the fraction of the injected actinide which reached the fetus was very small. The

TABLE I. Distribution of Actinides 24 hr After Injection in Pregnant Rats (% of injected dose/g wet wt)

	Adult	15-16 Fetus	19-20 Fetus
<u>Ionic ^{239}Pu</u>			
Liver	2.5	0.03	0.04
Femur	0.73	--	2.1
Kidney	0.43	--	0.20
Fetus	--	0.005	0.007
Membrane	--	1.9	3.2
Placenta	--	0.40	0.91
Amnionic fluid	--	0.005	0.01
<u>Colloidal ^{239}Pu</u>			
Liver	3.2	0.0011	0.017
Femur	0.16	--	0.056
Kidney	0.06	--	0.007
Fetus	--	0.0002	0.0004
Membrane	--	0.030	0.071
Placenta	--	0.009	0.023
Amnionic fluid	--	0.0009	0.0002
<u>Ionic ^{237}Np</u>			
Liver	1.4	0.03	0.01
Femur	1.9	--	0.41
Kidney	3.6	--	0.23
Fetus	--	0.016	0.02
Membrane	--	0.81	1.2
Placenta	--	0.04	0.18
Amnionic fluid	--	<0.01	<0.01
<u>Ionic ^{233}U</u>			
Liver	0.03	0.01	0.08
Femur	0.58	--	0.06
Kidney	5.2	--	0.06
Fetus	--	0.01	0.03
Membrane	--	0.13	0.33
Placenta	--	0.01	0.05
Amnionic fluid	--	<0.01	<0.01

concentration of actinide in the placenta varied widely depending upon the actinide injected, its physical state, and the stage of gestation. Concentration in the fetal membranes likewise varied but was, in all cases, at a substantially higher level than in the placenta.

Distribution of the actinides among the various fetal tissues was quite dissimilar to that observed in the comparable tissues of the dam. Of special interest is the finding that, with ionic plutonium, the concentration in the fetal femur was three times that found in the mother's femur.

Autoradiographic studies are in progress to determine the site of localization of plutonium within the

placenta and fetal membranes and within the fetal femur.

Conclusions

It is evident that the placenta and fetal membranes constitute barriers to the free passage of actinides from the maternal circulation to the fetus. The degree of exclusion from the fetus depends on the properties of the actinide and on the physicochemical state in which it is administered. Although the proportion of actinide reaching the fetus was low, it is not inconsequential because of the high radio-sensitivity of the fetus and high affinity of certain fetal tissues for these materials.

NEPTUNIUM-INDUCED FATTY LIVERS IN RATS: ELECTRON MICROSCOPIC AND BIOCHEMICAL STUDIES

The earliest observed morphological alterations in developing fatty livers induced by ^{237}Np were dilatations in the rough endoplasmic reticulum. These dilatations appeared to give rise to the lipid bodies characteristic of ^{237}Np -induced fatty livers. Morphological and biochemical evidence suggests that protein synthesis may be less markedly inhibited after ^{237}Np administration than after CCl_4 , puromycin, or ethionine administration.

Investigators:
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D. D. Mahlum

Technical Assistance:
R. R. Adee

Previous studies in this laboratory have demonstrated that the intravenous administration of ^{237}Np to female rats at dose levels above 6 mg/kg body weight induced the formation of a fatty liver (Annual Reports, 1962 et

seq.). Histopathological lesions were observed in the liver as early as 6 hr following ^{237}Np treatment, and lipid accumulation, as determined chemically, became significant at 12 hr. The present investigation was initiated to

determine the effect of ^{237}Np on the fine structure of rat hepatocytes in order to provide further insight into the mechanism of ^{237}Np -induced fatty liver development. In addition, protein synthesis was examined in ^{237}Np -poisoned rats since fatty livers induced by CCl_4 , puromycin, and ethionine have been associated with impaired synthesis of plasma and liver proteins.

Observations

For the electron microscope studies, female rats were injected with 12 mg/kg of ^{237}Np as the citrate. A similar citrate solution, without ^{237}Np , was administered to control animals. Because ^{237}Np -treated animals do not eat during the first 24 hr, all animals were fasted until sacrificed 2, 3, or 24 hr postinjection. Liver tissue was initially fixed in glutaraldehyde, subsequently fixed in osmium tetroxide, dehydrated with ethanol, and embedded in Epon. Sections were stained with uranyl acetate and lead citrate.

The fine structure of the hepatocyte in control rats was essentially similar to that described in the literature. The endoplasmic reticulum was primarily of the rough type and only occasional lipid bodies were present.

The first changes noted in hepatocyte structure of experimental rats were dilatations of the rough endoplasmic reticulum (Figure 1). These dilatations, or "crescents" as we have termed them, were routinely present 3 hr after ^{237}Np injection, but rarely after 2 hr. The crescents were approximately $0.2\ \mu$ wide and $1\ \mu$ long and

their interiors were electron-transparent by the methods of preservation used in this study. At these early times in fatty liver development the crescents were discretely localized in only a few cisternae of the rough endoplasmic reticulum and the general parallel orientation of cisternal stacks was maintained. There appeared to be a decrease in the ribosome population of the membranes limiting the crescents. In contrast, rough endoplasmic reticulum, continuous with and adjacent to the crescents, appeared morphologically unaltered with the usual complement of ribosomes being present. Crescents similar to those in the rough endoplasmic reticulum were also observed within the nuclear envelope. Hepatocytes from 2- and 3-hr

FIGURE 1. Crescent (C) in Rough Endoplasmic Reticulum of Hepatic Parenchymal Cell from Rat Treated with ^{237}Np , 3 hr Prior to Sacrifice [The collapse of the membrane limiting the crescent (arrows) is thought to be the result of dissolution of the crescent contents during specimen preparation. Note that the rough endoplasmic reticulum, continuous with and adjacent to the crescent, is morphologically normal in appearance.]

^{237}Np -treated rats did not contain increased numbers of typical lipid inclusions, although the crescents may represent an initial lipid accumulation.

A decrease in overall specimen contrast was routinely observed in liver samples obtained 24 hr after ^{237}Np injection. Crescents were only rarely found at this time, but those which were present were continuous with spherical lipid bodies of 0.1-2 μ diameter. These lipid bodies occurred in clusters as well as individually and were randomly distributed throughout the cytoplasm (Figures 2 and 3). The lipid bodies observed in 24-hr treated animals appeared to be of two types, i.e., electron-transparent (Figure 2) and electron-dense (Figure 3). We suggest that both types are lipoidal in nature with their different electron densities possibly reflecting different chemical characteristics. Electron-dense material, similar to the electron-dense lipid bodies, was found in the rough endoplasmic reticulum of 24-hr ^{237}Np -treated animals, but not in 2- or 3-hr treated rats. At 24 hr posttreatment, the rough endoplasmic reticulum was disoriented and the integrity of the parallel arrays disrupted; however, ribosomes could be found associated with the endoplasmic reticulum.

In the biochemical studies, female rats were injected with ^{14}C -leucine 5 or 24 hr after a dose of 12 mg/kg of ^{237}Np . Blood and liver samples were obtained 1 hr after administration of the ^{14}C -leucine. A 5-hr pretreatment with ^{237}Np did not affect the incorporation of ^{14}C -leucine into either liver or plasma proteins; however, a pretreatment time of 24 hr resulted in

FIGURE 2. Crescents (C) Within the Rough Endoplasmic Reticulum Are Shown to be Continuous with the Lipid Bodies (L) in Hepatocyte of 24-hr ^{237}Np -Treated Rat

FIGURE 3. Lipid Bodies (L) in Hepatocyte of 24-hr ^{237}Np -Treated Rat Occur Individually and in Clusters [Lipid inclusions (arrows) are shown in the rough endoplasmic reticulum which is frequently associated with mitochondria (M).]

a decreased incorporation of ^{14}C -leucine into liver proteins but not into plasma proteins (Table I).

These morphological and biochemical studies suggest that inhibited protein synthesis may be less important in fatty liver development after ^{237}Np administration than after CCl_4 , ethionine, or puromycin treatment. The early development of dilatations in the rough endoplasmic reticulum may represent deposition of lipoidal materials that later form the lipid bodies typical of fatty livers.

Conclusions

Several pertinent points about the hepatotoxic effects of ^{237}Np remain unanswered: (1) What are the respective quantitative contributions of increased fat mobilization and impaired secretion of triglycerides from the liver to the development of a fatty liver? (2) Does the impaired triglyceride secretion from the liver, without a concomitant decrease in synthesis of plasma proteins, suggest a defect in the conjugation of lipid with protein in the liver? If so, this system offers a unique opportunity to study lipo-

TABLE I. Effect of ^{237}Np on Incorporation of ^{14}C -Leucine into Liver and Plasma Proteins

Length of ^{237}Np treatment, hr	Specific activity, counts/min/mg protein			
	Liver		Plasma	
	Control	^{237}Np	Control	^{237}Np
5 (a)	147	156	410	505
24 (b)	98	52	133	159
24 (c)	31	24	78	86

(a) Approximately 10 μCi of $\text{D,L-}^{14}\text{C}$ -leucine were injected intramuscularly.

(b) Approximately 2 μCi of $\text{L-}^{14}\text{C}$ -leucine were injected intravenously.

(c) Approximately 5 μCi of $\text{L-}^{14}\text{C}$ -leucine were injected intravenously.

protein synthesis and secretion.

(3) Are there specific changes in certain plasma lipoproteins which were not noted in these studies? (4) What is the mechanism(s) of action of protective treatments such as hypophysectomy, adrenalectomy, and butylated hydroxytoluene administration (Annual Report, 1965)? It should be emphasized that these questions have fundamental significance for fatty livers caused by other substances, as well as for many basic processes occurring in the cell.

DISTRIBUTION OF CERIUM-144 IN THE FETAL AND NEWBORN RAT

Pregnant female rats were intravenously injected with ^{144}Ce after 15 or 19 days of gestation. Significant amounts were retained in the placenta and fetal membranes but only a small fraction of that administered reached the fetus. Relative to the dam, a very large fraction of the body burden of the fetus was contained within the developing skeleton and little was present in the fetal liver.

Investigators:

D. D. Mahlum

M. R. Sikov

Technical Assistance:

Joan O. Hess

This study was undertaken to obtain quantitative data on the extent of cross-placental transfer and fetal distribution of ^{144}Ce . Earlier studies had suggested that the primary site of deposition of cerium in the developing fetus was in the organic matrix of the skeleton.⁽¹⁾

Observations

A dose of 50 μCi of ^{144}Ce was intravenously injected into pregnant rats of the Charles River CD strain after 15 or 19 days of gestation. Animals were sacrificed at 1 or 3 days after injection. As indicated in Table I, only a small fraction of the administered ^{144}Ce was present in the fetus 24 hr after injection; the concentration was independent of the stage of gestation. The concentration of cerium in the placenta was substantially greater than that in the fetus, and the concentration in the fetal membranes was greater than that in the placenta.

The concentrations of ^{144}Ce in the liver, kidney, and femur of the fetus at 24 hr after administration were all substantially less than in the corresponding tissues of the dam.

The relative distributions in dam and fetus were grossly different. In the adult, the concentration in the liver was approximately 15-fold that in the femur, while in the fetus, the concentration in the liver was only one-half that in the femur (Table I). A similar distribution of activity was seen in the animals sacrificed at birth following injection at 19 days of gestation.

TABLE I. Distribution of ^{144}Ce 24 Hr After Injection in Pregnant Rats

	Percent dose/g tissue		
	Adult	15-16 Fetus	19-20 Fetus
Liver	7.0	0.06	0.05
Femur	0.49	--	0.10
Kidney	0.72	--	<0.01
Rib (vertebral)	0.13		0.16
Rib (sternal)	0.10		0.02
Fetus	--	0.02	0.02
Membranes	--	1.8	1.6
Placenta	--	0.36	0.39
Amnionic fluid	--	0.01	0.01

	Percent of body content		
	Adult	15-16 Fetus	19-20 Fetus
Liver	75	27	18
Femur	0.48	--	1.2
Kidney	1.7	--	0.18

The vertebral (ossified) end of the fetal rib contained a ^{144}Ce concentration approximately ten times that of the sternal end (cartilaginous). In contrast, relatively little difference between the two ends was noted in the adults. We have not yet ascertained whether the dissimilarity between the adult and the fetus primarily reflects differences in metabolism of the element or in the physicochemical state in which the element is presented to each. A technique has been developed for intracardiac injection in newborn rats which will permit us to study the metabolism of cerium during early postnatal development.

Conclusions

Only a small fraction of the ^{144}Ce administered to the pregnant female reaches the fetus, while substantially greater amounts are retained in the placenta and fetal membranes. The material which does reach the fetus is quite differently distributed than in the adult animal.

Reference

1. Asling, C. W., M. E. Johnston, P. W. Durbin, and J. G. Hamilton. *Localization of Cerium-144 in the Skeletal Tissues of Fetal Rats*, UCRL-8024. 1957.

AGE-RELATED DIFFERENCES IN THE METABOLISM OF CESIUM-137 BY THE RAT

Cesium metabolism in prenatal and neonatal rats was compared with that in the adult. The prenatal animals exhibited less tendency for selective accumulation in specific tissues than did the newborn and adult animals.

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

Technical Assistance:
Joan O. Hess
Barbara J. Schulz

A number of metabolic and physiological parameters change during the development and maturation of the rat. Cesium-137 was used to study some of these developmental factors because its biological behavior is similar to that of potassium and because of its presence in the biosphere as a product of nuclear fission. Moreover, previous studies in other laboratories have indicated^(1,2)

that the metabolism of cesium by the prenatal animal may be quite dissimilar to that of the adult.

Observations

Pregnant rats were either injected after 15 or 19 days of gestation with 50 μCi of ^{137}Cs chloride and sacrificed 24 hr later, or were injected

after 16 days of gestation and killed 4 days later.

The concentration of ^{137}Cs attained by the fetus was quite low and was independent of age (Table I). The concentration in each fetal tissue was significantly lower than in the comparable tissue of the dam. While some of the maternal organs evidenced a marked tendency to concentrate cesium, the distribution in the fetal tissues was more uniform. The concentration in the placenta was about twice that in the fetus and in the fetal membranes.

In contrast to the autoradiographic results reported by Nelson,⁽²⁾ no preferential accumulation of ^{137}Cs by fetal cartilage was noted in the present studies. This is exemplified by the essentially identical values seen in the cartilagenous and ossified portions of the ribs as well as the low ratios (not pre-

sented) in other predominantly cartilagenous structures such as the trachea and xiphoid process.

Conclusions

The placenta presents a barrier to the free exchange of ^{137}Cs between the maternal circulation and the fetus. Moreover, the ^{137}Cs entering the fetus is metabolized differently than that in the dam as shown by the failure of specific fetal tissues to concentrate ^{137}Cs .

References

1. Hood, S. L., and C. L. Comar. *Arch. Biochem. Biophys.*, vol. 45, pp. 423-433. 1953
2. Nelson, A., S. Ullberg, H. Kristoffersson, and C. Rönnbäck. *Acta Radiol.*, vol. 55, pp. 374-384. 1961.

TABLE I. Concentration (Percent Dose/g) of ^{137}Cs in Some Tissues of the Dam and the Fetus (a)

	Dam		Fetus		
	1 Day	4 Days	15-16 Days	19-20 Days	16-20 Days
Blood	0.08			0.03	
Liver	0.47	0.18	0.08	0.11	0.04
Kidney	0.70	0.26		0.10	0.05
Muscle-striated	0.30	0.31		0.09	0.04
Diaphragm	0.55	0.32		0.12	0.05
Femur	0.14	0.08		0.08	0.04
Rib-ossified	0.25	0.17		0.12	0.04
Rib-cartilagenous	0.25	0.27		0.12	0.05
Trachea		0.14		0.06	0.04
Larynx		0.21			0.06
Total fetus			0.09	0.08	0.04
Placenta			0.16	0.14	0.05
Fetal membranes			0.08	0.07	0.05
Amnionic fluid			0.01	0.01	

(a) Average values from 5-12 animals, except for only two "4-day" dams.

INTERRELATIONSHIPS BETWEEN ENVIRONMENTAL TEMPERATURE AND DIET IN THE DISTRIBUTION AND RETENTION OF CESIUM-137 IN THE RAT

Rats injected with ^{137}Cs were maintained either at 4 °C or 22 °C while being fed diets of varied potassium content. Retention of ^{137}Cs was inversely related to the potassium content of the diet and directly related to environmental temperature.

Investigators:

D. D. Mahlum
M. R. Sikov

Technical Assistance:

Joan O. Hess
Barbara J. Schulz

Studies by Furchner and Richmond demonstrated that ^{137}Cs is retained less tenaciously in animals maintained in a cold environment than those kept at normal room temperature.⁽¹⁾ This was partially explainable on the basis of increased food consumption and a resulting increased potassium intake by the animals in the cold environment, although it appeared that factors other than potassium intake might be involved. The present study was undertaken to determine the effect of environmental temperature on ^{137}Cs retention and distribution under conditions of controlled potassium intake.

Observations

Six groups of four female rats of the Charles River CD strain, weighing between 250 and 300 g, were used in this study. Three groups were housed at room temperature (22 ± 1.5 °C) and three groups at 4 ± 1 °C. All animals were conditioned for 1 week on a low-potassium diet supplemented with potassium to provide a daily intake of 0.74, 54, or 108 mg. The rats maintained at 4 °C consumed twice as much food so the concentration of potassium was adjusted to make

the potassium intake independent of environment. Following conditioning, the rats were injected intraperitoneally with 50 μCi of $^{137}\text{CsCl}$ and returned to their respective diets and environments for an additional 18 days, during which time their content of ^{137}Cs was periodically assayed in a whole-body counter (Figure 1). At the conclusion of the study, the animals were killed and a number of tissues analyzed for ^{137}Cs .

The retention of the administered ^{137}Cs was inversely related to potassium consumption. A substantial effect was produced by raising the intake from 0.74 mg/day to 54 mg/day; increasing the level to 108 mg/day produced relatively less additional effect. At each potassium level, the animals maintained in the cold environment retained less ^{137}Cs than did the comparable group maintained at room temperature.

The measured tissue concentrations of ^{137}Cs , shown in Table I, have been normalized to the concentration in muscle of the animals receiving the normal (54 mg/day) intake level of potassium at room temperature for ease of comparison. These values closely reflect the total-body content measured immediately before sacrifice at 18 days (Figure 1).

FIGURE 1. Effect of Alterations in Environmental Temperature and Potassium Consumption on the Retention of Injected ^{137}Cs

TABLE I. Effect of Alterations in Environmental Temperature and Potassium Consumption on ^{137}Cs Distribution at 18 Days Postinjection [Concentrations of ^{137}Cs normalized to a value of unity for the specific activity of abdominal muscle of normal rats (22 °C, 54 mg K/day)]

Tissue	22 °C			4 °C		
	Low K	Normal K	High K	Low K	Normal K	High K
Liver	0.27	0.25	0.20	0.40	0.26	0.15
Diaphragm	1.11	0.63	0.52	1.38	0.61	0.45
Abdominal muscle	1.88	1.00	0.85	1.70	0.99	0.72
Vertebral rib	0.68	0.36	0.30	0.58	0.38	0.25
Sternal rib	0.92	0.44	0.40	0.77	0.44	0.33
Larynx	0.47	0.28	0.22	0.60	0.25	0.17
Trachea	0.21	0.16	0.12	0.21	0.12	0.09
Skull	0.08	0.05	0.04	0.05	0.03	0.03
Femur	0.26	0.14	0.11	0.24	0.13	0.08

A considerable variation in the specific activity of the various elements of the skeleton was noted. In general, the rib contained a higher ^{137}Cs concentration than did the femur which, in turn, had a much greater concentration than did the skull. The concentration in the sternal or cartilaginous ends of the ribs was consistently higher than in the vertebral or ossified ends.

Conclusions

Increased potassium intake decreased the retention of injected ^{137}Cs . Re-

duced environmental temperature produced a further diminution of cesium retention in excess of that which can be accounted for on the basis of potassium intake. Neither factor altered the pattern of distribution among tissues.

Reference

1. Furchner, J. E. and C. R. Richmond. *J. Appl. Physiol.*, vol. 18, pp. 786-788. 1963.

AGE DEPENDENCE OF RADIOIODINE METABOLISM AND EFFECT IN RATS

From a study of the deposition and retention of radioiodine in the thyroid of prenatal, newborn, weanling, and adult rats, radiation doses to the thyroid were calculated for various radioiodine intakes. Comparison of the damage resulting from these various intakes with the dose delivered revealed a rather sharply decreasing radiosensitivity with increasing age.

Investigator:
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Technical Assistance:
Barbara J. Schulz
Joan O. Hess

There are numerous indications that the metabolism and toxicity of various radionuclides, as well as the sensitivity to external X- or γ -radiation, vary with animal age. There have been few systematic studies of these variations. The effects of internally deposited ^{131}I are of particular interest in this regard since Beach and Dolphin⁽¹⁾ have suggested that the child's thyroid appears to be more

radiosensitive, in terms of tumor induction, than that of the adult human. On the other hand, Doniach⁽²⁾ has reported that the radiation sensitivity of the weanling rat thyroid, as measured by the subsequent response to a goitrogenic challenge, was similar to that observed in adult rats and found no marked differences in radioiodine uptake or biological half-life.

Observations

Rats of four ages (19-day fetuses, newborns, weanlings, and young adults) were injected with 0, 0.5, 1.0, or 3.0 $\mu\text{Ci/g}$ of carrier-free ^{131}I as NaI (30 rats per age-dose group). The postnatal animals were injected intraperitoneally while the fetuses were exposed by intraperitoneal injection of the dam. One week prior to exposure, the animals were placed on a low-iodine diet and maintained on this diet until sacrifice or for one week after exposure. Randomly selected rats from each age group were sacrificed at 1, 3, and 7 days after injection and the concentration of radioactivity in the thyroid gland determined. The remaining animals were kept for four additional months on a normal stock diet. At that time, they were placed on a low-iodine diet for one week, then given 1.0 μCi of Na^{131}I intraperitoneally and killed 24 hr later for determination of incorporation in the thyroid gland and other tissues and observation of abnormalities of development induced by the prior exposure.

The degree of incorporation and the retention of the initial dose of ^{131}I in the thyroid gland was markedly influenced by the magnitude of the dose. The higher doses rapidly produced an effect on the thyroid which led to an early release of activity so that the integrated radiation doses were not proportional to the amount administered. Incorporation was less in the younger animals which tended to minimize this effect. The $\text{rad}/\mu\text{Ci/g}$ equivalent was

also less in the small glands, further decreasing the radiation dose. The integrated radiation doses relating to the three levels of administered ^{131}I dose at the four ages may be obtained from Figure 1.

In this Figure, the incorporation of the second or test-dose of 1 μCi (expressed as percent of control incorporation) has been plotted as a function of original radiation dose to the thyroid. Despite the lack of parallelism of these lines, it is possible to estimate the relative radiosensitivity of the thyroid of animals of these ages. Considering the dose required to reduce incorporation to 50% of control levels, values of approximately 18,000 rads were obtained for the adult, 12,000 rads for the weanling, 5,000 rads for the newborn, and 1,000 rads for the fetal rats. Although a somewhat different ratio of doses would be obtained at other levels of effect, the progression would be largely unchanged.

Distinct age-related alterations in overall body weight and in the relative size of some of the viscera were noted. It has not yet been established to what extent these are direct radiation effects and to what extent they are secondary to thyroid malfunction. Other age-dependent effects noted, which relate to the induced hypothyroid state, include differences in protein-bound radioactivity and extra thyroidal retention of ^{131}I .

Conclusions

It is apparent from these results that the metabolism of radioactive

FIGURE 1. Effect of Age on ^{131}I Dose-Effect Relationship in Rat Thyroid

iodine is different in very young animals than it is in the more mature and that the sensitivity of the thyroid to acute damage is greater in very early life. It is of some interest that the resistance of the thyroid approaches adult levels by the time of weaning, in confirmation of the results of Doniach,⁽²⁾ since the overall radiosensitivity of rats,

as measured by the $\text{LD}_{50/30}$, is well above adult levels at this age.

References

1. Beach, S. A. and G. W. Dolphin. *Phys. Med. Biol.*, vol. 6, pp. 583-598. 1962.
2. Doniach, I. *Brit. J. Cancer*, vol. 11, pp. 253-257. 1957.

INHALATION STUDIES

The 13 reports in this section attest to the intensive and varied program of inhalation studies in progress in the Biology Department. Four reports are concerned directly with plutonium inhalation studies. Ruthenium, cerium, and promethium inhalation studies are described in other reports. Three reports deal with the probable relationship between pulmonary irradiation, lymphopenia, and enhanced taurine excretion in rats, dogs, and man. Three reports detail biochemical and ultrastructural studies related to radiation effects in the lung. An additional report on inhalation studies is included in the section which follows on Space Nuclear Systems Studies.

By their nature, many of these studies are of long duration. Each passing year adds increased significance to the results of the 40-dog chronic toxicity study. Primary lymphatic tumors were observed in lungs and lymph nodes for the first time, during the past year. This observation, together with revised estimates indicating tumor production at lung doses as low as 3000 rads (assuming the dose to be averaged over the total lung which, of course, it is not), may necessitate substantial revision of our views on the toxicity of inhaled plutonium. With the availability of additional dog-holding facilities in 1967, we may look forward to an extension of this program at lower dose levels.

Accumulating evidence seems to rather clearly link the lymphopenia observed following chronic pulmonary irradiation with the destruction of circulating lymphocytes. The enhanced excretion of taurine would appear to be another manifestation of this destruction of lymphocytes.

The concluding reports in this section on biochemical and ultrastructural studies of lung tissue and the effects of radiation on lung tissue, while affording nothing like an adequate explanation for observed effects, may nevertheless offer the best long-range possibility for ultimate understanding.

CHRONIC EFFECTS OF INHALED PLUTONIUM DIOXIDE IN DOGS

Twenty dogs have died or were sacrificed when death was imminent 29-79 months postexposure. The plutonium burden at death ranged from 0.3-3 μ Ci. Respiratory insufficiency and lymphopenia were the primary clinical signs associated with severe fibrosis and metaplastic changes in the lungs and fibrosis of the tracheobronchial and mediastinal lymph nodes. Twelve of the dogs showed primary pulmonary neoplasia. One dog had a lymphangiosarcoma in a mediastinal lymph node and two dogs showed neoplastic changes in the vasculature of the lung.

Investigators:

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

Technical Assistance:

L. R. Richardson

Forty beagle dogs were given single 10-30-min exposures to $^{239}\text{PuO}_2$ aerosols 4-7 years ago for study of long-term translocation of $^{239}\text{PuO}_2$ and for observation of biological effects (Annual Reports, 1962, et seq.). To date, plutonium analyses and histopathologic examination have been completed on 25 of these. This report summarizes the present status of the experiment.

Observations

Ten dogs died or were sacrificed when death was imminent due to respiratory insufficiency. These dogs were 29-51 months postexposure and the plutonium burden at death ranged from 0.9-2.9 μ Ci (Table I). Pulmonary

neoplasia in these dogs was an incidental finding at necropsy or was seen a few months prior to death in thoracic radiographs. In eight other dogs, pulmonary tumors were seen in thoracic radiographs prior to clinical signs of severe respiratory insufficiency. These dogs were sacrificed 54-79 months postexposure and the terminal plutonium burden ranged from 0.5-1.9 μ Ci. One dog died with meningitis and another with cardiovascular disease, currently not considered to be associated with the plutonium exposure. Five additional dogs were sacrificed 28-55 months postexposure to determine the tissue distribution of plutonium in asymptomatic dogs.

In addition to clinical signs associated with the pulmonary pathology,

TABLE I. Dog Mortality and Tissue Distribution of Inhaled $^{239}\text{PuO}_2$

Dog number (a)	Time of death, months postexposure	Plutonium body burden, μCi		Distribution of Pu at death, percent of total				Average dose to lungs, (c) rads
		Deposited (b)	At death	Lungs	Bronchial & mediastinal lymph nodes	Liver	Bone	
182	29	4	2.7	74	21	2	1	7900
184	31	3.8	2.5	75	17	5	1	8400
272	32	4.6	2.9	63	17	14	4	8200
215	38	2.4	1.4	50	42	2	4	5300
83	40	3.1	1.8	59	27	6	2	7400
268 (d)	40	1.7	1.1	46	25	20	3	3400
173	44	3.8	2.1	46	48	2	2	11000
106	45	5.0	2.7	50	37	6	2	13600
183	45	1.7	0.9	52	38	6	3	4100
180	48	2.7	1.4	42	49	5	3	7500
76	51	2.9	1.4	47	45	3	4	8000
246 (e)	54	2.1	1.9	55	24	16	3	8400
255	54	2.1	1.0	42	24	26	3	4900
259 (f)	54	2.1	1.0	55	23	15	6	5500
213	56	2.7	1.2	49	27	15	5	7200
216	60	1.2	0.5	51	26	15	7	3100
85	66	2.9	1.1	36	30	21	6	8600
86 (g)	68	0.8	0.3	37	49	6	5	2500
81 (h)	74	2.6	0.9	23	54	19	4	7100
212	79	1.0	0.8	53	39	3	2	8400
Sacrificed for tissue distribution of plutonium								
104	28	1.2	0.8	68	26	2	1	3500
79	30	1.4	0.9	34	57	6	1	3000
158	30	0.2	0.1	33	53	10	1	450
257	44	1.0	0.6	41	32	21	4	2300
64	55	0.03	0.01	43	50	1	4	94

(a) Underscoring indicates dog with primary pulmonary tumor.

(b) Alveolar deposit estimated from burden at death assuming a whole-body retention half-time of 1500 days.

(c) Average dose to lung assuming uniform distribution of absorbed alpha energy from plutonium. (Previously published values for some of these animals were substantially higher because they were based on higher estimates of alveolar deposition and because translocation from lung was ignored.)

(d) Encephalitis - lung pathology present but apparently not cause of death.

(e) Also capillary hemangioma in lung.

(f) Also lymphangiosarcoma in lung.

(g) Cardiovascular death - lung pathology present but apparently not cause of death.

(h) Also lymphangiosarcoma in mediastinal lymph node.

lymphopenia was a consistent observation in these dogs (Figure 1). There was no significant difference in the degree or time of onset of lymphopenia in dogs with plutonium burdens at death of 0.5-2.9 μCi . The onset of lymphopenia in dog 86 (0.3 μCi plutonium burden) occurred later and the lymphocyte count was depressed less than in dogs with higher plutonium burdens. This dog showed some indication of recovery from the lymphopenia

and approached control lymphocyte levels prior to death. Dog 158 (0.1 μCi) and dog 64 (0.01 μCi) showed no significant lymphopenic response.

The plutonium burden at death ranged from 0.3-2.9 μCi with 23-75% of this plutonium in the lungs, 16-44% in the tracheobronchial lymph nodes, 1-32% in the mediastinal lymph nodes, 2-26% in the liver, and 1-7% in the bone. The highest concentrations

FIGURE 1. Lymphopenia in Dogs After Inhalation of $^{239}\text{PuO}_2$ (95% confidence interval shown for controls)

occurred in the tracheobronchial lymph nodes followed in descending order by the mediastinal lymph nodes, lung, and liver.

The lungs of these animals showed marked pleural, subpleural, parenchymal, alveolar, and septal fibrosis and sclerosis. In addition, other large areas appeared to be undergoing consolidative processes consisting of septal cell proliferation, giant cell formation, hemorrhage, and varying degrees of fibroplastic response. Scattered throughout, in association with some of these fibrosed and sclerotic areas, were numerous foci of alveolar and bronchiolar type metaplasia. Transition from metaplasia to neoplasia could be seen in many of these regions.

Twelve of the dogs evidenced primary pulmonary neoplasia. The tumors were multicentric in origin and metastases to the tracheal bronchial lymphatics, tracheobronchial lymph nodes, and mediastinal lymph nodes were seen in eight dogs. Metastases to the diaphragmatic parietal pleura were seen in two dogs. The neoplasms were classified as bronchiolo-alveolar carcinomas in nine dogs. The lungs of dog 255 had multiple tumors classified as bronchiolar adenocarcinomas and a capillary hemangioma. The lungs of dog 259 had multiple foci of bronchiolo-alveolar carcinomas, a bronchial carcinoma, and a lymphangiosarcoma. The lungs of dog 81 had multiple bronchiolo-alveolar carcinomas and bronchiolar adenocarcinomas.

Autoradiographs of lung sections showed radioactive particles associated with the desmoplastic and metaplastic

processes, especially in the subpleural and peripheral parenchymal regions. The irradiated proliferating cells, comprising the metaplastic response to lung scarring, appeared to be the nidus of tumor formation.

The tracheobronchial lymph nodes were composed of dense sclerotic connective tissue and were devoid of lymphoid elements. Serial autoradiographs of these sections showed heavy, diffuse distribution of radioactivity throughout the scarred parenchyma. The mediastinal nodes evidenced varying degrees of fibrosis and sclerosis, depending on their radioactive particle content. A mediastinal node in dog 81 showed a lymphangiosarcoma which appeared to originate in the capsule of the lymph node. The lymph node was completely destroyed and was composed entirely of necrotic tissue and collagen.

Because the amounts of ^{239}Pu deposited in the dogs were too low to accurately estimate clearance rates by whole-body-counting equipment available at the time of exposure, and because it was not practical to analyze all of the feces and urine for several years, initial alveolar deposition was estimated indirectly from clearance rates obtained in relatively short-term experiments. Dogs with minimal lung pathology, from 3 months to nearly a year postexposure, showed a mean whole-body retention half-time of approximately 1500 days. Employing this parameter, alveolar deposition for the long-term dogs was estimated (Table I). Using these estimates for each dog, the quantity of plutonium found in the lung at death, and assuming a single exponential rate for lung clearance, the total

accumulated radiation dose to the lung was calculated. These values ranged up to 13,600 rads. We had earlier estimated higher doses for some of these animals, based on higher estimates of initial deposition and neglecting the effect of translocation of plutonium from the lung.

Conclusions

Twelve of the 40 dogs exposed to plutonium have shown primary pulmonary neoplasia, an incidence of 30% compared to a reported canine primary lung tumor incidence of 0.2%. The tumors appeared multicentric in origin and metastases were evident in 10 dogs. The tumors were classified as bronchiolo-alveolar carcinomas, bronchiolar adenocarcinomas, and bronchial carcinomas. In addition, one dog showed a lymphangiosarcoma in a mediastinal lymph node and two other

dogs showed neoplasia of the vasculature of the lung.

The lungs of these tumor-bearing dogs at death contained 100-500 times, and the tracheobronchial lymph nodes 25-50,000 times, the amount of plutonium which would result in a mean dose of 0.3 rem per week to the tissues. In the dose levels studied to date, the clinical symptoms and pathology point to the lung as the critical organ causing death.

The significance of the lymphangiosarcomas is difficult to evaluate since only two have been seen, and because the concentration of plutonium in the lymph nodes was approximately 100 times greater than in the lung. This is, however, the first indication of primary neoplasia in the lymphatics resulting from plutonium deposition.

These results emphasize the need to expand the study at lower exposure levels.

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

Some evidence for a different biological behavior of plutonium and americium in the lung is provided by limited analyses on tissue samples from dogs exposed to plutonium oxide aerosols.

Investigators:
W. J. Bair
T. M. Beasley*

Because of the difficulty of measuring internally deposited ^{239}Pu by external counting, consideration has been given to estimating ^{239}Pu burdens from

external measurements of ^{241}Am , a 60 keV gamma-emitting daughter of ^{241}Pu , a common contaminant of ^{239}Pu . This procedure assumes that ^{241}Am remains in equilibrium with plutonium in the body. To test this assumption, ^{239}Pu -to- ^{241}Am ratios in tissue samples from dogs

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exposed to plutonium oxide aerosols were compared with the ratios in samples of the aerosol. The samples were all counted at the completion of the experiment, on platinum disks, with a high resolution silicon diode alpha spectrometer capable of distinguishing between the 5.15 MeV ^{239}Pu alpha and the 5.48 MeV ^{241}Am alpha.

Observations

Preliminary results from dogs sacrificed 3 months after inhalation of PuO_2 indicate that ^{241}Am re-

mained in equilibrium in all dogs with the possible exception of those which inhaled the oxide prepared from the delta form of the metal at 450 °C (Table I). In this case there were increased ratios of ^{239}Pu -to- ^{241}Am in both lung and lymph nodes.

Ratios of ^{239}Pu -to- ^{241}Am were also determined for tissues from dogs which died 3-1/2 to over 6 years after inhaling PuO_2 (Table II). There was a suggestion of disequilibrium in the bronchial lymph nodes but not in the lung. The ratios in the lymph nodes were, in this case, less than those

TABLE I. $^{239}\text{Pu}/^{241}\text{Am}$ Ratios in Aerosols and Dog Tissues (3-month study)

Origin of plutonium dioxide	Number of animals	Ratio: $^{239}\text{Pu}/^{241}\text{Am}$ (a)		
		Aerosol	Lung	Bronchial lymph nodes
Oxalate calcined at 1000 °C	3	13.8 ± 0.3	13.4 ± 0.3	11.6 ± 0.6
Oxalate calcined at 350 °C	3	12.0 ± 0.2	13.0 ± 1.2	14.2 ± 0.1
Metal oxidized at 450 °C	3	31.0 ± 1.9	37.8 ± 1.5	36.1 ± 1.2
Metal oxidized at 123 °C	3	31.0 ± 0.6	31.5 ± 1.0	30.0 ± 0.9

(a) Standard errors associated with counting are included.

TABLE II. $^{239}\text{Pu}/^{241}\text{Am}$ Ratios in Aerosols and Dog Tissues (Long-term study)

Dog number (a)	Time of death (months after exposure)	Ratio: $^{239}\text{Pu}/^{241}\text{Am}$ (c)		
		Aerosol	Lung	Bronchial lymph nodes
268	40	12.6 ± 0.2	12.8 ± 0.2	10.5 ± 0.3
246 (b)	54	13.1 ± 0.3	11.8 ± 0.4	9.8 ± 0.2
259	54	13.6 ± 0.2	13.4 ± 0.4	11.4 ± 0.4
212 (b)	78	13.2 ± 0.3	14.3 ± 0.84	9.0 ± 0.3

(a) All dogs showed primary pulmonary tumors.

(b) Metastatic tumors in bronchial lymph nodes.

(c) Standard errors associated with counting are included.

in the aerosol sample and in the lung, especially in dog No. 212 which had carried its plutonium burden for the longest time. That dogs No. 212 and 246 showed a greater disequilibrium relative to the aerosol than the other two dogs may be related to the fact that lymph nodes of these dogs contained metastatic tumors. All four dogs had primary lung tumors.

Conclusions

Preliminary results indicate that under certain conditions disequilibrium of ^{239}Pu and ^{241}Am may occur in lungs and bronchial lymph nodes of dogs following inhalation of PuO_2 . No clear pattern of change can be discerned from the limited number of dogs thus far studied.

COMPARATIVE DISPOSITION OF FOUR TYPES OF PLUTONIUM DIOXIDES INHALED BY DOGS

Groups of three dogs inhaled dry aerosols of one of four different plutonium dioxides. The lung retention half-time of oxalate calcined at 350 °C was about one year, half that of the other oxides tested. The greater lung clearance of this oxide was accompanied by greater accumulation of plutonium in the bronchial lymph nodes.

Investigators:

W. J. Bair
J. F. Park

Technical Assistance:

W. Skinner
M. D. Snyder
L. R. Richardson

The purpose of this study was to compare the disposition of four types of inhaled plutonium dioxides which were prepared by different methods and oxidized at different temperatures.

Observations

Four groups of beagle dogs were given a single exposure to one of four different plutonium dioxide aerosols. Two were prepared by calcining the oxalate at 1000 °C or at 350 °C. Another was produced by the ignition at 450 °C of the stabilized delta metal and the other by the slow oxidation of the pure metal at 123 °C.

The particle size characteristics of the four aerosols were similar, with count median diameters of about 0.5 μ . Mass median diameters were 2.8 μ for the calcined oxalates, 4.8 μ for the metal oxidized at 450 °C and 1.3 μ for the metal oxidized at 123 °C. The oxides prepared from the oxalates were about 0.03% ultrafilterable and the oxidized metals about 0.006%. X-ray diffraction analyses indicated that all four were pure crystalline plutonium dioxides.

In all dogs except two the alveolar deposition ranged between 20 and 45% of the total quantity of plutonium deposited. The whole-body retention

half-times ranged from about 1000 days to more than 3400 days, Figure 1. The largest values were observed for dogs which inhaled metal oxidized at 450 °C. The lung retention half-times ranged between 600 to 1000 days for all dogs except those which inhaled the oxalate calcined at 350 °C. For these dogs the retention half-time was 300-400 days.

Figure 2 shows the complete disposition of the alveolar deposited plutonium. The lungs contained more than 90% of the alveolar deposited plutonium 3 months after exposure in all dogs except those which inhaled the oxalate calcined at 350 °C. In these dogs the

lungs contained only 80 to 87%. The difference in lung burden is largely accounted for by the amounts found in the bronchial and mediastinal lymph nodes. The accumulation of plutonium in the bronchial lymph nodes was greatest after inhalation of the oxalate calcined at 350 °C. Fecal elimination of alveolar-deposited plutonium was least in the dogs which inhaled the metal oxidized at 450 °C. These dogs showed the longest whole-body retention half-times.

Translocation of plutonium to tissues outside of the lung and lymph nodes was least for the dogs which inhaled oxalate calcined at 1000 °C.

FIGURE 1. Whole-Body and Lung Retention of Inhaled PuO₂

Urinary excretion of plutonium as well as translocation appeared to be greatest for the oxalate calcined at 350 °C.

Conclusions

The results of this study provide further evidence for the relatively long-term pulmonary retention of inhaled PuO_2 , its gradual accumulation in bronchial and mediastinal lymph nodes, and the accompanying low rate of translocation and excretion. None

of the four types of PuO_2 deviated from this general pattern, although certain differences in their behavior were apparent, demonstrating the ability of biological processes to discriminate among compounds which were crystallographically indistinguishable.

Certain radiation protection considerations are also implied by these results. Because of its greater accumulation in lymph nodes, the oxalate calcined at 350 °C may be less hazardous than the other oxides.

W.J.B. - 8-1966
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FIGURE 2. Disposition of Alveolar Deposited ^{239}Pu (3 months after exposure)

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

Preliminary results suggest that inhaled $^{239}\text{PuO}_2$ is somewhat more effective than $^{238}\text{PuO}_2$ in causing acute mortality in rats.

Investigator:
B. O. Stuart

Technical Assistance:
J. C. Gaven

To help determine whether the permissible limits for ^{239}Pu are valid for ^{238}Pu , another alpha emitter but with a specific activity 280 times greater than ^{239}Pu , the acute toxicities of the two isotopes inhaled as oxide by rats were compared. The oxides were calcined at 350 °C and ground to a particle size of 0.1 μ (count median diameter). Groups of 12 rats were exposed to one of eight different aerosol concentrations of either of the two isotopes.

Observations

Acute mortality curves were drawn using estimates of the quantity of plutonium initially deposited in the alveoli as determined from whole-body counts and analyses of lung tissue. These results are preliminary since not all of the tissue analyses are complete. Estimated LD₅₀ doses for times after exposure ranging from 14-120 days are given in Table I. For early death, almost twice as much ^{238}Pu was required as ^{239}Pu . At longer survival times the acute lethal doses were about the same for the two isotopes.

Both isotopes at levels greater than 15 μCi caused death 6-8 days after ex-

posure, the earliest deaths observed in our laboratory for an inhaled oxide of a radionuclide. Death was characterized by pulmonary congestion, hemorrhage, and terminal bronchiole and alveolar flooding with proteinaceous fluid, apparently derived from capillary leakage. In the high-dose groups, lymphocytopenia occurred less than a week after exposure. Circulating lymphocytes dropped to 10-25% of pre-exposure and control levels. Neutrophil levels increased twofold. Lymphocytopenia was slight in the low dose groups.

Conclusions

Based on preliminary dose estimates, ^{239}Pu was more effective than ^{238}Pu at doses causing early mortality. This difference was not apparent at longer survival times, up to 4 months postexposure.

TABLE I. LD₅₀ Doses for Inhaled PuO_2 in Rats

Time for which LD ₅₀ was determined, days	LD ₅₀ in μCi	
	^{238}Pu	^{239}Pu
14	13	7.5
30	7.3	5
45	4	2.6
60	1.6	1.7
90	0.8	0.8
120	0.6	0.5

* This study was supported in part by U.S. Air Force Contract Delivery Order No. (29-601)-62-1507.

DISTRIBUTION AND EXCRETION OF $^{106}\text{Ru-RhO}_2$ INHALED BY BEAGLE DOGS

Three years after inhalation of calcined $^{106}\text{Ru-RhO}_2$, the whole-body and pulmonary retention half-times in dogs were 2.5-4.2 and 2.5-4 years, respectively. Fecal excretion decreased rapidly in the first few days after exposure; urinary excretion was negligible. After 2 years, 80% of the body burden was in the lungs and 16% in bone.

Investigator:
B. O. Stuart

Technical Assistance:
J. C. Gaven

This report presents continuing observations on the distribution and excretion of calcined $^{106}\text{Ru-RhO}_2$ particles [count median diameter (CMD) = $0.1\ \mu$] inhaled by six beagle dogs during single exposures 3 years previously (Annual Report, 1965). Initial alveolar deposition was 20-30 μCi total beta.

and indicate very rapidly decreasing rates of excretion of $^{106}\text{Ru-Rh}$ in feces. Excretion was predominantly by the fecal route for several months after

Observations

The whole-body retention of $^{106}\text{Ru-Rh}$ was followed by total-body counting. Retention half-times (corrected for decay) range from 900-1540 days. The estimated pulmonary retention half-times, based on longitudinal scans, ranged from 870-1550 days. Two animals were killed 7 months postexposure, and a third at 25 months. The results of radioanalyses on tissues from these animals are shown in Table I. Significant translocation from the lungs to skeleton occurred during the 7-25 months interval.

The very slow elimination of $^{106}\text{Ru-Rh}$ from the body was reflected in the excretion data. Constants of power functions fitted to the data are shown in Table II. The high negative slopes ("b" values) for fecal excretion were remarkably similar for all six animals,

TABLE I. Distribution of $^{106}\text{Ru-Rh}$ in Beagle Dogs after Inhalation of $^{106}\text{Ru-RhO}_2$ Particles (CMD = $0.10\ \mu$)

Tissues	Percent of total body burden	
	7 Months postexposure (two dogs)	25 Months postexposure (one dog)
Tracheobronchial lymph nodes	1.4	3.6
Lungs	98	79
Kidneys	0.006	0
Spleen	0.01	0
Liver	0.08	0
Bone	0.20	17
Muscle	0.13	0.001

TABLE II. Parameters of $^{106}\text{Ru-Rh}$ Excretion by Beagles After Inhalation of the Calcined Oxide (All data fitted to the power function expression $Y = Atb^*$)

Dog no.	Feces		Urine	
	A	b	A	b
348	6.8	-4.8	0.008	0.022
353	1.8	-3.6	0.028	-0.40
354	0.76	-4.6	0.010	-0.11
355	2.4	-4.9	0.007	0.10
356	1.2	-4.8	0.008	0.05
358	2.0	-4.9	0.008	0.30

* Y = percent body burden in μCi , t = time in days.

exposure. The rates of urinary excretion were extremely low and changed very little with time; the slightly positive slopes seen in some dogs were a result of unusually low urinary radioisotope excretion during the first few days after exposure.

Conclusions

This study shows whole-body and pulmonary retention times for inhaled

$^{106}\text{Ru-RhO}_2$ on the order of several years. Excretion of Ru occurred almost exclusively by the fecal route. After 2 months, daily excretion amounted to only 0.1% of the body burden in the feces and 0.01% in the urine. Translocation of Ru from the lungs to other tissues was very slow, 20% of the body burden moving to the tracheobronchial lymph nodes and skeleton by 2 years post-exposure.

RETENTION OF INHALED $^{144}\text{Ce-PrO}_2$ IN BEAGLE DOGS

Comparison of inhaled peroxide-prepared or calcined $^{144}\text{Ce-PrO}_2$ showed that the calcined oxide was less extensively excreted in the urine, more extensively translocated to lymph nodes, and somewhat less extensively translocated to liver. For both forms, at 30 months postexposure, the lungs contained about 10% of the retained body burden, the liver 40-50%, and the skeleton about 30%.

Investigator:
B. O. Stuart

Technical Assistance:
J. C. Gaven

Previous studies in our laboratory indicated that inhaled $^{144}\text{Ce-PrO}_2$ showed different patterns of translocation and susceptibility to removal by diethylenetriaminepentaacetic acid (DTPA) treatment, depending upon whether the oxide was prepared in the amorphous form by precipitation with peroxide or in the crystalline form by calcination (Annual Report, 1964). From one-half to two-thirds of either form inhaled was retained with half-times greater than 2 years. This report provides further data on the long-term distribution and excre-

tion of these two forms of $^{144}\text{Ce-PrO}_2$ in beagle dogs.

Observations

Figures 1 and 2 show curves obtained by fitting daily excretion values with the power function $Y = At^b$, where "Y" is the percent of existing body burden excreted in the urine or feces on a given day, and "t" is time after exposure in days. Ninety-five percent confidence limits are shown for each curve. Computer analyses of fecal excretion curves

indicate no significant difference in excretion rates between animals exposed to the peroxide-prepared oxide and those exposed to the calcined oxide. In contrast, urinary excretion curves were significantly different for the two cerium oxides.

Table I shows the distribution of inhaled peroxide-prepared or calcined $^{144}\text{Ce-PrO}_2$ in beagle dogs at 30 months postexposure. The only difference

between the two oxides was the higher fraction in the tracheobronchial lymph nodes, and correspondingly lower fraction in the liver, of dog 12 which inhaled the calcined oxide.

Conclusions

Fecal excretion rates were similar for inhaled peroxide-prepared and calcined $^{144}\text{Ce-PrO}_2$. Since urinary

FIGURE 1. Fecal Excretion of $^{144}\text{Ce-Pr}$ After Inhalation of Cerium Oxide by Beagle Dogs

FIGURE 2. Urinary Excretion of $^{144}\text{Ce-Pr}$ After Inhalation of Cerium Oxide by Beagle Dogs

TABLE 1. Distribution of $^{144}\text{Ce-Pr}$ in Beagle Dogs 30 Months After Inhalation of the Oxide (Percent of body burden at sacrifice)

Tissue	Peroxide-prepared oxide		Calcined oxide
	Dog 7	Dog 8	Dog 12
Tracheobronchial lymph nodes	0.12	0.54	7.9
Lungs	10	8.9	9.4
Spleen	0.21	0.07	0.06
Kidneys	1.1	2.2	0.90
Liver	47	53	40
Skeleton	33	27	34
Muscle	5.1	3.9	2.1

excretion and translocation to liver were greater for the peroxide-prepared oxide, it is concluded that the calcined oxide was slightly less soluble in vivo.

RETENTION AND TRANSLOCATION OF INHALED CALCINED PROMETHIUM OXIDE IN BEAGLE DOGS

Biological half-times for total-body retention of inhaled calcined $^{148}\text{m} + ^{147}\text{Pm}_2\text{O}_3$ particles were 450-590 days. At 5-1/2 months after exposure, 44% of the body burden was in the lungs, 24% in the skeleton, and 22% in the liver. Pulmonary retention half-times ranged from 210-540 days.

Investigator:
B. O. Stuart

Technical Assistance:
J. C. Gaven

In an earlier study, six beagle dogs were exposed to aerosols of calcined $^{147}\text{Pm}_2\text{O}_3$ which was neutron-activated to produce a 20-to-1 ratio of ^{147}Pm to $^{148}\text{m}\text{Pm}$, the latter providing a 0.6 MeV gamma emission for whole-body monitoring (Annual Report, 1965). However, the relatively high solubility (3-4% determined by dialysis) of the promethium oxide in the 0.1% pluronics suspension used for aerosol generation and

the apparent translocation of some radioactivity to the abdominal region (inferred from longitudinal scanning) within a few weeks after exposure suggested the need for further experiments. Accordingly, four additional beagle dogs were exposed to aerosols of promethium oxide (count median diameter = $0.06\ \mu$) prepared by recalcining the neutron-irradiated material used in the earlier study.

Observations

During the first week after exposure, 45-80% of the initially deposited promethium was cleared, almost exclusively by the fecal route. Figure 1 shows the whole-body retentions of inhaled $^{148}\text{m}\text{PmO}_2$. By 2 weeks after exposure, the early phase of clearance was virtually complete. Half-times for whole-body retention 14-120 days after exposure were 450, 510, 590, and 450 days for dogs 1, 2, 3, and 4, respectively. These compare with the earlier experiment (Annual Report, 1965) in which whole-body retentions on the order of a year or longer were found with six dogs.

Longitudinal scans of dog 1 at 2 weeks and 4 months after exposure are shown in Figure 2. While the radioactivity peak was virtually confined to the lungs 2 weeks after exposure, by 4 months it had extended partially into the abdominal region indicating a gradual translocation to other

organs. This translocation was verified by analyses of the tissues of dog 1 following sacrifice at 5-1/2 months postexposure, as shown in Table I. Promethium accumulated in the tracheobronchial lymph nodes, with about half of the body burden remaining in the lungs and 20-25% translocated to both liver and skeleton.

Regression curves for lung retention were obtained by planimeter measurement of the areas under the lung regions of longitudinal scans such as those in Figure 2. The effective half-times for $^{148}\text{m}\text{Pm}$ in the lung ranged from 35-39 days. These are equivalent to biological retention half-times for PmO_2 of 210-540 days.

Conclusions

Longitudinal scans of beagles after inhalation of recalcined promethium oxide showed localized lung radioactivity peaks for several months after exposure.

FIGURE 1. Whole-Body Counting Rates of Inhaled $^{148}\text{m} + ^{147}\text{Pm}_2\text{O}_3$ in Beagle Dogs

FIGURE 2. Longitudinal Scans of Inhaled $^{148}\text{m} + ^{147}\text{Pm}_2\text{O}_3$ in Beagle Dogs

TABLE I. Tissue Distribution of ^{147}Pm in Dog 1 at 164 Days After Inhalation of Calcined $^{148}\text{m} + ^{147}\text{Pm}_2\text{O}_3$

Tissue	Percent of body burden at sacrifice
Tracheobronchial lymph nodes	3.3
Lungs	44
Spleen	0.02
Kidneys	0.6
Liver	22
Bone	24
Gastrointestinal tract	0.3
Muscle	1.6
Skin	0.9
Other	4.0

This compares with a more rapid partial translocation to the abdomen seen following inhalation of the non-recalcined promethium oxide used in previous studies. However, long-term retention half-times of 1-2 years were observed for both promethium oxides. Translocation of ^{147}Pm equally to liver and skeleton reduced the lung burden to 44% of the total-body burden at one-half year after exposure, following the same general distribution pattern as inhaled promethium perchlorate (studied earlier in this laboratory) but with much greater pulmonary retention.

TAURINE EXCRETION IN BEAGLE DOGS AFTER INHALATION OF PLUTONIUM OXIDE

Beagle dogs excreted increased levels of urinary taurine within a 3-month period following the inhalation of $^{239}\text{PuO}_2$ aerosols. It is suggested that the increased urinary taurine levels are associated with the destruction of circulating lymphocytes.

Investigator:
J. V. Dilley

Technical Assistance:
K. E. McDonald

Elevated urinary taurine levels associated with a lymphopenia were previously observed in dogs 2 to 3 years after inhalation of $^{239}\text{PuO}_2$ (Annual Report, 1965). These animals also exhibited other clinical manifestations of pulmonary fibrosis and some degree of respiratory distress which could not be ruled out as factors contributing to either the lymphopenia or the increased taurine excretion. Further studies were initiated to clarify the relationship between increased urinary taurine and lymphopenia.

Observations

Pre-exposure lymphocyte levels were determined in beagle dogs acclimated to metabolism cages. The animals were then exposed to $^{239}\text{PuO}_2$ aerosols with 6-33 μCi being deposited in their lungs. Lymphocyte counts and 24-hr urine samples were taken periodically over the following 3-month period. There was an increase in the taurine excretion rate over the 3-month period following the aerosol exposure, and a simultaneous decrease in the number of circulating lymphocytes (Figure 1).

FIGURE 1. Lymphocyte Counts and Urinary Taurine Levels in Beagle Dogs Before and After Inhalation of $^{239}\text{PuO}_2$

Conclusions

In the absence of other clinical or pathological disorders, it would appear that increased excretion of taurine results from the destruction of lymphocytes. If this hypothesis is correct, destruction of lymphocytes rather than impaired production of lymphocytes could account for the lymphopenia occurring after inhalation of PuO_2 . With blood circulating

through the lung more than twice every minute, lymphocytes might well accumulate a critical radiation dose from a deposited radionuclide.

The metabolic conversion of cysteine to taurine is apparently unaffected by ^{239}Pu irradiation of the lung. Therefore, the utilization of cysteine is probably continuing in a normal manner and is not a contributing factor to the plutonium-induced lymphopenia.

TAURINE EXCRETION IN EXTRACORPOREALLY IRRADIATED LYMPHOCYTIC LEUKEMIA PATIENTS

Two patients treated for chronic lymphocytic leukemia by extracorporeal irradiation had increased urinary taurine levels which appeared to reflect the decrease in number of circulating lymphocytes. There was no significant change in the number of other circulating cells or in the plasma proteins.

Investigator:
J. V. Dilley

Observations

Two adult males with diagnosed lymphocytic leukemia were subjected to extracorporeal X-irradiation after they became refractory to chemotherapeutic agents.* One patient (B.C.) was exposed to X-irradiation for a period of 6 hr every third day via an arterio-venous shunt from the forearm.

The other patient (A.M.) was exposed in the same manner except that the irradiation exposure was carried out daily for 6 hr per day until a total of 174 hr of exposure time had accumulated. The only significant changes noted during the irradiation period was a fall in the circulating lymphocyte count. The red cell, leukocyte, and platelet count remained within normal limits as did the serum and urinary uric acid levels, BUN, and urea levels. Total urine samples were collected during the entire course of hospitalization and 10 ml aliquots of each 12-hr sample were frozen and saved for later determination of the taurine content. Cell counts, blood

* Samples were obtained through the cooperation of Drs. E. D. Thomas and R. Storb, Department of Medicine, University of Washington School of Medicine, Seattle, and were delivered, by air, through the courtesy of Mr. R. J. Wheat, Richland Flying Service, Richland, Washington.

chemistry, and urinalysis were performed on each patient every other day by the hospital clinical chemistry laboratory.

Figures 1 and 2 show the lymphocyte counts and urinary taurine values for these two patients. In patient A.M., elevated urinary taurine levels accompanied the decrease in lymphocyte counts with maximum taurine levels delayed until nearly a week following the first substantial decrease in lymphocytes. This is consistent with the observations of Cavellieri et al.⁽¹⁾ and Kretchmar⁽²⁾ who noted similar findings following therapeutic whole-body X-irradiation or accidental whole-body exposure. Results from patient B.C. differed from those obtained from patient A.M. in that maximum taurine levels coincided with the period of decreasing lymphocyte count. The intermittent X-ray exposure given patient B.C. might be expected to produce a different temporal response

than the more continuous exposure given patient A.M. The first of the three intermittent exposures undoubtedly destroyed lymphocytes in the circulating blood, but to a degree which perhaps could be replenished from lymphocytes stored in body pools. In patient B.C., taurine excretion returned to normal levels shortly following the last irradiation period, although lymphocyte levels were still decreasing. In patient A.M., taurine excretion remained elevated throughout the extended irradiation period and long after lymphocyte counts had reached minimal levels.

Conclusions

The destruction of circulating lymphocytes by extracorporeal irradiation is accompanied by a significant increase in the urinary excretion of taurine. In one patient the maximum elevation in urinary taurine was

FIGURE 1. Patient A.M. - Lymphocyte Counts and Daily Urinary Taurine Excretion

FIGURE 2. Patient B.C. - Lymphocyte Counts and Daily Urinary Taurine Excretion

delayed by about a week. This delay could indicate that irradiated lymphocytes released taurine precursors, i.e., cysteine, cysteine sulfinic acid, etc., rather than taurine per se; or that the lymphocytes were rapidly removed from the circulation and stored in spleen and/or lymph nodes where they were subsequently destroyed at a much lower rate.

References

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TAURINE EXCRETION IN RATS TREATED WITH LYMPHOPENIC AGENTS

Two lymphopenic agents, cortisone and methotrexate, produced a sharp increase in the excretion of urinary taurine when administered in a single dose to adult rats.

Investigator:
J. V. Dilley

Technical Assistance:
R. J. Franks
K. E. McDonald

Circulating lymphocytes are extremely sensitive to cortisone and methotrexate. In an effort to establish a relationship between the destruction of circulating lymphocytes and increased levels of urinary taurine, rats were treated with these two agents and their taurine excretion rate was determined.

Observations

Adult female Sprague-Dawley rats were acclimated to individual metabolism cages for 3 days. Twenty-four-hour urine samples were then collected from each animal for the next 3 days. Following this control collection period one-half of the animals were given 1 mg/kg of methotrexate and the other

half, 5 mg/kg of cortisone acetate by intraperitoneal injection. Urine samples were collected from each animal for the next 3 days.

Results of taurine analyses on the urine samples are shown in Table I. There was a marked increase in the taurine excretion of the methotrexate group on the first day following treatment. The response to cortisone was slower but clearly evident. A similar response to cortisone was previously noted in a dog (Annual Report, 1965).

Conclusions

These studies provide more evidence for the relationship between increased taurine excretion and the destruction

of circulating lymphocytes. The differences in the time of appearance of the taurine increase following drug

treatment remain unexplained, but may be due to a difference in the mechanism of action of the two agents.

TABLE I. Urinary Taurine Excretion by Rats Following Drug Treatment

Number of animals	Treatment	Taurine excreted per 24 hr ^(a) (mg)		
		Day 1	Day 2	Day 3
20	Control period	4.2 ± 2.1	4.7 ± 2.3	3.7 ± 1.2
10	Methotrexate, 1 mg/kg	21.8 ± 2.2	7.3 ± 3.1 ^(b)	
10	Cortisone, 5 mg/kg	5.8 ± 3.9	13.7 ± 7.3 ^(b)	

(a) Mean ± standard error.

(b) Values for pooled day 2 and 3 samples.

EFFECTS OF X-IRRADIATION ON LUNG BIOCHEMISTRY

Changes in the levels of hydroxyproline fractions and of lipids in lung tissue of rats were detected after thoracic X-irradiation at doses which produced no detectable histopathology.

Investigator:
E. G. Tombropoulos

Technical Assistance:
K. E. McDonald
Sandra T. Baker

Lung histopathology associated with thoracic X-irradiation has been described extensively. However, little is known of biochemical changes which occur. To identify and correlate biochemical changes with histopathology occurring after radiation, the lungs of female rats were analyzed after 800 R or 1000 R X-irradiation were delivered to their thorax. Some preliminary results from a portion of these studies were reported in last year's Annual Report.

Observations

Three groups of fifty 40-day-old Sprague-Dawley female rats were exposed to thoracic X-irradiation. Two of these groups received 800 R and one group received 1000 R. Unexposed groups of similar rats were kept as controls. The hydroxyproline and lipid contents of lung tissue fractions from rats killed up to the fiftieth day after exposure were determined as described previously (Annual Report,

1965). Two fractions were analyzed for hydroxyproline. One was a fraction which was water extractable after autoclaving the lung tissue, which is usually referred to as extractable collagen. The other fraction was water insoluble and includes elastin. In separate experiments, fatty acid synthesis in lung mitochondria from irradiated and nonirradiated animals was compared.

In all experiments, 4 days after exposure, the total hydroxyproline content of both control and irradiated animals dropped about 10%, rising slowly between the tenth and the eighteenth day (Figure 1). The initial drop may have been a result of reduced food intake during the first day following irradiation in both the control and irradiated rats. Fasting has been reported to cause a reduction in extractable collagen levels.

The total hydroxyproline content of rats given 800 R tended to increase, relative to controls, from the second to the fourteenth day postexposure. This increase, initially, was mainly in the collagen fraction. In all experiments, on the fourth day postexposure, levels of collagen in the irradiated rat lungs were greater than in the controls, and the difference was statistically significant at the 95% confidence level. The fraction containing elastin showed an increased hydroxyproline content in X-irradiated animals relative to controls from the tenth to the eighteenth day. This increase, which was statistically significant on the tenth day, could have been a reflection of the earlier increased collagen levels. It has been reported that collagen may undergo in vivo solubility changes which would result in its appearance in the insoluble fraction at later times.

FIGURE 1. Effect of 800 R Thoracic X-Irradiation on Lung Hydroxyproline Levels in the Rat

TABLE I. The Effect of Chronic Cyanide Ingestion on the Hydroxyproline Content of Rat Lung Collagen^(a)

	Body wt, g	Lung wt, g	Hydroxyproline content, mM/g wet lung			
			0.15M NaCl fraction	0.5M NaCl fraction	0.5M citrate fraction	0.3M TCA ^(b) fraction
Controls	397 ± 37	1.98 ± 0.75	6.2 ± 0.9	2.4 ± 0.2	10.7 ± 1.0	7.6 ± 0.2
Treated	462 ± 81	1.45 ± 0.23	7.2 ± 0.7	2.9 ± 0.4	10.7 ± 1.2	6.1 ± 0.8
P value, 5%	0.5	0.5	0.4	0.02	--	0.01

(a) Each value reported is the average and the standard error of the determinations from five animals.

(b) Trichloroacetic acid

FIGURES 1-3. Negative Stained Collagen Fibrils. (Magnification same for all three figures.) FIGURE 1. A normal reconstituted collagen fibril from an adult female rat. The banding is about 670 Å per each dark-light unit. FIGURE 2. A normal appearing reconstituted collagen fibril from a cyanide-drinking rat. The periodicity is considerably shorter than the comparable controls. Otherwise it appears entirely normal. FIGURE 3. An abnormal collagen fibril reconstituted from an extract of lung of an animal drinking cyanide in its water for several months.

Figure 1 summarizes the combined data from the two 800 R experiments. Similar results were obtained after 1000 R thoracic X-irradiation.

The lung lipid content tended to increase during the first two days after exposure to 800 R (Figure 2). After 1000 R, an increase of lipid content on the first day postexposure was followed by a reduction the second day. The incorporation of acetate into long-chain fatty acids by lung mitochondria of rats given 800 R or 1000 R was increased 10 to 50% above the control levels. This was consistent with the increase in total lung lipids.

Conclusions

After 800-1000 R thoracic X-irradiation, increased lung hydroxyproline levels and increased fatty acid synthesis were detected at a time when no histopathology was observable. The biochemical changes were consistent with the histologic changes seen after higher radiation doses. Biochemical changes, when more fully understood, may afford an earlier and more sensitive indication of radiation effects in the lung.

FIGURE 2. Effect of 800 R Thoracic X-Irradiation on Lung Lipid Content in the Rat

EFFECT OF $^{239}\text{PuO}_2$ INHALATION ON LUNG TISSUE

Inhaled $^{239}\text{PuO}_2$ in rats caused an increase in collagen associated with a decrease in elastin and neutral lipids of lung tissue.

Investigators:
E. G. Tombropoulos
B. O. Stuart

Technical Assistance:
J. C. Gaven
K. E. McDonald

Experiments were initiated to study possible biochemical changes in lung tissue after inhalation of radionuclides. This reports preliminary observations on rats which had inhaled $^{239}\text{PuO}_2$.

Observations

Ninety-six 4-month-old female rats were exposed to six different levels of plutonium oxide aerosols resulting in initial lung burdens ranging from 0.3 to 8 μCi . Twenty-four other rats were kept as controls. When half of the animals of each group died, the remaining animals were sacrificed. Within 2-1/2 months after exposure, lungs from 24 animals representing all groups were analyzed for total lipid, lipid phosphorus, collagen, and elastin content as previously described (Annual Report, 1965). Tissue samples were also taken for histopathology.

No relationship could be established between biochemical changes and the initial ^{239}Pu lung burdens because all rats were sampled at about the same stage of development of pulmonary pathology. Therefore, the results were combined for this initial report.

Lungs from the ^{239}Pu rats had 50% more collagen and less elastin and total lipids than lungs from controls (Table I). The ratio of lipid-to-lipid phosphorus indicated that the reduction of total lipids was due to a reduction of neutral lipids rather than to phospholipids. Histologically, lungs of the $^{239}\text{PuO}_2$ rats generally exhibited a thickening of alveolar walls and a deposition of fibrinous material within the alveoli. These observations agree with the biochemical findings.

TABLE I. Rat Lung Collagen, Elastin, and Lipid Content After Inhalation of $^{239}\text{PuO}_2$ (Percent of dry defatted tissue)

	$^{239}\text{PuO}_2$ treated	Controls
Collagen	7.0 $\pm$ 0.4	4.5 $\pm$ 1.2
Elastin	0.9 $\pm$ 0.1	1.4 $\pm$ 0.2
Collagen/ elastin	7.8	3.1
Lipid	8.1 $\pm$ 0.5	11.3 $\pm$ 1.8
Lipid phosphorus	0.26 $\pm$ 0.02	0.22 $\pm$ 0.01
Lipid/lipid phosphorus	32	50

Conclusions

Inhaled $^{239}\text{PuO}_2$ particles caused an increase in collagen and a reduction in elastin and neutral lipid

content of the lungs. These changes were similar to those seen in lungs of rats exposed to 1000 R thoracic X-irradiation.

EFFECT OF CHRONIC CYANIDE INGESTION ON LUNG COLLAGEN

Lungs of rats chronically fed cyanide for over a year showed increased salt-soluble collagen (protocollagen) and decreased acid-soluble (stable) collagen. Electron micrographs of the reconstituted collagen fibrils indicated that dietary cyanide may have induced fundamental structural defects.

Investigators:

J. V. Dilley
J. D. Berlin

Technical Assistance:

R. J. Franks

It was earlier reported that chronic cyanide ingestion altered the clearance of particles from the lung of rats (Annual Report, 1965). This suggested the possibility that cyanide altered the structure of the lung at the cellular or subcellular level. The purpose of the present study was to explore this possibility further by examining the effects of cyanide, a potent metabolic inhibitor, on lung collagen.

Observations

Adult female Sprague-Dawley rats were given 1 mg/ml of cyanide in their drinking water for over a year. One-half to 1 grain of saccharin was added to each water bottle in order to induce the animals to drink freely. Control animals were maintained under identical conditions but received no cyanide.

Groups of animals were sacrificed and the lungs assayed for collagen by the method of Hauck and Jacob.⁽¹⁾ This method depends on the solubility of the collagen precursors (protocollagen) in neutral salt solutions and the solubility of stable collagen (mature) in acid solutions. The hydroxyproline content of these solutions, determined by the method of Martin and Axelrod,⁽²⁾ was used as an estimate of the total collagen content. Typical results are shown in Table I.

Collagen fibrils can be readily reconstituted from salt and/or acid solution by dialysis or by neutralizing the acid solution. This affords an opportunity to study the structure of the fibrils by electron microscopy. In Figure 1 the normal fibril is seen with its alternate light and dark banding and a spacing which is approximately 670 Å for each dark-light band unit. Figure 2 shows a normal-appearing

fibril from a cyanide-treated animal, but the unit spacing is reduced to 490 Å. Figure 3 shows an abnormal fibril from a cyanide-treated animal. The dark bands from the normal dark-light band sequence are completely absent, and there is only a suggestion of normal periodicity among the light-appearing material that is present. Such abnormal fibrils were found in two of the three experimental animals studied and in none of the four controls.

Conclusions

Cyanide is a potent enzyme inhibitor. It may be inhibiting one or more enzymes involved in the synthesis of stable collagen from procollagen polypeptides, thus accounting for the apparent increase in the quantity of hydroxyproline in the salt-soluble fraction. Alterna-

tively, a metabolite of cyanide, 2-iminothiazolidine-4-carboxylic acid, may inhibit the synthesis of proline into the procollagen polypeptides. This would result in an accumulation of proline-hydroxyproline residues in the salt-soluble fraction of the tissue (as seen in these experiments) and could also lead to abnormal stable fibrils, since the amino acid sequence of the procollagen would be unable to form the necessary cross-linking. Further studies are clearly required to choose between these or other possible mechanisms.

References

1. Hauck, J. C., and R. A. Jacob, *Proc. Soc. Exptl. Biol. Med.*, vol. 105, pp. 324-326. 1960.
2. Martin, C. J., and A. E. Axelrod, *Proc. Soc. Exptl. Biol. Med.*, vol. 83, pp. 461-462. 1953.

SPACE NUCLEAR SYSTEMS STUDIES

The reports in this section deal with biological problems connected with the utilization of reactors or radionuclide power sources in space. Spread of radioactive contaminants from such operations could constitute a hazard and we are investigating several specific aspects of such problems.

We have been concerned principally with the plutonium oxide microspheres planned for utilization in radionuclide power sources. Are these relatively large particles inhalable? If deposited in the lung, will they be retained? If ingested, what is the probability of holdup in the gastrointestinal tract; what damage will they do if held up; and to what extent will plutonium be absorbed from these very insoluble particles?

Three reports deal quite specifically with the problems outlined above, as studied in dogs and miniature swine. A fourth report is concerned more generally with gastrointestinal passage time, as influenced by age, in rats. A fifth report deals in a preliminary fashion with the problem of environmental redistribution of these particles, as influenced by particle size and density, wind velocity, etc.

RETENTION OF PLUTONIUM OXIDE MICROSPHERES IN BEAGLE DOG LUNGS

Alveolar deposition was minimal in dogs which inhaled 50- μ $^{238}\text{PuO}_2$ microspheres dispersed in air, only one particle being found in the lung of a dog sacrificed immediately after exposure. Two dogs retained single 120- μ microspheres, given by pulmonary intubation, for more than 6 months.

Investigator:
D. H. Willard

Technical Assistance:
W. Skinner

To help evaluate the potential hazard of 50- μ or larger $^{238}\text{PuO}_2$ plasma jet-fired microspheres, it is necessary to determine whether such large particles are inhalable and, if so, whether they are retained in the lungs. Beagle dogs were exposed to 50- μ $^{238}\text{PuO}_2$ microspheres dispersed in air or given single 120- μ $^{238}\text{PuO}_2$ microspheres by pulmonary intubation. Whole-body scanning techniques were used to follow the course of the particles in the dog. Using a dog phantom, it was shown that one 50- μ microsphere in a dog lung could be detected by this method.

Observations

Eleven dogs inhaled air in which 50- μ microspheres were dispersed. Immediately following exposure, whole-body scans were interpreted to indicate

that ^{238}Pu was deposited in lung and stomach areas of six dogs. However, after 24 hr, all particles were in the stomach and intestinal regions of five of the dogs. The particles were excreted by the fecal route within 3 to 5 days. The sixth dog was sacrificed immediately after the initial whole-body scan. The anterior portion of the tongue contained 17% of the activity and the stomach contained 83%. One particle, considerably smaller than 50 μ , was located in a gross section of the lung by autoradiography. Seventeen dogs were given one or several 120- to 220- μ microspheres by pulmonary intubation. One dog retained a 120- μ microsphere for 88 days. Two dogs have retained the microspheres for 6 months, while three other dogs have retained particles for several weeks. The only biological effect

observed in the dogs which retained particles for 6 months was a possible reduction of circulating lymphocytes.

under the experimental conditions employed. However, the probability of pulmonary deposition and retention is apparently very small.

Conclusions

These preliminary results show that 50- μ $^{238}\text{PuO}_2$ microspheres are inhalable

PASSAGE TIME AND PATHOLOGY FOLLOWING FEEDING OF $^{238}\text{PuO}_2$ MICROSPHERES TO SWINE

The passage time of $^{238}\text{PuO}_2$ ceramic submillimeter particles administered intragastrically to nine Hanford Miniature Swine averaged 10.5 days for the excretion of 99% of the dose. The passage of 0.18 to 1.1 Ci of ^{238}Pu elicited only a mild, inflammatory response. Two cases of particle retention at the ileo-cecal junction produced local inflammation and small regions of necrotic tissue surrounding the particles.

Investigators:
V. H. Smith
H. A. Ragan
J. L. Beamer

In continuation of studies reported last year (Annual Report, 1965), $^{238}\text{PuO}_2$ ceramic submillimeter microspheres were administered intragastrically to six additional Hanford Miniature Swine. The object of these experiments was to measure passage time, to determine the incidence of long-term particle holdup, and to observe any pathology which might result.

Observations

The data on passage time for the nine pigs fed microspheres by gavage is summarized in Table I. The average time of first appearance of microspheres in the feces was 2.7 days. A delay of 1 day may be attributable to experimental trauma, since the animals usually failed

to defecate for about 24 hr after being snared and stomach-tubed. Fifty percent of the dose was excreted, on the average, by the 5.6th day and 99% by the 10.5th day. In more than half the animals, particles were still present in the tract on the day of sacrifice, 14 days after dosing. Pig 2170 had a few particles in the stomach as well as through the rest of the tract. These particles appeared to be mobile but closely associated with the mucus lining of the stomach and small intestines. At necropsy, these particles were visible on the mucosal surface; they could usually be washed off with the mucus and were not associated with any gross lesions. Particles in the spiral colon and the large intestine were usually in the intestinal contents and not on the

TABLE I. Passage Time of $^{238}\text{PuO}_2$ Microspheres in Swine

Pig number	^{238}Pu administered		Passage time (days postadministration)		
	Dose, Ci	Number of particles (calculated)	First appearance	50% Excreted	99% Excreted
1939	0.96	20,000	2	6	10
2006	1.1	23,000	3	6	9
2012	1.1	23,000	3	5	9
2111	0.27	5,600	2	3	6
2132	0.33	6,900	2	5	13
2163	0.18	3,700	4	6	12
2157	0.71	15,000	2	6	10
2169	0.30	6,200	3	5	12
2170	0.37	7,700	1	7	>14

mucosal surface. In the cecum, a few particles were against the wall but generally were mixed with the contents.

Particles were found imbedded in the crypts of lymphoid tissue at the ileo-cecal junction in two pigs. Surrounding the particles was an area approximately 1 cm in diameter which was grossly necrotic and inflamed. Histopathologic examination confirmed the necrosis. Autoradiography indicated about 10-20 particles were present in the involved region of pig 2132. Numerous large areas of complete mucosal necrosis extended well down into the lamina propria. In some instances this damage extended almost to the muscularis. Associated with these areas were increased numbers of granulocytes, plasma cells, lymphocytes, and many eosinophils. Mitotic activity was increased in the Peyer's patches and in the cells of the intestinal glands. Several particles were removed from the necrotic tissue at the ileo-cecal junction in pig 2012. Optical examination and sizing disclosed them to be of 95, 83, 49, and 8-18 μ diameter.

Microscopic examination revealed scattered areas of villar tip necrosis with numerous inflammatory cells and sloughing into the lumen of the small intestine with much less damage to the cecum and large intestine. The lamina propria contained increased numbers of lymphocytes, plasma cells, and eosinophils, typical of an inflammatory process. These observations are consistent with radiation damage and are general sequelae of an inflammatory condition. While pathologic conditions were observed throughout the small intestine, it was most severe in the ileum. The cecum, spiral colon, and posterior colon exhibited only slight necrosis and showed less damage than the small intestine. Histopathologic examination has not been completed on pigs 2157, 2169, and 2170.

Small lithium fluoride dosimeters were surgically implanted along the serosal surface of the digestive tract of pig 2157 two weeks prior to dosing. The maximum exposure found after dosing was 3.1 R at the ileo-cecal junction. The values shown in Table II are from

TABLE II. Evaluation of LiF Dosimeters Implanted in Pig 2157

<u>Location of dosimeter</u>	<u>Dose, R</u>	<u>Relative dose, ileo-cecal junction = 1</u>
Duodenum - 20 cm from stomach	0.4	0.13
Jejunum - 425 cm from stomach	0.7	0.23
Ileum - 475 cm from stomach	0.8	0.26
Ileum - 675 cm from stomach	1.0	0.32
Ileo-cecal junction	3.1	1.0
Cecum - blind end	0.7	0.23
Ansa spiralis	1.8	0.58
Distal colon - 25 cm from anus	1.7	0.55
Ovary - near uterus junction	1.0	0.32
Abdomen wall - near umbilicus	1.1	0.36

one animal and are probably accurate to within 50%. They are not representative of the dose to the mucosal surface, except in a relative sense, since the low-energy radiation involved will be mainly absorbed by the mucosal surface and gastrointestinal contents.

Conclusions

The passage times observed in these experiments are longer than were expected. Radiation from the particles might be a contributing factor in increasing passage time; however, a several-fold variation in the amount of radioactivity and the number of

particles (Table I) showed no correlation with passage time. Although apparently receiving the lowest radiation dose, the most extensive, generalized damage was seen in the small intestine, a consequence, no doubt, of its greater radiosensitivity.

The generalized radiation hazard from ingestion of $^{238}\text{PuO}_2$ microspheres by humans would seem to be quite negligible, considering the small number they are likely to ingest. Particle hold-up, while highly improbable, could lead to significant pathological changes. The long-term effects of such an event in humans and animals are unknown.

ABSORPTION OF $^{238}\text{PuO}_2$ FROM THE GASTROINTESTINAL TRACT OF SWINE

Nine Hanford Miniature Swine were given large numbers of $^{238}\text{PuO}_2$ microspheres by gavage. After 14 days the amount absorbed to tissues and urine was less than 10^{-6} of the dose. Absorption as a function of surface area of the microspheres was less than 2 mCi/m^2 .

Investigators:
V. H. Smith
J. L. Beamer

The possibility of ingestion is a potential hazard in the manufacture and utilization of $^{238}\text{PuO}_2$ microspheres. Subsequent absorption of plutonium from the gastrointestinal tract could conceivably lead to a significant deposition in body tissues. To evaluate this possibility, studies were conducted in Hanford Miniature Swine. Some preliminary results from this study were presented in last year's Annual Report.

Observations

Three batches of microspheres were used, all of production-run quality and supplied by Mound Laboratory. The third batch had purportedly been "cleaned" ultrasonically and contained less plutonium dust associated with the microspheres. Three swine were employed in studies with each batch of microspheres. The swine were maintained in metabolism cages for 14 days following administration of the microspheres by gavage. Total urine output was collected and various tissues sampled at necropsy. With the exception of a few samples still not analyzed, the results are summarized in Table I.

The results for any particular tissue are widely divergent. Within batches there is a somewhat greater consistency. One must be suspicious

of the very high plutonium concentrations found in the gastric, prefemoral and prescapular lymph nodes of pig 1939 since such concentrations did not occur in any of the other animals. Although the data from "Batch 3" animals are still incomplete, there is a strong suggestion that these microspheres were considerably more soluble than those from the other batches. Whether this difference should be attributed to the "cleaning" procedure is impossible to say.

Conclusions

Despite rather obvious and puzzling differences in the data thus far obtained, it is nevertheless quite clear that absorption is so low as to constitute an entirely negligible hazard. Assuming the maximum indicated absorption of about 10^{-6} , a single 200- μ diameter microsphere, containing about $67 \mu\text{Ci } ^{238}\text{Pu}$ would lead to a body burden of 67 pCi, which is less than one five-hundredth of the permissible limit for occupationally exposed personnel. The possibility of microsphere retention (as considered elsewhere in this Annual Report) with resultant damage to the gastrointestinal tract, and perhaps enhanced absorption, may constitute a more significant hazard.

TABLE I. Absorption of ^{238}Pu from $^{238}\text{PuO}_2$ Microspheres in the Gastrointestinal Tract of Miniature Swine

Batch no.	1			2			3		
Pig no.	1939 ^(a)	2006 ^(a)	2012 ^(a,b)	2111	2132 ^(a,b)	2163 ^(a)	2157	2169 ^(a)	2170 ^(a)
Dose, Ci	0.96	1.1	1.1	0.27	0.33	0.18	0.71	0.30	0.38
Tissues	Tissue plutonium content, nCi								
Liver	0.31	0.38	2.4	0.20	0.35	0.90	1.9	180	
Spleen	0.090	0.009	0.087	0.011	0.005	0.013	1.9	4.1	
Kidneys	0.16	0.47	0.80	0.007	0.065	0.013	0.17	19	
Lung	0.080	0.036	0.037		0.014		2.7		
Heart	0.080	0.002	0.038	0.005	0.002	0.007	0.009		
Muscle ^(c)	15	3.0	0.89		0.53	0.35	72		
Ovaries		0.003	0.21	0.001	0.003	0	0.001	0	
Skeleton	0.25	2.3	8.9	6.5	6.8	6.2	32	31	51
Other ^(d)	150	0.22	0.42	0.015	0.033	0.012	0.025	0.25	
Total tissue	170 ^(e)	6.4	14	6.7	7.8	7.5	110	230	
Urine	0.66	0.65	1.2	0.30	0.40	2.8			
Total absorbed	170 ^(e)	7.0	15	7.0	8.2	10	>110	>230	
Absorbed fraction of fed dose ($\times 10^8$)	18 ^(e)	0.64	1.4	2.6	2.5	5.6	>29	>77	
Absorption per unit area, $\mu\text{Ci}/\text{m}^2$	420 ^(e)	15	32	61	59	130	>690	>1800	

(a) Microspheres present in the gastrointestinal tract at necropsy.

(b) Microspheres embedded in the lymphoid tissue of the ileo-cecal junction at necropsy.

(c) Calculated assuming 42% of the pig's body weight to be muscle.

(d) Includes adrenals, thymus, parotid, pancreas, salivary glands, and sundry lymph nodules.

(e) Includes abnormally high Pu concentrations in the gastric, prefemoral, and prescapular lymph nodes.

EFFECT OF AGE AND PARTICLE SIZE ON PASSAGE TIME THROUGH
THE GASTROINTESTINAL TRACT OF THE RAT

The passage times of insoluble plutonium oxide particles and of a poorly absorbed soluble tracer, ^{106}Ru , were studied in adult and neonatal rats. Substantial differences in kinetics were seen, notably an increase in retention time in the stomach and ileum of the neonatal rats and a much reduced rate of passage through their shorter tract.

Investigators:

M. R. Sikov
D. D. Mahlum

Technical Assistance:

Alma L. Crosby
Joan O. Hess

Although the gastrointestinal tract is the critical organ for hazard evaluation following the ingestion of non-absorbed radioactive materials, relatively little is known of the kinetics of passage of these materials through the tract, especially in the immature animal. This report presents results from studies on the effect of age on passage time of insoluble particles and of a poorly absorbed soluble radionuclide.

Observations

Three nominally different size distributions of plutonium oxide particles [count median diameter (CMD): 0.13 μ , 0.15 μ , 1.8 μ] were administered intragastrically to adult and 6-day-old rats. The animals were sacrificed at 1, 3, 7, and 21 days after administration, and the retention and distribution in the gastrointestinal tract ascertained.

As shown in Table I, a substantial fraction of all three particle size distributions was still present within the tract at 1 and 3 days following administration. The smallest distribution tended to be excreted more com-

pletely than the other two, particularly in the newborn animals. The possible influence of coprophagy dictates caution in the interpretation of the data on the distribution within the gastrointestinal tract of the adult animals. Allowing for possible distortion due to coprophagy, it is nevertheless apparent that the fraction found in the ileum and colon was substantially greater, and the cecal content lower in the infant rats than in the adults. The rudimentary state of the cecum in the younger animals no doubt explains the latter observation. The data obtained at 21 days postadministration are omitted from Table I; in all instances, less than 0.1% of the administered activity was present in the gastrointestinal tract at 21 days.

A poorly absorbed radioactive tracer, $^{106}\text{Ru-Rh}$, was administered to adult and 6-day-old animals by stomach tube, and its distribution within the gastrointestinal tract determined by autoradiographic and counting techniques at times ranging from 1/2 to 24 hr after administration. The initial rate of release from the stomach was greater in the adult than in the infant rats. This rapid rate continued longer in

TABLE I. Retention of Administered Activity in Gastrointestinal Tract at Intervals After Gavage

Particle distribution, CMD in μ	Days post-administration	Percent retained	Relative distribution (percent of total activity in tract)					
			Stomach	Duodenum	Jejunum	Ileum	Cecum	Colon
<u>Adult</u>								
0.13	1	8.1	8	0.2	3	11	54	23
	3	0.4	5	<0.1	0.3	2	58	36
	7	0.1	9	0.1	3	11	50	26
0.15	1	17	1	0.5	0.4	3	59	37
	3	0.8	0.3	<0.1	0.8	0.6	46	52
	7	0.3	40	0.9	4	8	30	16
1.8	1	0.8	7	0.6	6	4	63	20
	3	1.2	4	0.1	1	2	33	60
	7	0.9	8	0.2	2	6	45	40
<u>Neonatal</u>								
0.13	1	3.2	1	0.8	0.8	2	2	93
	3	0.1	2	2	3	18	1	74
	7	<0.1	3	0.2	2	29	12	54
0.15	1	11	1	0.2	1	13	2	82
	3	3.4	0.1	<0.1	23	59	4	14
	7	0.3	0.9	0.1	7	74	3	15
1.8	1	8.4	0.3	0.1	3	2	0.8	94
	3	1.1	0.3	0.3	18	58	2	21
	7	0.3	1	0.1	3	52	2	42

the adults so that nearly 80% of the activity was cleared within 2 hr; in the neonatal animals, only about 50% was released in 2 hr (Figure 1). As shown in Figure 2, entry into the small intestine was rapid and the material passed on with little retention. The residence time in the last half of the small intestine was much greater in the juvenile animals than in the adults, so that the times of entry into and the patterns of passage through the large intestine were similar although the adults attained greater peak levels. There was little retention of ^{106}Ru in the cecum of the young animals which is in accord with our finding with plutonium particles.

FIGURE 1. Removal of $^{106}\text{Ru-Rh}$ from the Stomach as a Function of Time After Administration by Gavage to Adult and Neonatal Rats

FIGURE 2. Distribution of $^{106}\text{Ru-Rh}$ in the Gastrointestinal Tracts of Adult and Neonatal Rats at Serial Times After Administration by Gavage.

Conclusions

While the overall passage times did not differ markedly for juvenile and adult animals, the shorter tract length of the neonate resulted in a substantially reduced rate of passage and a concomitant increase in the average time of contact with any given length of the tract. There

was clear evidence for a longer retention of material in the stomach and in the ileum of the infant rat as compared to the adult. Smaller organ diameters would also have the effect of increasing radiation dose. All of these observations suggest a greater hazard from ingested radioactive particles in the infant and the need for more extensive study of this problem.

RESUSPENSION OF PARTICULATES FROM A SURFACE

Static critical wind velocities were measured for resuspension of particulates having varied size and density. In all cases tested, a minimum critical velocity is observed in the size range 20-60 μ .

Investigator:
J. J. C. Hsieh

Deposited particles may again become airborne through the action of wind force. Static critical wind velocity for a given size particle is a measurement of the ability of wind to resuspend an already deposited and undisturbed particle. The measurement has practical value in the prediction of inhalation and ingestion radiation hazards from already settled radioactive particles such as might result from the destructive reentry of nuclear reactor or radionuclide power sources from space.

Observations

Lead and aluminum particles were used to simulate plutonium and radioactive-fused silicate glass since they are of about the same respective densities. The particles were separated into various size fractions by sieving the coarser fractions and by air current separation of the finer particles. The particles of a given size range were allowed to fall onto glass slides at approximate terminal velocities. Two test slides, placed side by side on the bottom of a wind tunnel for each test run, were surrounded by other glass slides to minimize the effect of the slide edge on the wind-flow pattern. At least two test runs were conducted

for each particle size. The Hastings nondirectional thermocouple anemometer, type N-7 probe, was placed at the center of the tunnel which has a cross section of 9" x 9". Air velocity was increased in small increments until it appeared that about one-half of the particles on the slide were resuspended. This recorded wind velocity was taken as the static critical velocity for groups of uniformly sized particles. Although a few particles are dislodged at lower speeds and some remain attached even at higher speeds, the great bulk of the particles is dislodged with a slight increase in velocity. Data from these tests are shown in Figure 1.

FIGURE 1. Static Critical Wind Velocity Curve for Deposited Pb and Al particles (Temp. 18-21 °C, Relative Humidity 44-45%)

Five-micron lead particles and 15- μ aluminum particles were not dislodged from the slides by winds of 60 mph, the maximum achievable in this wind tunnel.

Conclusions

The critical wind velocity curves for both lead and aluminum have a minimum value in the mid-particle size range. This minimum is more sharply defined for lead than for aluminum. While the smallest particles studied are more difficult to resuspend than the larger ones, once suspended they

remain in the air for a longer time because of their lower settling velocity.

These findings offer an explanation for the lack of retention of larger particles by plant leaves exposed to close-in fallout during early bomb testing. They also offer a start toward a better definition of parameters affecting deposition and retention of particles on surfaces. Future emphasis will be on the natural surfaces of leaves and soil as well as on some structural surfaces.

COMBATING DETRIMENTAL EFFECTS OF RADIATION

Our efforts in this area have for many years been largely concerned with the removal of internally deposited radionuclides. Much of this effort, particularly that involving inhaled radionuclides, is inseparably connected with studies of deposition and retention and is incorporated in other sections of this Annual Report.

The first of the three reports in this section describes very practical attempts to evaluate treatment procedures through large animal studies. This effort will be expanded with the availability in 1967 of new facilities designed for handling grossly contaminated swine.

The second report represents a somewhat different approach to the study of plutonium removal which we feel offers considerable promise. By gaining a better understanding of the processes normally employed by the body in removing insoluble particulates from the circulation, it may be possible to stimulate these processes and improve their efficiency.

The third report concerns a peripheral study of the potential application of heavy metal chelates as radiologic contrast media.

PLUTONIUM-CONTAMINATED WOUND STUDIES IN SWINE

Four years after intradermal injection of plutonium nitrate, liver and skeletal burdens were 11% and 13%, respectively, of the administered dose. Plutonium was also present in lymph nodes. Tourniquet application appeared to reduce the absorption of subcutaneously implanted plutonium in the foreleg of swine. Chilling the contaminated foreleg did not reduce absorption.

Investigators:
Beatrice J. McClanahan
H. A. Ragan

Four years ago, ten swine were multiply injected, intradermally, with plutonium nitrate, at dose levels ranging from 0.0016 μ Ci to 5 μ Ci (Annual Report, 1962). One of these animals was sacrificed at 4 years postinjection for study of retention and redistribution of the plutonium. Additional short-term studies are described in which efforts were made to alter the translocation of plutonium from a subcutaneous injection site on the foreleg.

Observations

Table I lists the plutonium content of tissues measured 4 years after multiple intradermal injections. One-fourth of the injected dose was retained in the liver and skeleton. Substantial quantities were also

TABLE I. Retention of Plutonium
4 Years after Intradermal Injection

<u>Tissue</u>	<u>Percent of administered dose</u>
Skeleton	13
Liver	11
Kidney	0.020
Spleen	0.019
Ovaries	0.0064
Adrenal	0.0010
Lymph nodes	
Superficial inguinal	0.053
Calcified area from superficial inguinal	0.12
Prefemoral	0.54
Prescapular	0.61
Hepatic and internal iliac	0.0026
Bronchial	0.0045

present in certain lymph nodes. Plutonium retention at the injection sites ranged from 0.3-2.0%, except for the lowest level 0.0016 μCi sites where an average of 7.5% was retained.

A calcified area found in the superficial inguinal lymph node contained more plutonium than the rest of the node. Histologic examination of the prefemoral node revealed marked congestion and hemorrhage in the peripheral sinuses. The structure of the node, however, appeared normal. Other lymph nodes examined exhibited no abnormal characteristics.

Sections of skin from sites injected with 1 or 5 μCi showed areas of eschar formation, hyperkeratotic reaction, some necrosis, and epithelial downgrowth. In the deep dermis were regions displaying calcium salt deposition, consolidation of collagen fibers, and hyalinization. Plutonium was associated with tissue macrophages, dense collagen fiber, hyperkeratotic portions and connective tissue cells. At lower dosage levels, most of the sections appeared normal except for a few areas of epidermal hyperplasia and hyperkeratosis; plutonium was located in the tissue macrophages and connective tissue cells of the dermis.

Studies were performed on six pigs to determine the effect of the application of a tourniquet, or of chilling, on the rate of absorption of $\text{Pu}(\text{NO}_3)_4$ from subcutaneous injection sites on each foreleg.

Immediately after injection of three pigs, a tourniquet was placed on each foreleg of one pig and the animal was placed in a sling. Another pig had its forelegs immersed in an ice bath to produce deep chilling. The third pig served as an untreated control. Animals were held for 2 hr, then killed by exsanguination. Tourniquets were not removed until the animal was dead. Results of two such experiments are shown in Table II. In both experiments, the use of tourniquets appeared to effect some reduction in the plutonium content of the liver,

TABLE II. Effect of Treatments on the Absorption of Plutonium from a Subcutaneous Deposition Site

Tissue	Percent of administered dose		
	Untreated	Tourniquet	Ice bath
<u>Experiment I</u>			
Liver	0.040	0.012	0.029
Regional lymph nodes			
Right	1.4	15	4.8
Left	0.0074	0.049	0.97
Injection sites			
Right	64	66	120
Left	78	82	89
<u>Experiment II</u>			
Liver	0.0076	0.0040	0.014
Regional lymph nodes			
Right	0.076	0.13	--
Left	1.3	0.055	--
Injection sites			
Right	80	87	85
Left	86	87	85

in contrast to results obtained in previous experiments (Annual Report, 1963). The tourniquets had no apparent effect on retention at the injection sites or on translocation to regional lymph nodes. Chilling appeared to produce no consistent or marked effect. Samples of blood were withdrawn from indwelling catheters at 0.5, 1, and 2 hr yielding results that were very erratic, exhibiting no trends that could be correlated with the treatments employed.

Conclusions

The large fraction of plutonium retained 4 years after intradermal injection of the nitrate underscores the importance of prompt excision of accessible plutonium-contaminated wounds, and the need for other treatment measures which might reduce translocation. Neither application of a tourniquet nor chilling of the wound area appear to offer great promise as such a treatment measure.

PHAGOCYTOSIS OF $^{239}\text{PuO}_2$ PARTICLES

Phagocytosis of carbon particles by peritoneal macrophages of the rat was more rapid than phagocytosis of $^{239}\text{PuO}_2$ particles. The rate of phagocytosis and translocation of $^{239}\text{PuO}_2$ particles from the peritoneal cavity were dependent on particle size. DTPA inhibited phagocytosis when given simultaneously with particles.

Investigator:
C. L. Sanders*

The objectives of this study were to determine the phagocytic abilities of various cell types in the peritoneal cavity; to determine the extent of cell damage as a result of phagocytosis; to determine the rate of translocation of plutonium from the peritoneal cavity; to compare phagocytosis of carbon particles with $^{239}\text{PuO}_2$ particles; and to discern the effects of diethylenetri-

aminepentaacetic acid (DTPA) on phagocytosis.

Observations

Female, Sprague-Dawley rats (300-400 g) were injected intraperitoneally with 1 mg charcoal particles (approx. 20×10^6 particles; mean diameter, 1μ) or $2 \mu\text{Ci } ^{239}\text{PuO}_2$ particles (approx. 8×10^6 particles; count median diameter, approx. $0.1\text{-}0.2 \mu$). DTPA or

*A.E.C. Postdoctoral Fellow

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was given intraperitoneally, usually simultaneously with particles.

The phagocytic activity of cells in the peritoneal wash was determined by visual counts of particles or by autoradiography. The decreasing order of phagocytic effectiveness for carbon and $^{239}\text{PuO}_2$ particles was macrophages, monocytes, large lymphocytes, eosinophils, small lymphocytes, and mast cells. Phagocytosis of particles was confirmed using phase contrast microscopy of living cells.

Phagocytosis of carbon particles was rapid with nearly all macrophages and about 35% of all other cells having phagocytized particles within 1 hr. Phagocytosis of $^{239}\text{PuO}_2$ particles was maximal at 1 and 4 hr after administration, when determined by visual and autoradiographic counting, respectively. The number of cells with $^{239}\text{PuO}_2$ particles, as determined by autoradiography, decreased at 6 hr to values identical to those obtained by visual counts (Figure 1). Phagocytosis was negligible by 24 hr as determined by either method. There were wide variations in the percentages of macrophages and lymphocytes in the peritoneal cavity with no obvious trend toward a change in cell composition. Most of the administered $^{239}\text{PuO}_2$ particles were phagocytized after 4 hr.

The number of alpha tracks originating from a $^{239}\text{PuO}_2$ particle on an autoradiogram is related to the size of particle. Track counts from particles on slides of peritoneal cells after 2 weeks exposure indicated that 36% and 23% of all particles had 4-6 and >30 tracks per particle, respectively, during the first 2 hr postinjection; 8%

FIGURE 1. Phagocytosis of Carbon and $^{239}\text{PuO}_2$ Particles by Peritoneal Macrophages (Phagocytic index = % of cells containing particles)

and 61% had 4-6 and >30 tracks per particle, respectively, after 5-6 hr.

There was a slight decrease in ^{239}Pu activity of the peritoneal wash during the first 5 hr. Total activity of the wash at 24 hr was less than 0.001 μCi . Total ^{239}Pu excreted in the urine and feces during the first 48 hr was less than 0.001 μCi per day. Approximately 90% of the administered activity was found in the mesenteries and omenta and about 6% in the liver after 24 hr.

Many peritoneal cells, especially lymphocytes, exhibited cytoplasmic vacuolization after exposure to $^{239}\text{PuO}_2$ particles. Plutonium particles were sometimes visible within the vacuoles. A few nuclei exhibited vacuoles and dissolution of nuclear material around the particle (Feulgen's stain). Phagocytosis of carbon particles did not result in significant cell damage. There was a significant increase in pycnotic or karyorrhectic cells 24 hr after $^{239}\text{PuO}_2$ administration.

The threshold concentration for DTPA inhibition of carbon particle phagocytosis was 0.005 mmoles, when DTPA was administered simultaneously with particles. Injection of 0.1 mmoles DTPA at 2 min after carbon particles resulted in an insignificant decrease in phagocytosis.

Administration of calcium in amounts greater than 0.1 mmoles, with carbon particles, resulted in a very significant association of particles with agglutinated cell masses in the peritoneal cavity of such proportion that black flakes were readily visible on withdrawal of the peritoneal wash after carbon particle administration.

Conclusions

Preliminary evidence would indicate that an alpha-emitting particle does not deter the macrophage from accomplishing its mission (i.e., phagocytosis and translocation). Only lymphocytes and monocytes appeared to exhibit significant cell damage as a result of plutonium administration. Larger particles were more rapidly phagocytized than were smaller particles. Smaller plutonium particles were more readily cleared from the peritoneal cavity than were the larger particles.

CHELATES AS CONTRAST MEDIA: URANIUM-DTPA

The diethylenetriaminepentaacetic acid (DTPA) chelate of uranium produced useful angiograms when used in swine; however, acute toxicity, manifested by cardiac arrest, contraindicates its use in angiography.

Investigators:

V. H. Smith

H. A. Ragan

Radiologic contrast media for use in large animals or man require an elemental moiety with atomic number (Z) greater than 50 for reasonable efficiency in the X-ray diagnostic energy region. Most commonly used elements are iodine (Z = 53), barium (Z = 56), and thorium (Z = 90), although the latter's usefulness is severely cur-

tailed by long-range toxic side effects. Lead (Z = 82) and bismuth (Z = 83) chelates, tested as radiocontrast media, yield good radiographs but have low therapeutic indices. Uranium usually forms more stable chelates than either lead or bismuth and the chelate might therefore be expected to be less toxic. Since X-ray absorption

is mainly by the photoelectric effect in the diagnostic energy region (50-90 keV), the absorption varies mainly as Z^4 , and uranium ($Z = 92$) should have a clear advantage over iodine or barium.

Observations

Uranium hydroxide was precipitated by adding a slight excess of ammonium hydroxide to a uranyl nitrate solution, washed several times, and dissolved at pH 3-4 with DTPA before being brought to pH 7.28 with sodium hydroxide. A slight excess of DTPA was present to minimize free metal ions. The final concentration of uranium was 75 mg/ml. Twenty milliliters of this uranium chelate solution were rapidly injected into the anterior vena cava of anesthetized 70 kg Hanford Miniature Swine. Radiographs of the chest region were made 20 seconds later. The solution caused immediate cardiac arrest in three swine. When calcium gluconate-calcium glucoheptonate was administered intravenously with the chelate, the animals survived, but the additional

dilution resulted in poor diagnostic quality arterioradiograms, but adequate quality pyelograms. Chelate injected subcutaneously was readily discernible on the radiogram.

Under the conditions used, the uranium-DTPA chelate cannot be recommended over less toxic compounds, even though the resolution obtained is comparable to that obtained with sodium diatrizoate (50% aqueous solution). The initial concentration of iodine in the latter is 2.4M compared with 0.27M uranium in the DTPA chelate, but the X-ray absorption of the uranium is about ten times greater than iodine, so the two contrast media are roughly similar in effective X-ray absorption.

Conclusions

While this exploratory experiment was unsuccessful, favorable qualities of the uranium chelate could be utilized by lowering the toxicity with a preinjection of DTPA and calcium. The chelate might be more useful in pyelography or other types of radiography than in angiography.

MOLECULAR AND CELLULAR LEVEL STUDIES

The six reports included in this section represent a substantial expansion of effort in the molecular and cellular field, made possible by the assignment of an AEC Postdoctoral Fellow and by additional funding provided by the Battelle Institute, a Division of Battelle Memorial Institute, Columbus, Ohio. The Battelle-supported work is included in this report because of its very close relationship to AEC-supported programs.

Two reports are concerned with a major program of continuing interest on the control of RNA metabolism in Escherichia coli. Three reports deal with various aspects of tryptophan transport and synthesis in Neurospora crassa. The sixth report reflects a particular interest of the assigned AEC Postdoctoral Fellow in the area of sterol metabolism.

In addition to these reports, a substantial effort in the molecular and cellular field is reflected in other sections of this Annual Report, including several ultrastructural, biochemical, and microbiological studies connected with investigations of the intestinal radiation syndrome, temperature acclimation in fish, and basic studies in pulmonary physiology.

RIBOSOMAL PRECURSORS IN STEADY STATE CELLS

The steady state level of ribosomal precursors relative to mature ribosomes was shown to be low in cells with a high differential rate of synthesis of RNA and high in cells with a low differential rate of synthesis of RNA. These observations, which are essentially independent of the rate of growth of the cells, support the view that immature ribosomes participate in the regulation of RNA synthesis.

Investigator:
W. H. Matchett

Technical Assistance:
Laura S. Winn

Recent work in this laboratory (Annual Report, 1965) showed that addition of methionine to cultures of Escherichia coli growing in minimal medium caused the cells to increase their differential rate of synthesis of RNA without increasing their rate of growth. Methionine was further shown to stimulate the rate of conversion of chloramphenicol ribonucleoprotein particles to mature ribosomes in cells recovering from chloramphenicol inhibition. These results suggested that ribonucleoprotein particles might themselves participate in the regulation of synthesis of RNA by acting as feedback inhibitors. This interpretation predicts an inverse relationship between the differential rate of RNA synthesis and the relative abundance of ribonucleoprotein particles.

Observations

Cultures of E. coli were grown in minimal medium containing 2-¹⁴C-uracil (10 µg/ml) and either casamino acids (0.5%) or methionine (50 µg/ml). The control culture received only the radioactive uracil. The curves of Figure 1 show growth rates for each of these cultures. At the times indicated by the arrows on each curve, samples were withdrawn, chilled rapidly, and centrifuged. Extracts were prepared from the pelleted cells by standard methods.

These radioactive extracts were mixed with a large excess of a non-radioactive extract prepared from cells which had been experimentally enriched with respect to ribonucleoprotein particles and which serve as a source of carrier material. The presence of carrier materials in the

FIGURE 1. Growth of *E. coli* in Various Media (K = growth rate in generations per hour)

resulting mixture permitted the physical isolation of radioactive ribosomes and ribonucleoprotein particles by standard methods of differential ultracentrifugation. The ribosomes and ribonucleoprotein particles resulting from the isolation procedure were checked for homogeneity by the method of zone centrifugation through density gradients prepared from sucrose. The preparations were found by this method to be free from cross-contamination.

Since the radioactive extracts were mixed with a 25- to 50-fold excess of nonradioactive carrier extract, the contribution of material absorbing at 260 $m\mu$ by the radioactive extract was trivial. The specific radioactivity of the resulting pellet was therefore a measure of the amount of either ribosomal or ribonucleoprotein material contributed to the mixture by the radioactive extract. Further, the specific radioactivity was independent of minor quantitative variations in the recovery of pellets at various stages in the ultracentrifugal procedure.

In Figure 2, the specific radioactivities of ribosomal pellets obtained from the cultures described in Figure 1 are plotted as a function of increases in turbidity during the period of log-phase growth under investigation. The slopes of these curves are therefore direct functions of the differential rates of synthesis of ribosomes in the experimental cultures. The curves show clearly that the synthesis of ribosomes proceeded at a considerably higher relative rate in cultures supplemented with either casamino acids or methionine than in unsupplemented cultures.

In Figure 3, the specific radioactivities of the ribonucleoprotein pellets are plotted in the same manner. Similarly, the curves presented show the relative rates of synthesis of ribonucleoprotein particles in the three cultures. It was of interest to

FIGURE 2. Relative Rate of Synthesis of Ribosomes

FIGURE 3. Relative Rate of Synthesis of Ribonucleoprotein Particles

note that although the supplemented cultures showed a higher relative rate of synthesis of ribonucleoprotein particles, the percentage increase was considerably less in this case than was observed in the case of the ribosomes. The supplemented cultures showed a 60% increase in the relative rate of synthesis of ribosomes with only a 25% increase in the relative rate of synthesis of ribonucleoprotein particles.

The relative rates presented in Figures 2 and 3 are in effect expressions of the levels of ribosomes and ribonucleoprotein particles per unit of protein in the cultures. By the simple algebraic expedient of plotting the specific radioactivity of the ribonucleoprotein particles as a function of the specific radioactivity of the

FIGURE 4. Level of Ribonucleoprotein Particles Relative to Ribosomes

ribosomes, curves are generated whose slopes reflect the level of ribonucleoprotein particles relative to the level of ribosomes for each cultural condition. These relationships are depicted by the curves of Figure 4. From these curves it is clear that the unsupplemented culture with the lowest relative rate of synthesis of RNA showed a 30% higher content of ribonucleoprotein particles per unit of ribosomes than either of the supplemented cultures having higher relative rates of synthesis of RNA.

In similar experiments it was of interest to note that methionine stimulated the rate of synthesis of ribosomes in leucine-inhibited cultures to the same extent as in minimal cultures. In view of the proposed mechanism of leucine inhibition,⁽¹⁾ this

observation alone would seem to rule out any trivial mechanism for explaining the methionine-mediated stimulation of RNA synthesis in E. coli.

Ron and Davis⁽²⁾ have obtained similar results and have reached this same conclusion independently. In leucine-inhibited cultures, which have a considerably lower growth rate, the presence of methionine caused the cells to exhibit a reduced level of ribosomal precursors relative to mature 70S ribosomes. The effects reported therefore would seem to be essentially independent of the growth rates of the cultures. On the other hand, the trend toward an inverse relationship between the level of ribonucleoprotein particles and the differential rate of synthesis of RNA is apparent in these results.

Conclusions

The addition of the single amino acid methionine to log-phase cultures of E. coli causes the cells to exhibit an abrupt increase in their differential rate of synthesis of RNA. The rate of growth of the cells is not appreciably changed by the presence of methionine. The stimulatory effect of methionine on RNA synthesis is apparent in cultures inhibited either by amino acid analogs or by leucine. It seems clear therefore that the effect is not the result of relieving a growth limitation imposed on the cells by a less than optimal endogenous supply of methionine.

It was reported previously (Annual Report, 1964 and 1965) that methionine enhanced the rate of conversion of chloramphenicol ribonucleoprotein particles to ribosomes during the process of recovery from chloramphenicol inhibition. From this and other results, it was inferred that methionine acting as a donor of methyl groups relieved a rate limitation imposed on the process of conversion of ribonucleoprotein particles (immature ribosomes) to mature ribosomes. To explain the overall stimulation of RNA synthesis, it was postulated that immature ribosomes participate in the regulation of RNA synthesis and further that methionine reduced their availability for this function by increasing the rate of their conversion to mature ribosomes.

This model led directly to the prediction of an inverse relationship between the ratios of immature to mature ribosomes and of total RNA to total protein. The results presented here are in basic accord with the prediction and therefore may be interpreted as offering support for the idea that immature ribosomes participate in the regulation of RNA synthesis in E. coli.

References

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TURNOVER OF CHLORAMPHENICOL RNA DURING RECOVERY

Less than 10% of the labeled RNA formed in the presence of labeled methionine and chloramphenicol is degraded during the first hour of a subsequent period of recovery from the chloramphenicol inhibition. We conclude that although instability of chloramphenicol RNA may be demonstrated under special non-physiological conditions, it appears to be quite stable under conditions permitting recovery.

Investigator:

W. H. Matchett

Technical Assistance:

Laura S. Winn

Reports from several laboratories have shown that ribonucleoprotein particles accumulated by cells under various conditions behaved as if they were the precursors of mature ribosomes. Previous studies in this laboratory (Annual Report, 1965) have shown that methionine specifically enhances the rate of conversion of ribonucleoprotein particles to mature ribosomes.

The recent demonstration of metabolic turnover of chloramphenicol RNA provided by the experiments of Dubin and Elkort⁽¹⁾ raised a question concerning the role or turnover in the response of the recovering cells to methionine. Dubin and Elkort achieved their demonstration of turnover of chloramphenicol RNA by observing the stability of ¹⁴C-labeled methyl groups which had been incorporated into RNA in the presence of chloramphenicol. Loss of radioactivity from RNA, during a subsequent period in which net synthesis of RNA was inhibited, was the criterion of turnover. This experimental design takes advantage of the fact that methylated bases or nucleotides liberated by the degradation of RNA would probably not be reincor-

porated as such since methylation is known to occur in vivo at the polynucleotide level.⁽²⁾

Observations

The accompanying figure shows the results of an experiment, similar to that of Dubin and Elkort, performed to assess the extent of turnover of chloramphenicol RNA during recovery under conditions permitting net synthesis of RNA. For this experiment cells were labeled with L-methionine-methyl-¹⁴C in the presence of chloramphenicol. Samples of RNA taken at time zero and after 40 min of recovery in minimal, methionine, and casamino acids media were subjected to density gradient analysis. Panel A shows the distribution of radioactivity in the 23S and 16S peaks of the RNA sample at time zero of recovery. Panels B, C and D show the distribution of radioactivity in RNA samples after 40 min of recovery in the indicated media. A comparison of these specific radioactivities revealed that only trivial amounts of turnover occurred during the recovery

period of the experiment. It would therefore appear that extensive degradation and resynthesis of RNA are not involved in the apparent increase

in rate of conversion of ribonucleo-protein particles to ribosomes inferred from the results presented previously.

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FIGURE 1. Turnover of ^{14}C -Methyl-Labeled Chloramphenicol RNA During a Subsequent Period of Recovery in Various Media

[Minimal grown cells were inhibited by addition of chloramphenicol to a final concentration of 100 $\mu\text{g/ml}$. After 5 min L-methionine-methyl- ^{14}C (specific radioactivity 10 $\mu\text{Ci}/\mu\text{mole}$) was added to a concentration of 4 $\mu\text{g/ml}$. Incubation was continued for 60 min. The cells were then centrifuged, washed free of isotope and chloramphenicol and resuspended in fresh minimal medium. Four aliquots were treated as follows: one was chilled immediately and to it was added a 10-fold excess of carrier cells (A); another was incubated 40 min without further addition (B); another received methionine to a final concentration of 50 $\mu\text{g/ml}$ and was incubated 40 min (C); the fourth aliquot received casamino acids at a final concentration of 0.5% and was incubated for 40 min (D). After incubation the cells were chilled and added to a 10-fold excess of untreated carrier cells. RNA extracted from the cells was layered onto sucrose gradients and centrifuged 17 hr at 25,000 rev/min. After centrifugation, about 35 fractions of equal volume were collected. The radioactivity and OD_{260} of each fraction were determined. The 16S and 23S peaks for each sample are plotted. The shaded bars indicate fractions used to compute specific radioactivity (cpm/ OD_{260} unit) of the 23S region.]

Conclusions

Ribonucleoprotein particles are generally regarded as metabolically unstable precursors of mature, stable ribosomes. This belief stems from two lines of evidence. Ribonucleoprotein particles are exquisitely sensitive to digestion with ribonuclease. Further, ribonucleoprotein particles have been observed to undergo degradation in vivo under special conditions in which the net synthesis of RNA is prevented by the presence of drugs (for example, actinomycin D). The significance of the results presented in this report

would seem to be that, although ribonucleoprotein particles are unstable under certain circumstances, they appear to be metabolically quite stable under physiological conditions where net synthesis of RNA and protein are not inhibited by the presence of drugs or the absence of required growth factors.

References

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TRYPTOPHAN TRANSPORT IN NEUROSPORA: ATTACHMENT SITES*

Three indicated attachment points for the transport site(s) are the α amino, carboxyl and β CH₂-groups of tryptophan. It appears that the formation of an aldimine is not involved in the mechanism of tryptophan transport in Neurospora.

Investigator:
W. R. Wiley

Technical Assistance:
L. C. Cronk

Previous investigations in this laboratory have shown that the transport of tryptophan across the cell membrane of Neurospora is mediated by a distinct stereospecific transport system (Annual Report, 1965). The process is enzymic in nature and chemically specific for a family of electrically

neutral amino acids. Leucine, phenylalanine, methionine, and α amino butyric acid competitively inhibit tryptophan transport, which would suggest that these amino acids share a common transport site(s). By studying the effect of structural modifications on the ability of various solutes to competitively inhibit tryptophan transport, it should be possible to define the points of attachment of the transport site(s) to the tryptophan molecule.

*This study derived partial support from the Battelle Institute, A Division of Battelle Memorial Institute, Columbus, Ohio.

By the same approach it should also be possible to make some inferences relative to the biochemical mechanism of attachment.

Observations

Table I shows the effect of various amino acids and amino acid analogs on the initial rate of tryptophan uptake. The indole moiety is not an inhibitor of tryptophan transport, which indicates that it is incapable of reacting with the transport site(s) and suggests that the recognition points for the transport site(s) are localized in the alanine portion of the tryptophan molecule. Substitutions on the β carbon (e.g., isoleucine, valine) result in loss of inhibitory activity. This loss suggests that the β carbon is an attachment point for the transport site(s). Leucine, which has a configuration around the β carbon similar to that of tryptophan, is an extremely effective competitor of tryptophan uptake.

To examine the requirement for an amino group in the uptake process, indolepyruvic acid was tested for its capacity to inhibit the rate of tryptophan transport. As shown in Table I, indolepyruvic acid is not inhibitory, demonstrating the absolute requirement for an amino group. Moreover, the amino group must be present in the appropriate position relative to the carboxyl. As shown, L-[α] amino butyric acid is an effective competitor of tryptophan uptake; L-[γ] amino butyric acid, on the other hand, is completely noninhibitory. Placing the amino group on the β carbon re-

TABLE I. Minimal Structural Requirements for Reactivity with the Transport Site

Competitor ^(a)	Tryptophan uptake (μ moles/g/min)
None	10
Reactivity of indole moiety	
Indole	12
Effect of substitutions on carbon β to α carboxyl of amino acid	
L-Leucine	0.96
L-Isoleucine	9.6
L-Valine	9.0
Requirement for α amino group	
Indolepyruvic acid	12
L-[α] Amino butyric acid	1.96
L-[γ] Amino butyric acid	9.6
β -phenyl- β -alanine	4.6
Requirement for carboxyl group	
Tryptamine - HCL	12
Effect of electrically charged side-chain	
L-Glutamic acid	11
L-Lysine	10

(a) Inhibitor ($4 \times 10^{-4} M$) and tryptophan ($2 \times 10^{-5} M$) were added simultaneously to the culture.

stores its capacity for reactivity with the transport site(s). This is indicated by the competitive inhibition observed for β -phenyl- β -alanine. We infer from these results that the amino group serves as a recognition point for the uptake site(s) in either α or β positions but not in the γ position.

The requirement for the carboxyl group in the transport process is indicated by the lack of inhibitory effect of tryptamine on the initial rate of tryptophan uptake. Other factors contribute to the reactivity of the transport site(s). For example, lysine and glutamic acid are not inhibitors of tryptophan transport, which suggests the additional requirement for an uncharged side-chain.

Pyridoxal 5' phosphate (Vit B₆) has been implicated in the mechanism of amino nitrogen transport site(s) formation in both mammalian and bacterial cells. The pyridoxal phosphate-amino nitrogen complex occurs through the formation of a Schiff's base, with the primary amine. If this reaction mechanism occurs in the transport of tryptophan in Neurospora, N-formyl derivatives of amino acids should not be competitors, since an aldimine linkage of the pyridoxal type could not be formed. This question was examined by testing the inhibitory effects of N-formyl derivatives of phenylalanine, methionine, and L-[α] aminobutyrate on tryptophan transport. Table II shows that these N-formyl derivatives retain the capacity for reactivity with the tryptophan transport site(s). Kinetic experiments with N-formyl L-phenylalanine showed that the observed inhibition is competitive. This would seem to exclude the formation of an aldimine linkage between the amino nitrogen and the tryptophan transport site(s) in the transport of tryptophan in Neurospora.

TABLE II. Effect of N-formyl Derivatives on the Uptake of Tryptophan

Competitor	Percent inhibition (a)
N-formyl DL-methionine	64
N-formyl DL-phenylalanine	80
N-formyl L-[α] amino butyrate	70

(a) Inhibitor (2×10^{-4} M) and tryptophan (2×10^{-5} M) were added simultaneously to the culture.

Conclusions

Tryptophan transport across the cell membrane of Neurospora is mediated through the formation of a tryptophan transport site(s) complex comprised of a three-point attachment of the transport site(s) to the α amino, carboxyl and β CH₂-groups of the tryptophan molecule.

The formation of an aldimine is not involved in this mechanism. Studies on the mechanism of amino nitrogen attachment to the tryptophan transport site(s) will be continued. It is possible that, if the mechanism of attachment is understood, a chemical reaction may be discovered which would stabilize the bonding of tryptophan to the site and permit its isolation and characterization.

TRYPTOPHAN TRANSPORT IN NEUROSPORA: METABOLIC CONTROL*

Results were obtained which suggest that the metabolic control of tryptophan uptake in Neurospora is mediated through the maintenance of a delicate balance between the breakdown and synthesis of the tryptophan transport system.

Investigator:
W. R. Wiley

Technical Assistance:
L. C. Cronk

In recent years much progress has been made in the elucidation of enzyme control mechanisms for specific biosynthetic sequences; however, much less is known about the identity and mode of action of factors which control membrane transport processes. One of the reasons for this discrepancy has been the difficulty in demonstrating distinct membrane transport systems. Recent investigations (Annual Report, 1965) on the transport of tryptophan in Neurospora show that tryptophan is transported by a distinct transport system which is enzymic in nature. The rates of tryptophan transport increase or decrease over a large range of values depending on the conditions of growth and the physiological state of the cells. These observations led us to postulate that the tryptophan transport process in Neurospora is under some pattern of metabolic control. The experiments described in this report were carried out in an attempt to determine the identity and mode of action of factors which control the level or activity of the tryptophan transport system.

Observations

Figure 1 shows the effect of tryptophan starvation on protein synthesis, tryptophan pool utilization, and tryptophan transport in Neurospora (strain td-201). Protein synthesis (as measured by incorporation of lysine) occurred at the expense of the intracellular pool of tryptophan for approximately 2.0 hr, after which time intracellular tryptophan was exhausted. The level or activity of the transport system remained constant for the first hour but was decreased to essentially zero after 2 hr. Three explanations seem possible: (1) Tryptophan transport requires tryptophan to maintain

FIGURE 1. The Effect of Tryptophan Starvation on Transport Activity

*This study derived partial support from the Battelle Institute, a Division of Battelle Memorial Institute, Columbus, Ohio.

the system at its maximally induced level. This possibility is eliminated by the knowledge that wild-type cells of *Neurospora* transport tryptophan maximally even when grown in the absence of exogenous tryptophan. (2) Tryptophan participates in a carrier-mediated exchange between intra- and extracellular tryptophan. This possibility was eliminated, however, since exchange between intra- and extracellular tryptophan during tryptophan transport was shown to be essentially nil. (3) Continued protein synthesis is required for the maintenance of transport activity.

To test the third possibility listed above, cells were depleted of tryptophan transport activity by tryptophan deprivation and the restoration of transport activity was measured under conditions where protein synthesis was specifically inhibited. Cells were starved for tryptophan for approximately 2.5 hr, after which they were supplied a very high concentration of tryptophan (150 $\mu\text{g/ml}$) and the increase in tryptophan transport activity measured in the presence and absence of protein synthesis. Protein synthesis (measured by the incorporation of ^{14}C -lysine) was inhibited by the addition of cycloheximide to the culture medium. As shown in Figure 2, cells treated with cycloheximide synthesized protein at a very much lower rate than untreated cells. The rate of restoration of tryptophan transport activity was correspondingly lower in the cycloheximide-treated cells than in the untreated cells. These results tend to substantiate the contention that the protein synthesizing system

exerts some metabolic control on the activity or level of the tryptophan transport system.

If we assume that the depletion in transport activity during starvation reflects a decrease in the population of membrane transport sites, it should be possible to estimate the turnover rate of the transport system by specifically inhibiting protein synthesis. The results of an experiment along these lines are shown in Figure 3. Cells treated with cycloheximide showed a rapid decrease in the level of the transport system at 30 °C. Cells

FIGURE 2. Requirement of Net Protein Synthesis for the Neogenesis of the Tryptophan Transport System

treated with cycloheximide and kept at 4 °C, retained the pre-established level of the transport system throughout the period of the experiment, as did untreated cells, incubated at either 4 or 30 °C. The turnover half-time for the tryptophan transport system at 30 °C was approximately 15 min. The fact that turnover of the system did not occur at 4 °C suggests that the process is enzyme mediated.

Conclusions

These data suggest that the rate of tryptophan transport in *Neurospora* is regulated through the maintenance of a delicate balance between the synthesis and breakdown of the transport system. A functional protein synthesizing system is an obligate requirement for maintaining the level of this system. If the rate of protein synthesis is increased or decreased, the transport synthesizing system may react by increasing or decreasing the number of tryptophan transport sites. Since it is known that a balanced amino acid pool is required for maximal protein synthesis, the physiological signifi-

FIGURE 3. Rate of Turnover of the Tryptophan Transport System

cance of the control of amino acid uptake is evident. Future experiments involving a differential analysis of the behavior of the variables which play a role on the control of tryptophan uptake are expected to provide a better understanding of this process.

REGULATION OF ENZYME SYNTHESIS IN NEUROSPORA CRASSA

Derepression of tryptophan synthetase formation in mutant td-201 (unable to make tryptophan) occurs when this strain is grown in the presence of either 3-amino-1,2,4-triazole (3AT) or anthranilic acid. Tryptophan derived endogenously by synthesis from indole is more effective in repression of enzyme synthesis than tryptophan supplied in the growth medium. An interpretation of these results is that enzyme synthesis is controlled by the ratio of charged to uncharged tryptophan-sRNA and that metabolic intermediates which accumulate in the presence of 3AT or anthranilic acid cause derepression of enzyme synthesis by affecting this ratio.

Investigators:
J. R. Turner
W. H. Matchett

Technical Assistance:
Laura S. Winn

The experiments described here were performed in an attempt to gain information concerning the mechanism of control of tryptophan synthetase formation in Neurospora crassa. Mechanisms of metabolic control postulated for biological systems have been derived primarily from experiments with bacteria. Because of the greater genetic complexity of higher organisms, and because metabolic control is postulated to occur at the level of DNA transcription in the cell, it is of interest to determine if a higher organism utilizes the same mechanisms for control of cellular functions. The enzyme, tryptophan synthetase, offers a good system for study of protein synthesis in N. crassa because of the substantial amount of information available concerning the biosynthetic pathway of tryptophan, the availability of well characterized mutants, and the simplicity of measurement of the enzyme.

Observations

In the present work we have taken advantage of the fact that the herbicide, 3-amino-1,2,4-triazole (3AT) causes the accumulation in cells of imidazoleglycerolphosphate, an intermediate in histidine biosynthesis, and that under these conditions tryptophan synthetase becomes derepressed. All experiments employed a tryptophan auxotroph, td-201, of N. crassa which is blocked in the conversion of indoleglycerol phosphate to tryptophan, and can use either indole or tryptophan for growth. Cells were grown in minimal medium with 150 mg/liter tryptophan. After 16 hr incubation at 30 °C, mycelia were harvested by filtration, washed, resuspended in fresh medium, with various additions, and reincubated at 30 °C. Samples were taken hourly, the mycelia washed, lyophilized, and powdered. All enzyme measurements were made on extracts prepared from this lyophilized powder.

Figure 1 shows the effect of 3AT and anthranilic acid on the differential rate of synthesis of tryptophan synthetase in the presence of tryptophan. The change in units of enzyme is plotted against change in milligram dry weight, which gives a line that describes the rate of enzyme synthesis relative to the rate of total protein synthesis. The differential rate in the presence of tryptophan is taken as the normal differential rate. Derepression of tryptophan synthetase is evident when the differential rate increases over that found in the presence of tryptophan. Approximately a threefold increase in the rate of enzyme synthesis is produced in the presence of either 3AT or anthranilic acid. All three of these cultures contained L-tryptophan, therefore, derepression is occurring in the presence of the presumed repressor.

It was earlier demonstrated⁽¹⁾ that *Neurospora* forms two distinct pools of

tryptophan, a metabolic pool and an expandable pool, and that internally synthesized tryptophan which enters the metabolic pool directly is preferentially used for protein synthesis. It was suggested that biosynthetic enzymes were under control of the metabolic pool and that enzymes of the tryptophan cycle were under control of the expandable pool.

Figure 2 shows that indole, alone or in the presence of 3AT, causes repression of tryptophan synthetase until the indole concentration in the medium has been reduced. In the presence of indole and anthranilic acid (Figure 3), tryptophan synthetase is repressed whereas with tryptophan and anthranilic acid derepression occurs. Since the presence of indole under all conditions tested caused repression of tryptophan synthetase formation, it appears that internally synthesized tryptophan is a more effective repressor than is externally supplied tryptophan.

FIGURE 1. Derepression of Tryptophan Synthetase in the Presence of Tryptophan

FIGURE 2. Inhibition of Tryptophan Synthetase Derepression by Indole

The tryptophan cycle in *Neurospora* involves the conversion of tryptophan to anthranilic acid by a set of enzymes separate from those of the biosynthetic sequence. One of the enzymes of this cycle, kynureninase, is inducible by tryptophan. Figure 4

FIGURE 3. Effect of Anthranilic Acid on Derepression of Tryptophan Synthetase

FIGURE 4. Inhibition of Kynureninase Induction

shows the induction of kynureninase by tryptophan and the complete inhibition of this induction by 3AT. The same effect of 3AT on the induction of kynureninase appears in cells grown with indole. The effect of 3AT on the control of tryptophan biosynthetic enzymes appears to be, at least in part, an alteration of the ability of tryptophan to act as a small molecule effector.

Conclusions

From this work it would appear that tryptophan per se does not cause repression of tryptophan synthetase. Its effect on the level of this enzyme may be due to its control of the level of indoleglycerolphosphate in the cell. It has been reported recently by Crawford and Gunsales that *Pseudomonas putida* mutants, which are capable of accumulating indole glycerol phosphate when incubated in the presence of anthranilic acid and tryptophan, become derepressed for tryptophan synthetase.⁽²⁾ They propose that indoleglycerol phosphate is the inducer of tryptophan synthetase and that tryptophan does not repress tryptophan synthetase formation. Our results show that, although tryptophan of external origin does not repress tryptophan synthetase formation, tryptophan arising endogenously by synthesis from indole does cause repression.

In the proposed mechanism for the function of the operon, the end product or co-repressor complexes with the repressor substance which then causes the operator gene to shut off transcription of the structural genes. It

is not clear, however, whether an inducer is required to turn transcription back on or that simply the absence of the co-repressor allows transcription to proceed. Since endogenously synthesized tryptophan does cause repression of tryptophan synthetase and indoleglycerolphosphate appears to induce this enzyme, one might speculate that an inducer is required and that in this system, indoleglycerolphosphate is that inducer.

A second possibility which we are now in the process of examining is that the ratio of charged to uncharged tryptophan-sRNA is the controlling factor in tryptophan synthetase formation and that indoleglycerolphosphate is involved in determining the charging ratio. The most dramatic derepression of tryptophan synthetase is seen where imidazoglycerolphosphate or indole glycerol phosphate accumulate, that is, in the presence of 3AT or anthranilic acid. The similarity of the structure of these two accumulation products suggests that their

effects are analogous in mechanism. According to this hypothesis of control by charging ratios, the presence of high levels of charged sRNA would cause repression of tryptophan synthetase and induction of kynureninase, while at lower levels of charged sRNA derepression of tryptophan synthetase would occur and kynureninase would not be induced. The derepressive effects of accumulated indoleglycerolphosphate and imidazoglycerolphosphate would then be due to their action as inhibitors of sRNA charging.

These compounds, indoleglycerolphosphate and imidazoglycerolphosphate, are being isolated and their effect on charging reactions will be tested directly in vitro.

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CONTROL OF STEROL SYNTHESIS IN YEAST

During a period of rapid ergosterol synthesis in yeast, there was no change in the rate of synthesis, indicating the absence of effective feed-back inhibition under the conditions investigated.

Investigator:
J. R. Turner

Siperstein et al.⁽¹⁾ reported that the synthesis of cholesterol in rat livers was influenced by feed-back control in which cholesterol inhibited the conversion of mevalonic acid to hydroxymethyl glutaric acid. These experiments were carried out with intact animals. The response of the synthetic system to added cholesterol in these experiments with intact animals was of such a nature that it was difficult to determine the type of feed-back control operating, i.e., repression or feed-back inhibition.

Yeast produce large amounts of ergosterol and the reactions leading to sterol synthesis in mammals and yeast are identical in the region where regulation is postulated to occur. Yeast are not subject to such complexities as hormonal control, cellular differentiation, etc., found in mammals. It was therefore hoped that yeast would constitute a system in which the control of sterol biosynthesis could be more precisely studied.

Observations

Yeast grown anaerobically are incapable of synthesizing ergosterol; however, upon subsequent aeration of anaerobically grown cells, sterol synthesis commences and proceeds at a high rate. The experiments described

here were carried out during such a period of aerobic growth and rapid ergosterol synthesis. Cells were grown anaerobically in standing culture for 40 hr at 30 °C, then harvested by centrifugation, washed, and resuspended in 0.1M KH_2PO_4 and 2% glucose for aerobic growth. At intervals during the period of aerobic growth, aliquots of the suspension were withdrawn for study of their ergosterol synthesizing ability as measured by incorporation of labeled sterol precursors. Incorporation of radioactivity into sterols was measured by incubating the cell suspension with the radioactive compound for 15 min, saponifying the cells, extracting the sterol, and assaying for radioactivity by scintillation counting.

Since acetate serves as the major source of carbon in the ergosterol molecule, any inhibition of synthesis should be reflected in the ability of the cell to incorporate ^{14}C -acetate into sterol. Figure 1 shows the rate of incorporation of ^{14}C -acetate into sterol for subcultures removed at intervals during a 10-hr period of aerobic growth, and the total sterol build-up in these cells as measured by a colorimetric method. There was little change in the rate of incorporation of acetate into sterol over a range of total intracellular sterol

FIGURE 1. Incorporation of Acetate- ^{14}C into Sterol

FIGURE 2. Incorporation of Methionine-Methyl- ^{14}C into Sterol

from 0 to 8 $\mu\text{g}/\text{mg}$ dry weight. All attempts to increase the intracellular sterol level by addition of sterol to the aeration medium were unsuccessful, presumably because of the relative impermeability of the cell membrane to ergosterol.

An alternative approach to this problem is the measure of the incor-

poration of ^{14}C -methyl groups into ergosterol from methionine-methyl- ^{14}C . Since this reaction is a terminal step in synthesis, it should reflect any inhibition of synthesis which occurs. Figure 2 shows the results of this type of experiment. As with incorporation of acetate- ^{14}C , there was no apparent decrease in the ability of the cell to synthesize ergosterol as the total ergosterol content of the cell increased.

Conclusions

Two different measures of overall synthetic ability, conversion of acetate- ^{14}C to ergosterol and incorporation of methyl groups from methionine-methyl- ^{14}C , were carried out during a period of increasing intracellular sterol content. Neither of these measurements gave evidence for the operation of feed-back regulation in this system. A possible explanation of this seeming anomaly is that the system is already maximally inhibited and that no further increase in the amount of end product, ergosterol, in the cell can further reduce the rate of synthesis; or, alternatively, one may speculate that the critical level of intracellular sterol required for control is greater than that achieved in these experiments.

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ENVIRONMENTAL RADIATION STUDIES - TERRESTRIAL

The first report in this section summarizes the past year's effort in Alaska, particularly with regard to the unusual accumulation of ^{137}Cs along the lichen-caribou-Eskimo food chain.

Five reports follow which deal with various aspects of the ecology of the Hanford Reservation and surrounding territory. Most of these reflect a continuing interest in specific features of the local and regional ecology, e.g., bird and beetle populations, cheatgrass vernalization, and ^{137}Cs mobilization from semi-aquatic environments. A new study which may point the way toward interesting future developments is concerned with the radiation sensitivity of native rodents captured in the Hanford environs. This work was stimulated by the assignment of an AEC Postdoctoral Fellow.

Five reports deal with laboratory studies of radionuclide and trace element uptake by plants. Three of these are concerned with cesium, one with lead, and one with iodine.

The final report in this section describes a preliminary study of the effect of radiation on growth and dormancy of potato tubers. This work was supported by funds from the AEC Division of Isotopes Development, but is included here for general interest.

CESIUM-137 IN THE FOOD CHAIN OF ALASKAN NATIVES

Cesium-137 body burdens of Anaktuvuk Pass, Alaska Eskimos during the summer of 1966 were about 20% greater than during 1965, confirming predictions made previously. Periodic measurements were begun at Anaktuvuk Pass, Arctic Village, and Ambler to define the magnitude of seasonal variations in human ^{137}Cs body burdens in peoples of contrasting caribou utilization patterns.

Investigators:

W. C. Hanson
L. L. Eberhardt

Technical Assistance:

G. W. Johnson
W. P. Neal
H. A. Sweany
Dorothy D. Wade

Measurements of fallout radio-nuclides, particularly ^{137}Cs , in the Alaskan lichen-caribou-man food chain continued during 1966. Whole-body counts were made with the same equipment as previously described (Annual Report, 1965). The instrument was checked (at Anaktuvuk Pass) with the shadow-shield counter and found to be properly calibrated.

Observations

Five measurements of ^{137}Cs body burdens in Eskimo residents of Anaktuvuk Pass were made between May and December 1966 (Table I). Values were about 20% greater than during 1965, reflecting the steady increase of ^{137}Cs in lichens and caribou noted since the study began in 1962. A slight decrease in ^{137}Cs content of

lichens occurred during the summer of 1966 at Anaktuvuk Pass, suggesting that the long-term trend of ^{137}Cs body burdens in caribou and man may also be expected to turn downward. However, unusual situations such as occurred during 1964, when unexpectedly high ^{137}Cs values in caribou flesh during the spring migration resulted in

TABLE I. Average Adult ^{137}Cs Body Burdens of Anaktuvuk Pass, Arctic Village, and Ambler Residents During 1966 (All values expressed as nanocuries)

Month	Location		
	Anaktuvuk Pass	Arctic Village	Ambler
May	880		
July	1090		
August	900		
October	880	480	480
December	640	350	600

comparable increases in Eskimo ^{137}Cs body burdens, might override this anticipated decrease.

Periodic measurements of human ^{137}Cs body burdens were begun during October 1966 at Arctic Village and Ambler to define the magnitude of seasonal variations of ^{137}Cs in peoples of contrasting caribou utilization patterns. Initial results (Table I) were obtained at the beginning of a major change in the seasonal dietary cycle following the arrival of the southward caribou migration; Anaktuvuk Pass values normally decrease during the first part of the winter and those of Arctic Village and Ambler were expected to increase throughout the winter. A departure from the expected pattern occurred at Arctic Village, where much of the fall caribou kill was lost by spoilage during an unusually warm

period and the caribou remained beyond the hunting range of the village. The resultant decrease in native ^{137}Cs body burdens from October to December reflected the food shortage experienced by the village.

Conclusions

These data emphasize the basic ecological relationships that influence the transfer of fallout radionuclides through arctic food chains. Continuing uncertainty as to causes of the unusual pattern of body burdens in 1964 emphasizes the need for more basic information about caribou migration patterns and extended sampling of lichens from winter caribou ranges. Periodic measurements of ^{137}Cs body burdens in native residents of the three villages will continue through 1967.

POPULATION STUDIES OF HANFORD GEESE AND GULLS

About 300 pairs of Canada geese which annually nest within Hanford boundaries have been studied since 1950. About 4000 pairs of California and ring-billed gulls which occupy two Hanford nesting colonies were studied during the period 1956-1966.

Investigator:
W. C. Hanson

Technical Assistance:
G. W. Johnson
H. A. Sweany

Studies of Hanford wildfowl began in 1950 as an adjunct of a biological monitoring program designed to survey radioactive materials in resident biota. During 1950, 1951, and 1952, cursory nesting surveys and banding (or ring-ing) of Hanford Canada geese (Branta

canadensis moffitti) were conducted in cooperation with State and Federal wildlife agencies. Since 1953, the nesting surveys have been comprehensive, with complete coverage of all 20 islands in the Hanford area and complete histories of all nests. Banding of flightless

birds was conducted each summer through 1965 in conjunction with governmental agencies.

California and ring-billed gulls established two nesting colonies on islands within Hanford boundaries during 1954. The colonies were first censused the following spring and, beginning in 1956, fledgling young were banded each year (except for 1959) to study dispersion and migration routes.

Observations

The Hanford population of nesting Canada geese has remained stable at about 300 adult pairs throughout the study and has produced about 800 to 1000 goslings per year, depending on nesting success. Average clutch size of 5.6 eggs and 98-99% fertility of adults compares favorably with other areas of the United States and indicates no deleterious effect of Hanford operations upon the population. About 100 to 125 flightless adult geese and goslings were banded each year during the period 1955-1965. Band recoveries have varied from 16-26% for each banding year, mostly recovered by sportsmen during waterfowl hunting seasons. Results indicate that most of the goslings remain near the breeding ground during their first autumn and then move northward with most of the other subadult (<3 years old) and a few adult birds during the spring waterfowl migration. Crossing the Rocky Mountains near the United States-Canadian border, they continue into northern Canada to spend their second summer. On their return southward in the autumn, about 10% of band recoveries have been made by hunters in northern Alberta and the

remainder throughout the western United States, mostly in eastern Washington, eastern Oregon, and northcentral California. They return to the breeding ground as non-breeding "unemployed" geese during their third summer, presumably paired, and most become permanent residents from then on. The number of "unemployed" geese is usually about 150 birds.

The two gull colonies are similar in structure, with California gulls occupying the higher ground in the center of the colonies and the ring-billed gulls nesting on the periphery. The latter species suffers greatest nest mortality due to inundation during the Columbia River freshet period. The higher ground apparently represents the nesting location of greatest survival value. Approximately 3000 adult birds occupy the Coyote Rapids colony compared to about 5000 at the Ringold colony. Nests averaged 2.8 eggs and there was considerable, but as yet unmeasured, mortality of eggs and nestlings. A total of 10,120 fledglings of both species were banded in 10 years of study. Band recovery has averaged 1.8%, considered exceptionally good for this species. Recovery has been highest for the Ringold birds compared to Coyote Rapids birds (2.7% versus 1.6%) and for ring-billed compared to California gulls (2.3% versus 1.3%). Recovery patterns show the young gulls emigrate during August and September, moving northward through eastern Washington and into southcentral British Columbia, thence to the Pacific Coast near Vancouver, British Columbia, where they remain until late autumn. They then follow the coastline southward to southern California and northwestern

Mexico, where they may reside for a considerable time before returning to the breeding grounds.

Conclusions

The extensive basic information obtained about the Hanford Canada goose population provides a very good foundation for further study. Having shown that Hanford operations are not injurious to the population--perhaps actually beneficial in providing some measure of protection--there is a

strong basis for studies of goose behavior, population dynamics, and possible effects of hydroelectric dam projects upon the goose population inhabiting what will soon be the only unimpounded section of the Columbia River.

The two gull colonies provide potential sites for gross observations of colony structure, survival value of nest sites, inter- and intra-species behavior, population dynamics, and related aspects.

EFFECTS OF RADIATION ON NATIVE RODENTS

The Great Basin pocket mouse, Perognathus parvus, was less resistant to acute ionizing radiation than either indigenous deer mice, Peromyscus maniculatus, or more southern members of the genus Perognathus. Secondary effects of radiation included prolonged testicular atrophy in deer mice, and extensive depilation and loss of muscle tone in the cheek pockets of Perognathus.

Investigator:

T. P. O'Farrell*
G. E. Cosgrove**

Technical Assistance:

G. W. Johnson
W. P. Neal

Recent interest in the responses of native mammals to acute ionizing radiation was stimulated, in part, by observations indicating that certain wild rodents were more resistant to radiation than their laboratory counterparts.⁽¹⁾ The present study sought to determine LD_{50/30} values for mammals native to the Hanford Reservation.

These values, in addition to their inherent interest, are needed for planning field studies designed to test the combined effects of radiation and environmental stresses on natural populations of indigenous species.

Observations

A total of 90 deer mice, Peromyscus maniculatus, and 180 Great Basin pocket mice, Perognathus parvus, were captured in the Hanford environs. All animals

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** Consultant Pathologist, Biology Division, Oak Ridge National Laboratory

were housed singly and received rat pellets, mixed seeds, oatmeal, and water ad libitum. After a 2-week acclimation period, mice were confined to ventilated plastic vials and irradiated with a 10,000-Ci ^{60}Co source. All deer mice and 90 pocket mice were irradiated at a dose rate of approximately 590 rads/min, while the remaining pocket mice were irradiated at about 90 rads/min. Weights were taken at treatment and subsequently at 7, 14, 21, and 30

days postirradiation. Dead animals were weighed, necropsied, fixed in formaldehyde, and tissue samples were prepared for histological examination.

At comparable dose rates pocket mice were more sensitive to acute radiation than deer mice. The estimated $\text{LD}_{50/30}$ values were 800 and 890 rads, respectively (Figure 1). At the lower dose rate, the $\text{LD}_{50/30}$ for pocket mice was substantially higher (950 rads). Mean

FIGURE 1. Survivorship Curves for Two Species of Native Rodents (Curves fitted to values obtained by probit transformation. Sample sizes were ten animals per point.)

survival times for the decedents were directly related to the $LD_{50/30}$ values (Figure 2). Higher lethality values were paralleled by greater survival times at all dose levels below the supralethal doses.

Irradiated mice that survived 30 days lost 3-10% of their initial weights during the first 7-14 days postirradiation. Degree of weight loss was usually directly related to radiation dose. Recovery was reflected in weight gains to pre-treatment values by 21-30 days postirradiation. Animals that died during the experiments usually lost 10-20% of their initial weight, often within a short period of time.

Histopathological findings indicated comparable damage at similar doses for both species. The animals showed evidence of hemato-poietic death with secondary infections, and hemorrhages of varying degrees. Damage to the epithelium

of the gastrointestinal tract was transient and healed very rapidly. The villi were not denuded at any dose level. Survivors showed good recovery in all other tissues examined except the gonads. Even low doses caused marked testicular and ovarian atrophy which had not recovered at 30 days postirradiation.

Sublethal doses of radiation caused immediate depilation in Perognathus. The loss of hair varying from slight slippage to total loss was apparent within 7 days after treatment. New hair, white to light gray in color, appeared by the second week. Low doses also decreased muscle tone in the cheek pockets; consequently, treated animals often moved about with the linings everted. Survivors displayed apparently normal pockets after 10-14 days.

Conclusions

The $LD_{50/30}$ values determined for Perognathus parvus were substantially lower than those reported for two southern species, Periognathus formosus, 1300 R,⁽¹⁾ and Perognathus longimembris, 1520 R,⁽¹⁾ suggesting that relatively high resistance to radiation is not a characteristic of all members of the genus. Deer mice, Peromyscus maniculatus, had an $LD_{50/30}$ of 890 rads, which is comparable to the $LD_{50/30}$ of approximately 1000 rads determined for Peromyscus leucopus, under similar colony and treatment regimens, at the Oak Ridge National Laboratory.

Sublethal radiation doses cause extensive depilation and loss of muscle tone in the pockets of

FIGURE 2. Mean Survival Times of Decedent Mice (The three scattered points represent single animals)

Perognathus. These effects could be important mortality factors under natural conditions. Loss of hair would immediately upset a part of the hygro-thermo regulatory capabilities which are so important to survival of desert species. Loss of control over pockets needed to transport seeds would force Pero-gnathus either to rely on available

underground caches of seeds, or spend more time above ground eating seeds as they are found, thus exposing the species to greater predation.

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ELEVATIONAL DISTRIBUTION OF ELEODES HISPILABRIS ON THE HANFORD RESERVATION

The ground-dwelling beetle Eleodes hispilabris was more abundant at low elevations than at high elevations. The period of seasonal activity at the highest elevation was only about 15 weeks as compared to 30 weeks or more at low elevations.

Investigator:
W. H. Rickard

Technical Assistance:
W. P. Neal

One of the most conspicuous ground-dwelling beetles of the natural vegetation mosaic of the Hanford Reservation is Eleodes hispilabris. The purpose of this investigation was to determine the relative abundance and seasonal distribution of E. hispilabris along an elevational gradient ranging between 500 and 3100 ft above mean sea level.

Observations

Abundance was determined by weekly catches from 40 pitfall traps, five placed at each of eight sites along an elevational transect from the basal plain to near the crest of the Rattlesnake hills. Trapping was conducted during the time of year when the beetles were active on the soil

surface from early spring to autumn. The total catch of beetles is shown in Table I. More beetles were captured at sites below 2700 ft than from those 2700 ft and higher in elevation. The greatest catch of 99 beetles was made at site number 6 located at about 2000 ft. Site number 5 had fewer beetles than did adjacent higher and lower sites, which may be attributable to the disturbance in this site created by a range fire in the summer of 1958. The fire destroyed the shrub component of the vegetation but the bunchgrasses (Agropyron spicatum, Stipa comata and S. thurberiana) survived the burning.

The seasonal distribution of the catch is shown for four representative sites in Figure 1. These data show a peak catch in early spring at

TABLE I. Total Catch of *Eleodes hispilabris* at Eight Sites Along an Elevational Gradient on the Hanford Reservation, 1966

Site no.	Approx. elev. (ft)	Vegetation-type	Total catch
1	500	<u>Artemisia-Purshia/Poa</u>	75
2	500	<u>Artemisia-Purshia/Poa</u>	48
3	800	<u>Artemisia/Poa</u>	75
4	1000	<u>Artemisia/Agropyron</u>	60
5	1300	<u>Artemisia/Agropyron</u>	33 ^(a)
6	2000	<u>Artemisia/Agropyron</u>	99
7	2700	<u>Artemisia/Agropyron</u>	16
8	3100	<u>Artemisia/Agropyron</u>	14

(a) Site now without *Artemisia tridentata* because of range fire in 1958.

elevations of 2000 ft and lower. A peak catch is also recorded at the higher elevations but later in the year, i.e., early summer. At elevations below 2000 ft there was a summer low followed by a small secondary peak of abundance in the fall. The secondary peak may be the result of increased activity as temperature becomes more ameliorated in early fall, or may indicate the emergence of a new generation of adults from the soil.

Conclusions

The chief interest in these data lies in the reduced population of *E. hispilabris* at the high elevations. Here beetles are active for only 15 weeks as compared to 30 weeks or more at lower elevations. Seasonal temperature changes play an important role in the ecological distribution of ectothermic animals. Temperatures decrease with increase in elevation up the slope of the Rattlesnake hills, but thus far these temperatures (soil and air) have not been measured with sufficient frequency and accuracy to assist in physiological interpretations of beetle response to seasonal temperature change.

FIGURE 1. Trap Catch of *Eleodes hispilabris* at Four Locations Along the Elevational Gradient of the Hanford Reservation During 1966 Activity Period

EFFECTS OF MOISTURE AND TEMPERATURE ON THE PHYSIOLOGY OF CHEATGRASS

The effect of cold night temperatures and soil moisture levels on the flowering response in cheatgrass was studied under controlled conditions. Five night periods at 3 °C were not sufficient to vernalize seedlings to induce flowering. Variations in soil moisture were also ineffective.

Investigators:
R. L. Uhler
W. H. Rickard

Technical Assistance:
Frances A. DeMers

Cheatgrass (Bromus tectorum), since its introduction from Europe, has become widespread and is of importance ecologically and agriculturally on semiarid lands of the western United States. This aggressive winter annual is one of the dominant grasses on the Hanford Reservation. It is normally a winter annual but under adverse conditions, it will act as a spring annual. Seeds germinate in the fall if moisture is sufficient; growth ceases during the cold winter and recommences in the spring. Flowering is in April or May. On the Hanford Reservation, this grass grows at elevations from approximately 300 to 3000 ft. The date of flowering is later as the elevation increases. Soil, moisture, and temperature also change with elevation. Since seeds produced in the spring crop are viable and have had no cold treatment, a reasonable assumption is that it is the seedling which requires vernalization. This report will discuss the flowering response to temperature and soil-moisture treatments.

Observations

Seven nonvernalized seeds were planted in each pot containing 1 kg of Warden silt loam soil. Germination

was 93% after 6 days. Cultures were thinned to three plants and grown under the following conditions: light (tungsten and fluorescent), 1800 fc; photo period, 16 hr; photo-temperature, 26.6 °C; nycto-temperature, 21.1 °C; relative humidity, 45-60%. Treatments commenced 19 days after planting. Seedlings were transferred to a growth chamber at 3 °C during the nycto-period for 0, 1, 2, 3, 4, or 5 nights and then returned to normal growth conditions. The cold-treated plants were maintained at 20% soil moisture. Other plants were maintained under a moisture regime of 20, 18, 16, 14, 12, 9, or 6% soil moisture by daily irrigation. The 20% level is near field capacity and the 6% level approximates 15 bars suction. The experiment was terminated 40 days after the first indication of flowering. All treatments were replicated five times.

At least one plant from each treatment flowered with the exception of those maintained at the 6% moisture level. The flowering response was so weak, however, that it was not considered to be a normal expression. Cold-treated plants showed a tendency toward more flowering than did those under the moisture regime, but the

response was not considered significant. None of the plants held at the lowest moisture level flowered but other moisture treatments had no effect on flowering.

Conclusions

Floral morphogenesis in cheatgrass is not induced by soil moisture tension; however, high tension will inhibit

morphogenesis. Although the flowering response was weak, and for the purpose of this study considered insignificant, it is noteworthy that the plant can survive under such adverse conditions. This adaptability is probably a major factor contributing to the widespread distribution of cheatgrass. Vernalization of seedling, if necessary for reproduction, was not satisfied by five cold nights.

CESIUM-137 IN THE VEGETATION OF A MOUNTAIN BOG

Sedges of a mountain bog show mobilization of ^{137}Cs from surrounding substrates, the greatest mobilization occurring in sedges growing in open water as compared to those growing in a less aquatic situation.

Investigator:
W. H. Rickard

Technical Assistance:
H. A. Sweany
Dorothy D. Wade
G. W. Johnson

Radioactive debris resulting from nuclear explosions conducted in the atmosphere has become widely distributed by meteorologic processes. The long-lived radionuclides of this debris have the potential to persist in the environment for many years. Once gleaned from the atmosphere by precipitation or by dry deposition, an initial site of attachment is the earth's vegetation cover. Uptake of ^{137}Cs by vascular plants from soil substrates is low but an increased uptake by emergent aquatic plants such as domestic rice and Polygonum persicaria has been reported. This paper reports the accumulation of fallout derived ^{137}Cs by aquatic emergent sedges of a mountain bog.

Observations

The bog studied is in a high rainfall area in the Cascade Mountains of Washington located near the summit of Snoqualmie Pass. The emergent vegetation of the bog consists mostly of sedge (Carex sp.) and horsetail (Equisetum sp.). Moss (Sphagnum spp) provides an understory to the larger plants of sedge and horsetail on raised organic tussocks and along shore margins not ordinarily covered by standing water.

Sampling was done by clipping all standing new growth vegetation from ten, 1 x 1 m quadrats arranged along a line transect running from near the bog margin to open water, i.e., without

emergent vegetation. Mosses plus organic detritus were plucked from these same plots by hand immediately after clipping. Analyses of dried vegetation samples were done by gamma-ray spectroscopy.

The data show a general decline in ^{137}Cs in new growth sedges from open water to the bog margin (Figure 1). At the same time the ^{137}Cs in moss + detritus samples increased from open water to the bog margin. Moss + detritus samples from quadrats 7 and 9 (Figure 1) were tussocked samples. The mosses of these quadrats have not been covered by water for as long a time as adjacent plots. The mosses of quadrats 17 and 19 probably were not covered by water at all. The higher ^{137}Cs content of these samples appears to be related to their longer time of aerial exposure.

The data shown in Table I clearly show the increase in ^{137}Cs in total vegetation from open water toward the

bog margin. This increase is due to increased biomass near the bog margin as well as long-term accumulation of ^{137}Cs by the aerial parts of long-lived mosses.

Conclusions

The significance of the new growth sedge data is the apparent mobilization of ^{137}Cs by sedges growing in open water as compared to those plants growing in a less aquatic situation. The short-lived shoots of sedge, one summer only, would appear to contribute to a more rapid biologic turnover of minerals than would the long-lived organs of the mosses. Because there appears to be a low exchange of water in the bog, it seems logical to expect that ^{137}Cs will continue to build up in the bog system at least for the next few years even without additional testing of nuclear weapons in the atmosphere.

FIGURE 1. Cesium-137 in the Vegetation Samples from a Mountain Bog, July, 1966

TABLE I. The Accumulation of ^{137}Cs in the Vegetation of a Mountain Bog, July, 1966

Distance from open water, meters	^{137}Cs pCi/m ²		
	Sedge	Moss + detritus	Total vegetation
1	1,800	300	2,100
3	2,300	160	2,400
5	2,900	2,100	5,000
7	Missing	7,100	-
9	2,700	4,200	6,900
11	2,100	5,600	7,700
13	820	8,500	9,400
15	620	8,200	8,900
17	560	9,500	10,000
19	490	13,000	13,000

CESIUM-137 MOVEMENT IN A ROOT-SOIL PROFILE

Young barley plants were grown in a surface-contaminated soil. A small downward movement of ^{137}Cs in the soil profile was not related to method of watering or to presence of plant roots.

Investigator:
J. F. Cline

Previously observed movement of ^{137}Cs into a plant-soil profile occurred at a faster rate than could be explained by the leaching action of water. Roots of most perennial plants are known to accumulate ^{137}Cs and K at the end of a growing season. This experiment was designed to show whether young fast-growing roots would move ^{137}Cs into a soil profile during early growth periods.

Observations

Barley plants were grown in soil columns 3 inches in diameter and 6 inches in depth. Eight hundred grams of oven-dry sandy loam soil were added to each column and topped with 200 g of soil contaminated with 10 μCi of carrier-free $^{137}\text{CsCl}$. The soil was irrigated with 200 g of water, and seeds were planted within the surface-contaminated layer. Soil moisture was maintained by daily addition of water such that the original 1200 g weight was maintained. Additions were by either surface or bottom water application. Half of the cans were not planted and maintained as controls.

Cans were sampled weekly for 4 weeks. In all cases, roots had

penetrated the length of the soil column. The soil columns were frozen and sawed into five equal sections, moving from the bottom to the top of each column to minimize cross contamination. The two top layers were combined for analysis because it was impossible to cut on a line that divided the upper, initially contaminated, soil layer from the soil below. Cesium-137 was measured by scintillation spectrometry.

Results are shown in Table I. No effect was seen which could be attributed to transfer by the plants, nor was there any apparent effect resulting from the method of adding water.

Conclusions

The slight movement of ^{137}Cs into the lower soil profile could not be related to either the presence of plant roots or to the effect of surface watering. Movement of ^{137}Cs did not increase with time so must be presumed to be associated with the initial wetting of the soil. Greater movement to the root zone might have occurred if the barley had grown to maturity.

TABLE I. Cesium-137 Location in the Soil-Root Profile

Harvest day	Soil section	Percent of ^{137}Cs in each section			
		Barley growing in soil		Soil only	
		Top watered	Bottom watered	Top watered	Bottom watered
7	1 & 2	99.2	98.3	99.2	98.8
	3	0.6	1.3	0.7	1.0
	4	0.2	0.4	0.1	0.2
	5	0.01	0.03	0.01	0.03
14	1 & 2	98.9	98.4	98.6	98.7
	3	0.9	1.3	1.2	1.2
	4	0.2	0.3	0.2	0.1
	5	0.01	0.01	0.01	0.01
21	1 & 2	98.9	98.6	98.9	99.2
	3	0.9	1.2	0.9	0.6
	4	0.2	0.2	0.2	0.2
	5	0.01	0.02	0.01	0.01
28	1 & 2	98.9	98.6	99.2	98.6
	3	0.9	1.2	0.7	1.1
	4	0.2	0.2	0.1	0.3
	5	0.01	0.02	0.002	0.01

AVAILABILITY TO PLANTS OF CESIUM-137 ADSORBED TO CLAY

Drying and heating of ^{137}Cs -contaminated clay does not significantly reduce ^{137}Cs uptake by plants below that observed when the ^{137}Cs is in ionic form. The extent of direct clay-root contact may affect the amount of ^{137}Cs taken up by the plant.

Investigator:
J. F. Cline

It is well recognized that ^{137}Cs is firmly bound to clay and thus is usually considered relatively unavailable for uptake by plants. However, when ^{137}Cs was mixed with bentonite and subsequently added as a suspension to a nutrient solution containing roots, uptake of the ^{137}Cs was as extensive as if the ^{137}Cs had been present in ionic form (Annual Report, 1965). The present study examines the effect of washing and rigorous

drying of the ^{137}Cs -soil mixture prior to its addition to the nutrient solution of the root environment.

Observations

A bentonite clay slurry contaminated with carrier-free ^{137}Cs was dried in a forced-air oven for 48 hr at 100 °C. One gram of dry clay was resuspended and added to 6 liters of nutrient

solution containing growing bean plants, *Phaseolus vulgaris*. Similar groups of control plants were grown in solutions containing the same clay which had not been subjected to drying. Each treatment was replicated four times. After 4 days exposure to the suspensions, the leaves, stems, and roots were harvested separately and counted on a gamma scintillator. Table I shows no significant differences in ^{137}Cs concentrations in the tissues of plants grown in the two suspensions.

Cesium-137-contaminated clay particles were washed three times in

distilled water to remove ^{137}Cs not adsorbed to the clay. The washed clay was then suspended in nutrient culture. An equal amount of ^{137}Cs , on 2 g of unwashed clay, was added to another nutrient solution as control. Each treatment was replicated four times. The two suspensions contained equal amounts of ^{137}Cs , but there was 15% less clay in the control. As shown in Table II, plants grown in the nutrient solution containing the washed clay particles accumulated significantly more ^{137}Cs in the leaves than did those grown in the unwashed control clay suspension.

To demonstrate the importance of solution turbulence to ion movement in the root environment, a millipore filter was placed between two glass reservoirs. A ^{137}Cs -contaminated clay suspension was placed on one side of the membrane and distilled water on the other side. Glass bubblers were placed in both reservoirs. Figure 1 shows that rapid transfer occurred when the solutions were agitated by bubbling whereas little movement took place in the absence of turbulence.

Conclusions

Availability of ^{137}Cs to plants was not reduced by rigorous drying of a

TABLE I. Effect of Drying Clay on the Availability of Adsorbed ^{137}Cs

Carrier Cs, mg/liter	$\mu\text{Ci } ^{137}\text{Cs/g dry tissue}$	
	Leaves	
	Dried clay	Untreated clay
0	0.84 $\pm$ 0.08 ^(a)	0.95 $\pm$ 0.09
3.3	0.85 $\pm$ 0.09	0.90 $\pm$ 0.14
6.7	0.90 $\pm$ 0.03	0.93 $\pm$ 0.04
10	0.92 $\pm$ 0.03	1.01 $\pm$ 0.02
13	0.90 $\pm$ 0.04	1.02 $\pm$ 0.03
Stems ^(b)		
	Dried clay	Untreated clay
0	0.40	0.45
3.3	0.40	0.45
6.7	0.45	0.44
10	0.44	0.47
13	0.40	0.50
Roots		
	Dried clay	Untreated Clay
0	1.06 $\pm$ 0.05	1.03 $\pm$ 0.12
3.3	0.83 $\pm$ 0.03	0.88 $\pm$ 0.05
6.7	0.91 $\pm$ 0.03	0.93 $\pm$ 0.03
10	1.08 $\pm$ 0.13	0.92 $\pm$ 0.07
13	1.01 $\pm$ 0.06	0.97 $\pm$ 0.10

(a) $\pm$ One standard deviation.

(b) Stem samples were pooled.

TABLE II. Effect of Washing Clay on the Availability of Adsorbed ^{137}Cs

Plant part	$\mu\text{Ci } ^{137}\text{Cs/g dry tissue}$ ^(a)	
	Washed clay	Unwashed clay
Leaves	7.6 $\pm$ 0.1	6.6 $\pm$ 0.2
Stems	5.0 $\pm$ 0.1	4.6 $\pm$ 0.3
Roots	15.4 $\pm$ 3.0	10.8 $\pm$ 0.3

(a) $\pm$ One standard deviation.

FIGURE 1. Transfer of ^{137}Cs from Clay Suspension Through Membrane to Distilled Water.

^{137}Cs -clay mixture prior to addition to a nutrient culture. This result, together with the increased movement of ^{137}Cs through a solution with agitation, suggests that ^{137}Cs can move rapidly from clay, through solution, to root. However, in the test comparing washed ^{137}Cs -clay with unwashed ^{137}Cs -clay, uptake by plants was greater from the washed clay suspension. This apparently anomalous result might be explained by the larger amount of clay in the washed series. This would imply that direct contact of the ^{137}Cs -bearing particles with the plant roots is of greater importance than exchange through the solution. Contact exchange would appear to be an important mechanism in the uptake of ^{137}Cs from clay suspensions.

TOXIC EFFECT OF CESIUM IN THE CELLULAR DEVELOPMENT OF PLANTS

Early effects of cesium toxicity in plants reduced concentrations of both low-molecular-weight DNA and ribosomal bound tRNA. At the same time, percentage of cells in interphase increased with a corresponding decrease in other stages of mitosis.

Investigators:
J. F. Cline
Mary W. Magula

Gross toxic symptoms in plants grown on a substrate containing cesium were described previously in terms of morphological changes in the leaves and roots (Annual Report, 1961). In further studies, mitotic indices in pea root cells were shown to be altered by cesium (Annual Report, 1963). Cell division stopped, and the nuclei were broken in root cells grown in a nutrient solution containing 10 mM cesium. Cells in the

interphase stage increased as the cesium concentration was changed from 0 to 5 mM.

Observations

Pea seedlings (*Pisum sativa* L.) were grown in a modified Hoagland's solution with potassium concentration reduced to 0.5 mM and cesium concentration varied from 0 to 10 mM. Tritiated thymidine and uridine were incorporated in the

solutions to label DNA and RNA, respectively. DNA separations were done on methylated-albumin-Kieselguhr (MAK) column chromatograms. Ribosomal binding of aminoacyl-tRNA was determined using a special cellulose nitrate filter.

Table I shows that significantly less tRNA was bound to the ribosomes in root cells grown on a substrate containing cesium.

Figure 1 is a representative curve of the molecular weight distribution of DNA isolated from pea root cells. Typical experiments showed a loss of the low molecular weight DNA when cesium was present in toxic amounts.

Conclusions

These results may be interpreted to indicate that cesium blocks the attachment of tRNA to the ribosome. From other data it seems clear that cesium is competing with potassium and disrupting the normal metabolic pattern.

The elimination of a low molecular weight DNA fraction seen in root cells grown in a cesium substrate is similar to observations reported to result from other stresses such as excess aluminum or lithium ions, or excess drought. The disappearance of this low molecular weight fraction may be a sensitive indicator of incipient or potentially toxic conditions affecting growing plants.

TABLE I. Effect of Cesium on Percentages of Ribosome-Bound tRNA in Pea Roots (Average of determinations $\pm$ standard deviation)

	Bound tRNA	Unbound tRNA
Cesium-treated (2 mM)	26 $\pm$ 4	74 $\pm$ 4
Controls	37 $\pm$ 3	63 $\pm$ 3

FIGURE 1. Effect of Nutrient Cesium (2 mM) on DNA in Pea Roots

HEVESY REVISITED: THE ABSORPTION AND TRANSLOCATION OF LEAD BY PLANTS

The absorption, translocation, and distribution of lead in beans were examined. Lead is not readily absorbed and that which is remains in the water-conducting vessels. There is little movement to tissues separated from the veins by cellular membranes.

Investigator:
R. L. Uhler

Technical Assistance:
Frances A. DeMers

In 1923, George Hevesy described one of the earliest, if not the first, radiotracer study in the biological field.⁽¹⁾ It is discouraging to note that although techniques have become more sophisticated and terminology has changed, advancement toward an understanding of the nature of the salt transport mechanism during the intervening years has been minimal. The lack of progress is due to the complexity of the mechanism rather than to lack of interest or lack of investigations. The purpose of this study was to confirm the early experiment and examine in greater detail the internal distribution of Pb within plants.

Observations

Certain experimental variables differ from the Hevesy study; i.e., Phaseolus vulgaris var. Red Kidney instead of Vicia faba; the absorption period, 25 days vs. 1 day; absorption from 1.8 liter aerated, pH 5, Hoagland's solution rather than 0.5 liter double salt solution. Lead-210 was added as the nitrate to a concentration of 10 μ Ci/liter. The distribution of lead was examined autoradiographically as well as radiochemically in individual leaflets, petioles, pods, beans, and stem segments of wood (xylem), and bark (phloem).

The magnitude of root sorption of lead is evident in Table I. This is in agreement with Hevesy and, as he demonstrated, the bulk of this lead is adsorbed in an exchangeable form--up to 95% depending upon the counterion used. The plant root is exposed to a relatively constant source of lead and a barrier to its translocation is quite evident. Hevesy concludes "that it is not the transpiration current which transmits the assimilated lead" (his italics). Nevertheless, the primary pathway of lead in the plant is in the water-conducting vessels.

During ascent in the wood, some lead moves into the bark. The amount or rate of this transfer is much less than in the case of other divalent ions, e.g., Ca or Sr, which show wood-to-bark ratios close to unity. The degree of equilibration between wood and bark increases during ascent as evidenced by the wood-to-bark ratios in Table I. Autoradiographs show the major concentration of lead at the nodes. This is characteristic not only of the nodes of wood and bark but also of the petioles at stem and leaflet junctions. If the nodal areas had been removed from bark segments, the wood-to-bark ratios would have been much higher.

TABLE 1. Distribution of Lead in Bean Plant Tissues (Vertical order of tissues corresponds to position on plant axis)

	Tissue dry wt, mg	²¹⁰ Pb		Tissue ratio: wood/bark
		Total tissue, 10 ³ counts/min	Tissue conc. counts/min/kg	
Apex: Pods	0.84	0.74	0.88	
Beans	0.17	0.14	0.85	
4th Trifoliate	3.0	40	13	
Petiole	0.30	10	34	
Internode: Wood	0.19	3.8	20	2.3
Bark	0.09	1.7	19	
3rd Trifoliate	2.2	37	17	
Petiole	0.32	9.6	30	
Internode: Wood	0.31	9.4	30	3.5
Bark	0.13	2.7	21	
2nd Trifoliate	2.2	23	11	
Petiole	0.30	12	40	
Internode: Wood	0.14	7.0	51	4.1
Bark	0.064	1.7	26	
1st Trifoliate	2.3	18	8.0	
Petiole	0.29	14	49	
Internode: Wood	0.13	9.3	70	4.5
Bark	0.05	2.1	41	
Primary leaves	1.6	36	23	
Petioles (2)	0.16	4.3	28	
Internode: Wood	0.26	16	61	6.8
Bark	0.09	2.4	25	
Cotyledons	--	--	--	--
Internode: Wood	0.25	11	43	11
Bark	0.07	0.93	13	
Roots	4.7	2.15 x 10 ⁴	4.6 x 10 ³	

Concentrations of lead in the three individual leaflets of a given trifoliate did not differ; leaf concentrations are therefore expressed for the trifoliate. Both total activity and activity per gram are shown in Table I. Lead is preferentially translocated to the youngest leaves. Autoradiographs show activity predominantly in the veins of younger leaves. In older leaves, there is generally more lead in the interveinal area and the veins are less dense than in the newer leaves.

The activity of lead in the fruit is very low, which is in marked contrast to Hevesy's data. In his experiment, the root solution was 0.1N Pb(NO₃)₂ which is toxic and may have drastically altered the lead distribution. In autoradiographs of the fruit, the pod-petiole node is highest in activity. The exterior of the pod is faintly outlined as are the junctions between individual beans and the pod. The seed coat has some activity;

however, the endosperms were too low in activity to produce film darkening.

Conclusions

The extremely low availability of lead to plants from solution cultures or soil is quite evident from Hevesy's study, other experiments in this laboratory, and the present report. Lead, once it enters the water-conducting system, is transported up the stem. It would appear to be restrained by a series of barriers during this ascent. The barrier is believed to be the plasmalemma.

The toxicity of lead and its apparently increasing abundance in the environment has aroused concern. The data from this and other experiments indicate that entry of lead into the food chain through the soil-to-plant step will be very limited; certainly of less significance than entry via direct deposition on aerial plant tissues.

References

1. Hevesy, G. *Biochem. J.*, vol. 17, pp. 439-445. 1923.

CUTICULAR FOLIAR SORPTION OF IODINE

Experiments with begonia and coleus leaves which have stomata only in the lower leaf surfaces show that iodine can readily penetrate both surfaces. The diffusion coefficient of iodine "gas" through isolated begonia and tomato fruit cuticles is of the order of $10^{-3} \text{ cm}^2 \text{ hr}^{-1}$.

Investigator:
J. J. C. Hsieh

It has been frequently assumed that the rate of iodine gas absorption by leaves was proportional to the degree to which stomata were open, and that the route of foliar sorption of iodine gas was presumably through the stomata. There is a lack of convincing experimental evidence to substantiate such an assumption and the present studies were performed to investigate this problem.

Observations

Begonia and coleus were chosen for experimental plants since they have stomata only on the lower surface of the leaf. A circular area 5 cm in diameter on the upper (non-stomatal) surface of an intact begonia leaf was exposed to $^{131}\text{I}_2$ for a period of 30 min. To collect iodine passing through the leaf, a cup with 2N NaOH

solution was sealed to the underside of the leaf. A positive air pressure of about 2 cm of water was maintained in the air space between the leaf and NaOH solution to minimize the possibility of a gas leak. A leaf was placed in the vicinity of the exposed leaf for the detection of $^{131}\text{I}_2$ leakage. Various plant parts were sampled for ^{131}I at the termination of the experiment. Also, a small portion of the exposed leaf was taken for autoradiography.

Iodine-131 was sometimes, but not always, detected in the NaOH solution; however, without exception, ^{131}I was found inside the leaf and on the bottom leaf surface not directly exposed to iodine gas. The distribution of ^{131}I among various plant components in a typical experiment is shown in Figure 1. Iodine on the leaf surface is arbitrarily defined as the amount which can be stripped off by collodion films. Values for this presumably adsorbed iodine may be high since electron microscopic observation of leaf wax surface replicas show that collodion may strip off more iodine than that which would be adsorbed on the surface. Autoradiograms of the exposed leaf surface as well as cross sections of the treated leaf show that the penetration of iodine was randomly spotty and the route of movement tortuous.

The lipoidal cuticle has been thought to be the chief barrier to foliar uptake of iodine. An evaluation of the diffusion coefficient was conducted to determine rates of movement of iodine through this cuticle barrier.

Pectinase was used to digest the pectinous material binding cuticle to

epidermis and free the cuticle film from begonia leaves and tomato fruit. The isolated cuticles were laid over 7 mm (diameter) holes and sealed with paraffin at edges. Silicone rubber disks contaminated with a total of 40 μCi of ^{131}I were enclosed in an exposure chamber as the I_2 source. The silicone rubber disks were held so that no physical contact was made with the cuticles. Glass cups containing NaOH as the iodine absorber were placed directly beneath the cuticle surfaces. The distance between the cuticle and the NaOH solution was ~ 3 mm. All cuticles used were microscopically examined just before the experiment to ensure freedom from physical defects, i.e., tears, holes, etc. The $^{131}\text{I}_2$ concentration inside the exposure chamber was found to be 163 pCi/40 cm^3 at the end of the experiment.

Location		nCi ^{131}I
A.	Exposed leaf surface	408
B.	Opposite leaf surface	199
C.	Leaf interior at exposure site	404
D.	Upper epoxy seal surface	182
E.	Lower epoxy seal surface	19
F.	Leaf between seals	441
G.	Unexposed portion of leaf	32
H.	Remainder of plant	3

FIGURE 1. Distribution of ^{131}I in Begonia Plant 47 Hr after Exposure of a Nonstomatal Upper Leaf Surface for 30 Min

The diffusion coefficients (Table I) were calculated using Fick's first diffusion equation:

$$F = -K \frac{d(C_1 - C_2)}{dx}$$

where F is defined as the $^{131}\text{I}_2$ flux passing through the cuticle in units of $\text{pCi cm}^{-2} \text{ hr}^{-1}$; C_1 and C_2 are the ^{131}I concentrations (pCi cm^{-3}), respectively, inside the exposure chamber and in the air space between the cuticle and the NaOH solution (the measured value of 4 pCi/cm^3 was used for C_1 , and C_2 was assumed to be zero); x is the thickness of cuticle in cm; and K is the diffusion coefficient in $\text{cm}^2 \text{ hr}^{-1}$.

Conclusions

The results of these experiments quite conclusively demonstrate that iodine can penetrate the waxy cuticle of leaf epidermis, enter the leaf

TABLE I. $^{131}\text{I}_2$ Diffusion Coefficient Through Cuticles of Begonia Leaves and Tomato Fruit

<u>Cuticle species</u>	<u>Exposed surface</u>	<u>Diffusion coefficient at 22 °C ($\text{cm}^2 \text{ hr}^{-1}$)</u>
Begonia (stomata)	Outer	10×10^{-4}
Begonia (stomata)	Inner	2×10^{-4}
Begonia (stomata)	Inner	9×10^{-4}
Begonia (no stomata)	Outer	16×10^{-4}
Tomato (no stomata)	Outer	61×10^{-4}
Tomato (no stomata)	Inner	52×10^{-4}

proper, and even exit from the leaf on the opposite side. The lack of appreciable difference in diffusion rates through the cuticles with and without stomatal openings is a powerful argument that the cuticle is not an effective barrier to I_2 movement.

EFFECT OF IRRADIATION ON GROWTH AND DORMANCY OF POTATO TUBERS*

No evidence of stimulation of sprouting, yield, or number of stems per seed piece was observed in field tests when exposures up to 2.1 krad were applied to year-old Russet potatoes. Freshly harvested tubers were shown to sprout more rapidly when irradiated but this effect was confounded by defoliant treatments.

Investigator:
R. L. Uhler

Technical Assistance:
Frances A. DeMers

The detrimental effects of irradiation on plants have been well documented, especially at higher exposure levels. Some stimulatory effects have been claimed but the parameters affecting stimulation have not been identified. This report contains the results of field trials to verify the stimulatory and dormancy-arresting effects of irradiation on potatoes as reported in Russian and European literature.

Solanum tuberosum var. Russet Burbank, certified seed potatoes, stored on ice for the 2 weeks just prior to use, were exposed to ^{60}Co gamma radiation at 2.1 krad/hr to give exposures shown in Table I. The tubers were immediately cut to uniform seed pieces and planted on 2-foot centers in the field. Planting date was July 1, 1966. Each radiation level was replicated 80 times. Nonirradiated seed pieces (248) were also planted two rows deep around the plot. Individual hills were scored for days to emergence, days from emergence to bloom, number of stems, number of tubers, and yield at harvest, October 10, 1966.

The first response noted was a delay in emergence. Within 15 days, 50% of the control plants had emerged. Irra-

diated plants were delayed 5-6 days for each krad given. Ultimately, 91-98% of the plants emerged irrespective of treatment.

Flowering, with respect to time from planting or emergence, was not significantly influenced by the seed irradiation treatments. The number of plants which did bloom decreased as radiation exposure increased. Only 27% of plants grown from the highest exposure level flowered, whereas 60-70% of controls flowered (Table I).

The effect of irradiation on tuber development was in general detrimental (Table I). While the number of tubers per plant was not markedly changed, the yield and growth rate was. The most consistent effect was delayed emergence, a delay which gave a shorter growing season for the plants having higher exposure. While the detrimental effects are not striking, there was no response which could be considered stimulatory.

Freshly harvested potato tubers normally require a rest period prior to sprouting. To determine the effect of ^{60}Co irradiation on this dormancy requirement, tubers were obtained from the commercial 1966 Russet crop just prior to harvest. Some tubers were from fields which had been previously

* This study supported by funds from the U.S. Atomic Energy Commission's Division of Isotopes Development.

TABLE I. Harvest Data from Potato Plants Grown from ^{60}Co -Irradiated Seed Pieces

Exposure, krad	Tubers				Stems per plant	No. plants flowering
	Grams per plant	Tubers per plant	Grams per tuber	Growth rate g/day ^(b)		
0.0 (Border) ^(a)	564	5.3	106	6.5	2.5	157
0.0 (Control)	545	4.6	119	6.1	2.1	55
0.6	534	4.6	115	6.0	2.0	51
0.9	407	4.0	101	4.9	1.9	35
1.1	471	4.5	105	5.4	2.1	41
1.3	421	4.1	102	5.1	1.6	32
1.5	432	3.8	113	5.2	1.7	36
1.8	429	4.7	91	5.2	2.1	35
2.1	355	4.1	86	4.4	1.6	22

(a) Nonirradiated (248) plants surrounding the plot.

(b) Rate of tuber growth from emergence to harvest.

(12 days) sprayed with an arsenical defoliant (Atlas 30). Others were taken from actively growing but mature plants. Tubers from defoliated plants had, in effect, a short-storage period. Care was taken to exclude tubers from plants having tubers exposed above the soil surface. Tubers from defoliated and nondefoliated plants were irradiated (Table II) and planted August 19 in field plots. Treatments were replicated 20 times.

The emergence data of seedlings from irradiated seed pieces are shown in Table II. The 1.5-krad exposure clearly increased the emergence of tubers from defoliated fields and there is a suggestion of an increase with 1.8 krad to the tubers from actively growing plants. The significance of this alteration in dormancy is confounded with the 12-day "storage." As indicated earlier, potatoes normally will sprout and emerge in 2-3 weeks. It is unfortunate that these tests were initiated very late in the season. Cool soil un-

TABLE II. Effect of Irradiation on Emergence of Freshly Harvested Tubers (Irradiation delivered in 30 min, 20 replicates per treatment)

Exposure, krad	Plants emerged			
	Defoliated		Not defoliated	
	No.	Percent	No.	Percent
0	1	5	0	0
0.6	1	5	1	5
0.9	0	0	0	0
1.1	0	0	1	5
1.3	0	0	0	0
1.5	8	40	1	5
1.8	2	10	3	15
2.1	1	5	1	5

doubtedly delayed sprouting and frost hit before full sprouting was achieved.

Conclusions

No stimulatory effect of radiation on rate of sprouting or yield was noted in well-aged tubers chilled to near 0 °C just prior to irradiation. A suggestion of stimulation was obtained from somewhat ambiguous data on tubers freshly dug and irradiated immediately after removal from the field.

ENVIRONMENTAL RADIATION STUDIES - AQUATIC

The rather large number of reports in this section reflects some expansion of effort last year, particularly in the area of Columbia River studies. Several reports, as indicated in footnotes, cover studies which were supported by AEC funds from sources other than the Division of Biology and Medicine.

The first four reports describe quite basic studies dealing with temperature stresses in fish. This problem area is of vital concern, not only from the standpoint of reactor operations, but more generally in terms of the increasing threat of thermal pollution from many sources.

The remaining reports, except for the final one, are concerned directly or indirectly with specific observations of radionuclide, temperature, or other environmental factors in the Columbia River. Increased emphasis on Columbia River studies reflects both the general national climate of concern about pollution and the availability of refined analytical techniques. An additional organizational factor which has helped to encourage a broad approach to river problems was the merger during 1966 of the former Aquatic Biology and Ecology Sections into a single Ecology Section.

METABOLISM OF ACETATE AND PALMITATE BY TISSUES OF WARM AND COLD ADAPTED TROUT

Oxidation of acetate and palmitate to CO₂ was more rapid in tissues from cold adapted fish. Incorporation of acetate into lipids was higher in liver from cold adapted fish. These results are interpreted as signifying a reduced oxidative capacity in tissues from warm adapted fishes.

Investigator:

J. M. Dean

Technical Assistance:

Diana G. Bauer

Alice M. Russell

The relationship between water temperature and physiological capabilities of fish may be the most important single factor controlling the distribution and survival of a given species. Although the deleterious affects of increased temperature on fishes is well documented, very little is known about the mechanism of thermal toxicity. The present report summarizes studies on the metabolism of acetate-1-¹⁴C and palmitate-1-¹⁴C in the tissues of rainbow trout (Salmo gairdneri) adapted to high and low temperatures.

Observations

Homogenates of liver, red muscle, and white muscle from trout acclimated to 5 °C and 18 °C were incubated at three different temperatures with acetate-1-¹⁴C or palmitate-1-¹⁴C and the respiratory CO₂ trapped and counted.

The incorporation of radioactivity from acetate-1-¹⁴C into total liver lipids was also determined.

Liver from fish acclimated to 5 °C showed significantly greater conversion of acetate-1-¹⁴C to ¹⁴CO₂ when incubated at 5 °C than did liver from fish adapted to higher temperatures (Table I). Incubation at 11.5 °C and 18 °C resulted in a similar trend, but the differences noted were not significant at the P < 0.05 level.

Liver tissue from cold acclimated fish also incorporated more radioactivity into lipids than did liver from fish adapted to 18 °C. This effect was most marked, and of statistical significance, at an intermediate incubation temperature of 11.5 °C.

Since red muscle tissue of fishes has higher oxidative enzyme activity than white muscle, it is capable of oxidizing labeled substrates to ¹⁴CO₂

at a greater rate (Table II). Red muscle from cold acclimated fish incorporated more ^{14}C from acetate and palmitate into respiratory CO_2 than corresponding muscle from fish adapted to 18 °C, the difference being significant at the 11.5 °C and 18 °C incubation temperatures. Similarly, con-

version of acetate-1- ^{14}C to $^{14}\text{CO}_2$ by white muscle was greater in cold adapted fish than in warm acclimated fish, but enhanced oxidation of palmitate-1- ^{14}C by white muscle from cold acclimated fish was observed only with incubation at 18 °C.

TABLE I. Incorporation of Acetate-1- ^{14}C into Respiratory CO_2 and Lipids by Rainbow Trout Liver Homogenates (a)

Incubation temperature, °C	Respiratory CO_2		Lipids	
	5 °C fish	18 °C fish	5 °C fish	18 °C fish
5	9.6 ± 1.0 (23) (b)	5.6 ± 0.4 (22)	11.6 ± 0.9 (22) (b)	9.9 ± 0.8 (20)
11.5	15.0 ± 1.2 (17)	13.3 ± 1.5 (22)	13.9 ± 1.3 (15)	9.6 ± 0.5 (22)
18	16.7 ± 1.1 (20)	14.2 ± 0.9 (22)	16.9 ± 1.3 (21)	16.1 ± 0.8 (20)

(a) Results expressed as mean pCi/mg protein/hr ± standard error. The number of experiments is shown in parentheses.

(b) Cold and warm acclimated fish differences significant at the $P < 0.05$ level.

TABLE II. Incorporation of Acetate-1- ^{14}C and Palmitate-1- ^{14}C into Respiratory CO_2 by Rainbow Trout Red Muscle and White Muscle Homogenates (a)

Incubation temperature, °C	Incorporation from acetate (a)		Incorporation from palmitate (a)	
	5 °C fish	18 °C fish	5 °C fish	18 °C fish
<u>Red muscle</u>				
5	25.3 ± 1.9 (11)	15.8 ± 1.7 (6)	2.0 ± 0.2 (6)	2.0 ± 0.4 (4)
11.5	49.0 ± 3.1 (11) (b)	23.4 ± 1.8 (9)	3.7 ± 0.6 (5) (b)	2.0 ± 0.4 (5)
18	49.4 ± 3.2 (11) (b)	36.6 ± 4.6 (7)	4.6 ± 1.4 (4) (b)	1.3 ± 0.1 (2)
<u>White muscle</u>				
5	19.0 ± 1.4 (11)	13.0 ± 1.1 (6)	1.0 ± 0.1 (5)	1.6 ± 0.3 (3)
11.5	29.4 ± 1.8 (11) (b)	11.6 ± 0.8 (16)	1.4 ± 0.2 (5)	1.6 ± 0.2 (6)
18	37.9 ± 3.8 (12) (b)	19.4 ± 2.6 (7)	2.5 ± 0.5 (6) (b)	0.9 ± 0.2 (4)

(a) Results expressed as mean pCi/mg protein/hr ± standard error. The number of experiments is shown in parentheses.

(b) Cold and warm acclimated fish differences significant at the $P < 0.05$ level.

Conclusions

These results suggest changes in the enzyme systems responsible for the oxidation of acetate and palmitate and for the incorporation of acetate into lipids in fish acclimated to higher temperatures. Such reduction in oxidative or tricarboxylic acid

cycle activity is in line with observations of other investigators of decreased oxygen uptake by tissue preparations from fishes adapted to high temperatures. The differential response to incubation temperature suggests that liver, red muscle, and white muscle each respond differently to the high adaptation temperature.

TEMPERATURE-INDUCED DIFFERENCES IN ACID PHOSPHATASE LEVELS OF RAINBOW TROUT LIVERS

Livers from cold acclimated rainbow trout were found to contain twice as much acid phosphatase activity as livers from warm fish. Cytochemical examination at the electron microscope level showed acid phosphatase to be localized in lysosomes in trout hepatocytes, but not in the Golgi complex.

Investigator:
J. D. Berlin

Technical Assistance:
R. R. Adee

Previous morphological observations suggested the importance of examining the distribution and content of acid phosphatase in livers of warm and cold acclimated rainbow trout. Because the Golgi apparatus has in some cases been associated with the formation of acid phosphatase-rich secretory vacuoles, it was desirable to determine if the enlarged Golgi regions observed in cold acclimated fish (Annual Report, 1965) contained acid phosphatase. Because lysosomes contain acid phosphatase, the apparent increase in lysosomal-like bodies observed in the hepatocytes of cold acclimated fish also focused attention on this enzyme.

Observations

Levels of acid phosphatase in livers of 5 and 18 °C acclimated rainbow trout were determined by the method of Berthet and de Duve.⁽¹⁾ The in situ localization of acid phosphatase in hepatocytes of rainbow trout acclimated at 5 °C was accomplished by hydrolyzing β glycerophosphate with the acid phosphatase in the cell and precipitating the cleaved phosphate with lead. The lead phosphate was the electron-dense reaction product.

Figure 1 shows that acid phosphatase levels in 5 °C fish are approximately twofold higher than in 18 °C

FIGURE 1. Acid Phosphatase Activity in Livers of Cold and Warm Acclimated Rainbow Trout (P_i = inorganic phosphate)

fish. An Arrhenius plot was prepared for the enzyme occurring at each temperature in order to calculate its energy of activation. The temperature of acclimation had no effect on this parameter.

Figure 2 shows that the Golgi complex does not contain acid phosphatase since it lacks the reaction product. However, in this same figure, the reaction product appears closely associated with a lysosome.

Conclusions

The energy for activation of acid phosphatase is not markedly influenced

FIGURE 2. Subcellular Localization of Acid Phosphatase in Hepatocytes from Rainbow Trout Acclimated at 5 °C [The reaction product is not found in the Golgi complex (G), but is found in a lysosome (L).]

by acclimation temperature. Acid phosphatase levels are approximately twofold higher in livers of cold acclimated fish as compared to livers of warm acclimated fish. This finding suggested that the enlarged Golgi complex found in hepatocytes of cold acclimated fish might contain accumulations of acid phosphatase, but no such accumulation was observed in either cold or warm acclimated fish. It appears that the decreased activity

of acid phosphatase at low temperatures may be compensated for by increased amounts of acid phosphatase. Whether the levels of other enzymes are altered in a similar manner remains to be determined.

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THE EFFECTS OF TEMPERATURE AND X-IRRADIATION ON THE INTESTINAL EPITHELIAL CELLS OF JUVENILE SILVER SALMON

Cell kinetics of the intestinal epithelium of the juvenile silver salmon were shown to be temperature dependent. DNA-synthesis was temperature and X-irradiation dependent. Cell damage from irradiation was minimal at levels below 1000 R, but severe at 8000 R and higher levels.

Investigators:

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F. P. Conte**
R. E. Nakatani

The intestinal epithelial cell population of animals undergoes continuous renewal. This cell system is useful for the interpretation of environmental changes, which may significantly influence cellular dynamics. Data on cellular dynamics in poikilotherms are very limited. The present study on juvenile silver salmon (*Oncorhynchus kisutch*) is concerned with the effects of temperature and X-irradiation on

DNA-synthesis, cell kinetics, and structural damage.

Observations

To observe mitotic figures in the intestine, salmon acclimated at 5, 10, and 18 °C were injected intraperitoneally with a colchicine solution (1.4 mg/100 g body wt) and killed serially. The upper descending intestine was examined histologically. Arrested mitotic figures occurred in the basal region of the intestinal folds and the mitotic index was temperature dependent. If the results

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for the 5 °C fish are used as reference, the mitotic indices were 1, 1.5, and 2.3 for 5, 10, and 18 °C, respectively.

In a second experiment, tritiated thymidine was injected (1.5 μ Ci/g body wt) followed by sequence sampling at each temperature level, from 1 hr to 40 days postinjection. Initial incorporation of tritium, as measured autoradiographically, was in the basal region and onto the fold proper, with subsequent migration up the fold and ultimate sloughing into the lumen. The turnover times for the intestinal epithelium were temperature dependent, with estimates of 13-15 days, 23-25 days, and in excess of 35 days for 18, 10, and 5 °C, respectively. Labeling indices are being studied at the present time.

The combined effects of temperature (5, 10, and 18 °C) and whole-body X-irradiation (1000 R, 2000 R, and 4000 R)

were studied in a similar experiment with tritiated thymidine. The nucleoprotein fraction of the descending intestine was isolated by differential centrifugation and the samples processed through Schoniger combustion and liquid scintillation counting. Figure 1 shows the temperature dependence of thymidine incorporation in the unirradiated control. Figure 2 shows the effect of radiation on thymidine incorporation for the 10 °C salmon. The long-term data of Figure 1 confirm the autoradiographic tritiated thymidine estimates of cell turnover times.

The effect of whole-body, 250 kvp X-irradiation (140-16,000 R) was studied in 10 °C salmon by light and electron microscopy. Only the light microscope observations are fully interpreted at this time. Slight cell damage was evident at the 1000 R level

FIGURE 1. Incorporation and Retention of Tritiated Thymidine in Nucleoprotein of Intestinal Epithelial Cells of Juvenile Silver Salmon at Three Environmental Temperatures

FIGURE 2. Incorporation of Tritiated Thymidine in Nucleoprotein of Intestinal Epithelial Cells of Juvenile Silver Salmon at 10 °C Following X-Irradiation

by 21 days. Fish receiving 2000 R had swollen vesicular epithelial cells on the lower one-third of the intestinal folds by 7 days, and pronounced cellular damage over 85% of the folds by 19 days postexposure. The 4000 R group showed increasingly severe cell damage, while the 8000 R and 16,000 R

groups showed prominent cellular damage as early as 3 or 4 days post-exposure with complete loss of intestinal folds by 23 days postexposure.

Conclusions

Juvenile silver salmon intestinal epithelial cells proliferate in basal regions of the intestinal folds as shown by colchicine arrested mitotic figures and the initial incorporation of tritiated thymidine. Cell migration appears to occur by transposition of the cells up the folds, with ultimate sloughing into the lumen. Renewal times are temperature dependent, with estimates of 13-15 days, 23-25 days, and in excess of 35 days for 18, 10, and 5 °C, respectively. Incorporation of tritiated thymidine into intestinal nucleoprotein was temperature and radiation dependent. Microscopically evident damage to the intestinal epithelium of 10 °C fish following 250 kvp, whole-body X-irradiation was increasingly severe and evident at shorter postexposure intervals as dose was increased beyond 1000 R.

TEMPERATURE OF TISSUES OF FRESHWATER FISHES

The temperature of the gastrointestinal tract of five Columbia River fishes were not different from the ambient water temperature, but the deep muscles were 0.1 to 1.2 °C higher than the water. Swimming exercise reduced the deep muscle temperature of trout to ambient water temperatures.

Investigator:
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Technical Assistance:
Diana G. Bauer
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The body temperature of fish is often assumed to be the same or very similar to the ambient temperature of the water environment. In vivo measurements, however, have shown some striking differences. Recently, Carey and Teal⁽¹⁾ reported that bluefin tuna may have deep muscle temperatures which are 10 to 12 °C greater than the ambient environmental temperature. There have been conflicting reports on temperature measurements of freshwater fishes. Some preliminary results of in vivo measurements on some common Columbia River fishes are reported here.

Observations

A hypodermic thermistor temperature probe was inserted into the body of the fish to measure the temperature of the deep muscle and gastrointestinal tract. All measurements were obtained by direct read out and recorded continuously. Species measured were whitefish (Prosopium williamsoni), squawfish (Ptychocheilus oregonensis), carp (Cyprinus carpio), white sturgeon (Acipenser transmontanus), and rainbow trout (Salmo gairdneri). Table I summarizes the temperature measurements. In all cases, the deep muscle temperature was higher than the ambient temperature; difference ranged from about

0.1 to 1.2 °C. No difference was observed between the water and gut temperatures.

The effect of exercise on muscle temperature was also explored. Adult trout weighing about 1500 to 1800 g were induced to swim vigorously in a hydraulic test flume with a nominal water velocity of about 3 fps, for about 30 minutes. A thermistor probe implanted in the deep muscle just ventral to the dorsal fin recorded the temperature continuously during the test. A typical observation is illustrated in Figure 1. Muscle temperature did not increase with exercise, but, surprisingly, was reduced to ambient temperature. The exact mechanism of

FIGURE 1. Effect of Exercise on Temperature in Trout

TABLE I. Comparisons Between *in vivo* Tissue Temperatures of Five Freshwater Fishes and Ambient Water Temperature

Species	Number of fish	Range of body weight, g	Mean temperature, °C			Temperature difference between water and muscle, mean $\pm$ SE
			Water	Gut	Deep white muscle	
Whitefish <u>Prosopium williamsoni</u>	11	150-300	16.3	16.3	16.5	0.18 $\pm$ 0.02
Squawfish <u>Ptychocheilus oregonensis</u>	8	90-1210	16.5	16.5	16.8	0.33 $\pm$ 0.08
Carp <u>Cyprinus carpio</u>	9	2070-4720	16.7	16.7	17.0	0.32 $\pm$ 0.07
White sturgeon <u>Acipenser transmon-tanus</u>	6	350-3580	16.5	16.5	16.8	0.27 $\pm$ 0.12
Rainbow trout <u>Salmo gairdneri</u>	8	240-2000	17.0	17.0	17.9	0.71 $\pm$ 0.10

the temperature change in muscle cannot be explained at present, but it seems reasonable to assume that the greater water volume flowing past the body and irrigating the gills of the fish during exercise is a critical factor.

Conclusions

More temperature measurements of freshwater fishes subjected to differ-

ent environmental temperatures are clearly needed, but the limited data presented here suggest that common notions about body temperatures of fish may be incorrect. We plan to study the response of cold or warm acclimated trout to challenges of thermal changes.

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RADIONUCLIDES IN COLUMBIA RIVER ORGANISMS

The definition of seasonal variations in concentration of reactor-produced radionuclides in Columbia River biota was interrupted by closure of all reactors for a six-week period. Reactor shutdown reduced levels of ^{32}P and ^{51}Cr by two orders of magnitude in primary producers. Changes in radionuclide burden were much less in fish. Near equilibrium levels of radioisotopes in river organisms were reached within 2 weeks to 1 month after reactors resumed operation.

Investigators:

D. G. Watson
C. E. Cushing
C. C. Coutant
W. L. Templeton

Technical Assistance:

G. W. Johnson
Dorothy D. Wade

A program to define the seasonal variability in concentration of specific reactor-produced radionuclides in Columbia River biota was begun in February. Its purpose was to update the information obtained in earlier ecological work in 1950 to 1955 and to take advantage of improved instrumentation for radionuclide measurement. This study was to serve as a basis for future ecological work in the Columbia, but was interrupted by the shutdown of all production reactors for a 6-week period in July and August. Although this interruption prevented the definition of "normal" seasonal patterns of radionuclide accumulation in the river biota, it provided an unprecedented opportunity to study changes in isotope concentration during and immediately following shutdown.

Observations

Plankton, algae, invertebrates, and fish were sampled every 3 weeks during most of the year, but the frequency of samples increased to daily and weekly periods during and

immediately following reactor shutdown. High water levels due to the spring-summer freshet prevented extensive sampling of bottom organisms during June and July. Analyses were made for total beta, ^{32}P , ^{46}Sc , ^{51}Cr , ^{54}Mn , ^{59}Fe , ^{60}Co , ^{65}Zn , $^{95}\text{Zr-Nb}$, $^{106}\text{Ru-Rh}$, ^{137}Cs , $^{140}\text{Ba-La}$, and $^{144}\text{Ce-Pr}$.

The radioisotopes of phosphorus, chromium, manganese, zinc, scandium, zirconium-niobium, barium-lanthanum, and iron were found most consistently and in greatest quantities in the river biota. Cesium-137 was usually present in fish muscle in amounts of 0.5 to 3.0 pCi/g wet wt. Chromium-51 was dominant in plankton and algae, but diminished in the higher trophic levels.

Figures 1 to 4 show the concentrations of ^{32}P , ^{65}Zn , ^{51}Cr , and ^{59}Fe in three representative river organisms, net plankton, caddis fly larvae (*Hydropsyche* spp.), and juvenile shiners (*Richardsonius balteatus*). Concentrations of most isotopes diminished in the biota in May or June, probably as the result of increased reactor effluent dilution during this period of seasonally high river flow.

FIGURE 1. Phosphorus-32 in Columbia River Organisms

FIGURE 2. Zinc-65 in Columbia River Organisms

FIGURE 3. Chromium-51 in Columbia River Organisms

FIGURE 4. Iron-59 in Columbia River Organisms

Shutdown of the reactors caused a rapid loss of radioisotopes in the plankton, attached algae, invertebrates, and juvenile fish. Concentrations declined at least one and sometimes two orders of magnitude in these forms. Greatest changes were in the amounts of ^{51}Cr and ^{32}P , which dropped to background levels during the shutdown. Zinc-65, ^{54}Mn , ^{59}Fe , and $^{140}\text{Ba-La}$ declined much less and remained at measurable levels during shutdown. Differences in rate of loss of radionuclides are controlled by several factors including the different effective half-life of radioelements in the organisms and the degree of translocation from other parts of the ecosystem, such as the river sediments.

After reactor startup net plankton, algae, and invertebrates showed a rapid radionuclide uptake and approached equilibrium levels in about 2 weeks. Both ^{32}P and ^{51}Cr increased approximately two orders of magnitude within 1 week following startup. The large surface area-to-volume ratio of these organisms makes adsorption a major route of uptake and is no doubt responsible for the rapid response to changes in concentration of radionuclides in their environment. The caddis fly larvae and juvenile fish lagged a few days behind the plankton and algae because of the time required for transfer of radionuclides through the ingestion of food.

The higher levels of radionuclides in the river biota in September and October, as compared to the period just prior to shutdown, are not the result of the reactor shutdown but

are a seasonally expected increase related to more favorable growing conditions and to the increased effluent concentration resulting from lower river flow. In Figure 5, the seasonal trends in levels of total beta activity in river organisms for 1952, a typical year of uninterrupted reactor operation, are compared with values in 1966. The effects of shutdown are obvious. At a time when levels of radionuclides are normally increasing, the concentrations during shutdown decreased significantly.

The seasonal variability in radionuclide concentration in adult fish was much less than in other river organisms and the changes produced by reactor shutdown were not as extensive. The concentrations of ^{32}P and ^{65}Zn , the dominant radionuclides in adult fish, are shown in Figures 6 and 7. Phosphorus-32 decreased about one order of magnitude during shutdown in the muscle of the herbivorous large scale sucker (Catostomus macrocheilus). Levels of ^{32}P in other fish, and of ^{65}Zn in all fish, were little changed during the period of reactor shutdown. This lack of change, particularly in the case of the physically short-lived ^{32}P , must represent a reduction from levels that would have prevailed if reactor operation had been uninterrupted. Such a deduction is supported by the establishment, after resumption of reactor operation, of ^{32}P levels substantially higher than those prevailing before shutdown. Comparable data with respect to species, radionuclide measured, and location of collection are not available for past years. The rate of

FIGURE 5. Radionuclides in Columbia River Organisms - Reactor Shutdown vs. "Normal"

change in concentration of ^{32}P and ^{65}Zn in herbivorous fish gut contents was similar to that of the algae and plankton.

Conclusions

Reactor shutdown during the summer markedly altered the normal seasonal

pattern of radionuclide concentration in the river biota. During normal reactor operation, lowest levels of effluent isotopes in many river organisms occurred in early summer during time of maximum river flow; maximum levels were in the early fall when environmental conditions such as light and temperature were most

FIGURE 6. Phosphorus-32 in Columbia River Fish Muscle

FIGURE 7. Zinc-65 in Columbia River Fish Muscle

favorable, and when river flows were reduced. Zinc-65 and ^{32}P were the dominant radionuclides in most organisms; ^{51}Cr was dominant in plankton and algae.

Reactor shutdown caused a decrease of one to two orders of magnitude in levels of radionuclides in plankton, algae, invertebrates, and juvenile fish. Uptake after resumption of reactor operation was rapid, reaching

stable levels in about 2 weeks. The radionuclide loss by adult fish during shutdown and the uptake after reactor startup were not as extensive as in the lower trophic levels. The slower turnover of radionuclides in adult fish and the importance of adsorption in the fixing of radionuclides by the plants and invertebrates may explain these differences.

RETENTION OF RADIONUCLIDES IN COLUMBIA RIVER BOTTOM ORGANISMS

Effective retention half-lives were estimated for several radionuclides in Columbia River bottom organisms. Two-component systems were most frequently observed. There was evidence of seasonal variation and community senescence.

Investigator:
C. C. Coutant

The purpose of this study was to estimate rates of elimination of radionuclides from river bottom organisms. Exchange of radionuclides between organisms and their environment can be calculated from elimination rates and seasonal data on radioisotope concentration in the organisms. Elimination rates were estimated by determining retention at various times following cessation of radionuclide input. Input was denied by transplanting individuals or intact communities from an area of the Columbia River downstream from reactors to an uncontaminated site upstream from the reactors.

Observations

Retention experiments were undertaken three times during 1966 for the limpet snail, Fisherola nutalli Haldeman, twice for the green filamentous alga, Ulothrix zonata (Wey. & Mohr) Kutz, once for rock surface periphyton of mixed species composition, and once for rock surface periphyton composed mainly of the diatom Gomphonema parvulum (Kutz) Grun. Limpets collected downstream from reactors were placed on rocks held in polyethylene buckets on the river bottom at the upstream site. Intact communities of Ulothrix and periphyton on

river rocks were placed on steel trays upstream from the reactors.

Estimated values for effective half-life of slow components are shown in Table I. These elimination rates are very approximate and further observation is required before any firm conclusions can be reached. The half-lives of fast components were in most instances not determined, but were of the order of hours rather than days.

Conclusions

Two-component systems of radionuclide retention were commonly observed. An initial rapid decline was followed by a more gradual loss to the end of the experiment. In limpet bodies, the first component may be the result of voidance of the gut. The second component is at times very close to the physical decay rate of the isotope. There appear to be seasonal differences in the elimination rates from the limpet snail. These differences may be related to increase in size of the organisms.

In rock surface periphyton, only a newly established community exhibits much biological elimination. The Gomphonema periphyton population became established following high water in summer, and was relatively "pure"

at the time of the experiment. The community studied in April was of mixed species composition, including consid-

erable detritus, and its elimination rates were essentially those of physical decay.

TABLE I. Retention of Radionuclides in Columbia River Bottom Organisms

Organism	Slow component (a)				Slow component (a)		
	Radio-nuclide	T 1/2, days	% of total		Radio-nuclide	T 1/2, days	% of total
<u>Limpet - shell</u>				<u>Limpet - body (contd)</u>			
February collections	⁴⁶ Sc	11	63	November collections	³² P	6	100
	⁵¹ Cr	26(b)	55		⁵¹ Cr	4	100
	⁵⁴ Mn	56	95		⁵⁴ Mn	3	100
	⁶⁵ Zn	17	80		⁵⁹ Fe	6	100
March collections	⁵¹ Cr	27(b)	50		⁶⁵ Zn	8	100
	⁵⁴ Mn	52	100		⁹⁵ Zr-Nb	7	65
	⁶⁵ Zn	245(b)	50		¹³⁷ Cs	10	100
November collections	⁵¹ Cr	7	100	<u>Ulothrix</u>			
	⁵⁴ Mn	19	100	March-April collections	³² P	3	100
	⁵⁹ Fe	17	40		⁴⁶ Sc	8	75
	⁶⁵ Zn	8	100		⁵¹ Cr	8	85
	⁹⁵ Zr-Nb	12	55		⁵⁴ Mn	290(b)	55
<u>Limpet - body</u>					⁵⁹ Fe	10	75
February collections	³² P	10	60		⁶⁰ Co	8	60
	⁴⁶ Sc	5	40		⁶⁵ Zn	18	60
	⁵¹ Cr	12	62	<u>Periphyton</u>			
	⁵⁴ Mn	11	72	April collections	³² P	14(b)	100
	⁶⁰ Co	18	62		⁴⁶ Sc	84(b)	100
	⁶⁵ Zn	21	75		⁵¹ Cr	28(b)	100
March collections	³² P	3	100		⁵⁴ Mn	290(b)	100
	⁴⁶ Sc	7	50		⁵⁹ Fe	12	100
	⁵¹ Cr	7	80		⁶⁵ Zn	245(b)	100
	⁵⁴ Mn	31	65	<u>Gomphonema</u>			
	⁵⁹ Fe	9	100	November collections	³² P	10	30
	⁶⁵ Zn	30	100		⁴⁶ Sc	84(b)	35
					⁵¹ Cr	27(b)	45
					⁵⁴ Mn	290(b)	50
					⁵⁹ Fe	24	35
					⁶⁵ Zn	9	40

(a) Half-life of fast component (when present) was in most instances not determined, but was of the order of hours rather than days.

(b) Measured half-life approximates physical decay rate of radionuclide.

TISSUE BINDING OF ZINC-65 IN FISH AND OTHER VERTEBRATES

Preliminary studies have compared the ^{65}Zn concentration and stable zinc content of Columbia River fishes and terrestrial species from the Hanford Reservation.

Investigator:
D. R. Buhler

Technical Assistance:
R. G. Genoway

A considerable body of data is available on seasonal changes in radionuclide levels of Columbia River fishes and on the kinetics of uptake and excretion of selected radionuclides by fishes. However, investigation of the factors which control trace element deposition and turnover in fishes has received little attention. Thus, while ^{65}Zn can be detected in the tissues of fish sampled from the Hanford environs and marked interspecies differences in ^{65}Zn tissue concentrations are noted, a precise explanation for these differences is not available.

Therefore, a biochemical investigation of radionuclide metabolism and distribution in aquatic organisms was initiated to determine the significance of tissue radionuclide levels and to identify tissue-binding sites at a molecular level. Later phases of this investigation will also assess the biochemical consequences of such radionuclide deposition. This report is concerned with a comparison of the ^{65}Zn concentration and stable zinc levels of tissues from several species of Columbia River fishes.

Observations

Columbia River fishes accumulate ^{65}Zn primarily through the food web,

and relatively small amounts are absorbed from the water via the gills. The importance of the gastrointestinal tract as the primary route of absorption by fishes is indicated by the high specific activities (pCi ^{65}Zn /mg stable zinc) of stomach and intestinal contents. Tissues associated with digestion, reproduction, or excretion (i.e., intestine, stomach, caeca, liver, kidney, gall bladder, ovaries, and testes) had relatively high specific activities. The specific activities of other tissues were lower and those of nervous tissue and fat were particularly low. The specific activities of the eye, muscle, and intestinal contents of Columbia River fishes, arranged by species, showed the following order of decreasing activities: chiselmouth, squawfish, whitefish, perch, largescale sucker, finescale sucker, sturgeon, and smallmouth bass.

Eyes exhibited the highest concentration of ^{65}Zn in a given animal, however, the specific activities of eyes were quite comparable with those of muscle and blood. Thus, the high concentration of ^{65}Zn in eyes was directly correlated with the high stable zinc content of these tissues. Radioautographs and dissection and assay confirmed that the primary sites of ^{65}Zn deposition in the eyes of fishes were in

the highly melanized argentea, choroid, and iris tissues (Table I). The relatively high specific activity of cornea was apparently derived from a high ^{65}Zn concentration in the outer layer of corneal tissue. Melanized tissues from fish eyes which were high in ^{65}Zn also showed a significant concentration of $^{140}\text{Ba-La}$ and ^{59}Fe , suggesting that such melanin-rich tissues are capable of concentrating many heavy metals. Examination of other melanin-rich tissues (scales, skin, and peritoneal lining) from fishes also indicated an appreciable concentration of ^{65}Zn by such tissues.

Studies of terrestrial species which derive an appreciable portion of their diet from the Columbia River indicate that highly melanized tissues from these animals, such as feathers, hair, and eyes (Table I) bind appreciable quantities of ^{65}Zn . These same tissues also accumulate appreciable quantities of ^{51}Cr , ^{59}Fe , and $^{140}\text{Ba-La}$.

Conclusions

Preliminary studies with Columbia River fishes and terrestrial species, which depend on the river as a source

TABLE I. Distribution of ^{65}Zn and Stable Zinc in Eye Tissues^(a)

Tissue	Coarsescale sucker (14)			Chiselmouth (10)			Smallmouth bass (10)			Coyote ^(b) (2)		
	^{65}Zn	Zn	S.A.	^{65}Zn	Zn	S.A.	^{65}Zn	Zn	S.A.	^{65}Zn	Zn	S.A.
Argentea	6.8	2.6	2.6				2.5	1.7	1.6			
Choroid	10	5.1	2.0	27	6.4	4.3	7.1	4.5	1.6	0.40	1.2	0.34
Cornea	1.8	0.6	3.1	1.6	0.5	3.4	0.2	0.2	1.0	0.13	0.1	0.20
Corneal outer layer							1.5	0.4	3.4			
Iris	13	5.2	2.5	29	12	2.4	5.6	3.3	1.7	0.43	0.8	0.51
Lens	0.1	0.0	2.0	0.1	0.0	3.8	0.0	0.0	0.01	0.0	0.0	0.10
Retina	1.1	0.7	1.6	1.6	0.9	1.7	3.3	2.3	1.5	0.15	0.3	0.57
Sclera	0.5	0.2	2.3	1.5	0.4	3.3	0.1	0.1	2.0	0.04	0.1	0.35
Tapetum lucidum										9.9	33	0.29
Vitreous humor										0.0	0.1	

(a) Results presented as mean pCi of ^{65}Zn (^{65}Zn) or milligrams of stable zinc (Zn) per gram dry weight of tissue, and as specific activity (S.A., pCi $^{65}\text{Zn}/\text{mg Zn}$). Tissues from a given fish species were pooled without regard to sex. Number of eyes dissected is shown in parentheses.

(b) Tissues from both eyes of an adult female coyote were pooled.

of food, show a fairly good correlation between accumulation of ^{65}Zn and the stable zinc content of tissues. Tissues high in zinc, notably those tissues rich in melanin, accumulate appreciable quantities of ^{65}Zn . Specific activities of such tissues, however, are quite comparable to those of

blood and muscle indicating a ready equilibration with the zinc pool. Other radionuclides are bound to such melanin-rich tissues, especially those of the eye. The consequences of such highly localized tissue deposition of radionuclides are not known.

ZINC-65 METABOLISM IN FRESHWATER MUSSELS

Zinc-65 was accumulated in the following tissues of the freshwater mussel, listed in descending order of concentration: gills; mantle and palps; body mass, including the digestive gland, digestive tract, and gonad; adductor muscles; and foot.

Investigator:
G. B. Pauley
R. E. Nakatani

Technical Assistance:
J. D. Maulsby

Certain marine mollusks are known to accumulate ^{65}Zn from seawater and may serve as biological indicators of this isotope in the marine environment. Very little work has been done on the metabolism of radionuclides in freshwater bivalves. Because ^{65}Zn is one of the biologically significant gamma emitters found in the Columbia River below Hanford, an experiment was designed to determine the uptake and distribution of ^{65}Zn by freshwater mussels (*Anodonta californiensis*), one of the dominant macroinvertebrates in the Columbia River near Hanford.

Observations

Mussels were maintained in river water containing 100 $\mu\text{Ci/liter}$ of ^{65}Zn and sampled at intervals over a

period of 36 days. After 36 days, ^{65}Zn accumulation in the soft tissues was approximately 100 μCi , and in the shell of the animals, approximately 300 μCi . The pattern of uptake in the soft tissues is shown in Figure 1. About 75% of the tissue burden was present in the gills, mantle, and palps. In other experiments, the uptake of ^{65}Zn was shown to be proportional to the ^{65}Zn concentration in the water over a range of 1-100 $\mu\text{Ci/liter}$.

Autoradiographs showed some ^{65}Zn in all tissues, except the Leydig (connective tissue) cells and the basophilic glandular cells just beneath the epithelial surface of the foot. Heavy concentrations of ^{65}Zn were localized at the tips and

FIGURE 1. Total Tissue Burden of ^{65}Zn in the Soft Parts of the Freshwater Mussel, *Anodonta californiensis*

base of the outer mantle epithelium; in a basophilic tissue located at the junction of the mantle and gills; in the gonads and the gonadal ducts; between the muscle fibers; in the blood cells; in the epithelium of the intestine, style sac, and rectum; in the epithelial cells of the kidney; and in the lumen of the kidney near the epithelial surfaces. In epithelial cells, ^{65}Zn was concentrated in an area between the nucleus and the tip of the cells (Figure 2).

The distribution of stable zinc was found to be very similar to that measured for ^{65}Zn .

Conclusions

Most of the ^{65}Zn accumulated by the soft tissues of the freshwater mussel

FIGURE 2. Epithelium of Freshwater Mussel Intestine, Showing Localization of ^{65}Zn Between the Nuclei and Cell Tips (Note ^{65}Zn present in the blood cells in the lighter connective tissue)

was deposited within the tissues, rather than being adsorbed onto the surface. Tissues showing the highest concentrations of ^{65}Zn were the leucocytes present in the heart, the kidney epithelium, and the basophilic tissue (present in both the mantle and gills). This is in general agreement with previous indications in the scallop,⁽¹⁾ that excretory tissues (kidney and blood) accumulate ^{65}Zn . There is no structure in marine

bivalves analogous to the basophilic tissue of freshwater mussels. The high concentration of ^{65}Zn in this unnamed tissue of freshwater mussels suggests that it may have an excretory function.

Reference

1. Chipman, W. A., T. R. Rice and T. J. Price. U.S. Fish and Wildlife Service, Fish. Bull., vol. 58, pp. 279-292. 1958.

APPARATUS FOR MEASURING VOLUNTARY SWIMMING ACTIVITY: EFFECT OF ZINC-65 IN TROUT

An automatic recording apparatus was developed to measure voluntary swimming activity of fish. Limited tests with ^{65}Zn -burdened trout showed no significant differences among treatment groups and controls, but demonstrated the utility of the apparatus.

Investigators:

R. C. Erickson*
A. H. Seymour**
R. E. Nakatani

Technical Assistance:

R. G. Genoway

Voluntary locomotion and behavioral responses to ionizing radiation have been little investigated in fish. Recent evidence points to a functional sensitivity of the nervous and endocrine systems which is far greater than may be evidenced morphologically, and behavioral studies have shown themselves useful in detecting such effects. The purposes of this study were to (1) devise an apparatus and technique that would define and quantify units of movement in trout,

and (2) compare the pattern and amount of movement in yearling rainbow trout, Salmo gairdneri, following chronic feeding of ^{65}Zn .

Observations

The testing apparatus consisted of a doughnut-shaped swim channel, four equally spaced photoelectric cells, a central light source, and a chart recorder. Fish were scored in terms of "activity units" (number of passes by a photocell per hour), and "rest periods" (number of 10-min periods per hour with essentially no swimming activity). Figure 1 shows a fish

* AEC Richland Graduate Fellow.

** Professor, College of Fisheries, University of Washington.

passing by a photoelectric cell. Comparisons made between visual counts and chart counts showed the recording system was reliable and accurate within 5% of true values.

Three treatment groups were involved: a control group, and groups fed ^{65}Zn , 5 days a week, at the rate of 1.0 and 0.01 $\mu\text{Ci/g}$ of fish, for 16-18 weeks. Twelve weeks after the last ^{65}Zn feeding, ten fish from each group were tested for pattern and

total amount of swimming activity. Fish were tested singly at intervals over a 3-day period during which no food was given.

Estimates of absorbed dose, from gamma radiation only, were based on radioanalysis of serially killed members of the treatment groups. The average fish in the 1.0 $\mu\text{Ci/g}$ group ingested a total of 16,000 μCi and had an average whole-body absorbed dose of 1200 rads. Maximum dose rate

Neg 36992-18cr

FIGURE 1. Yearling Rainbow Trout Swimming Past a Photoelectric Cell on the Outer Swim-Channel Wall [The selenium-coated photosensitive cells (350 micro amp) in conjunction with an Esterline Angus Chart Recorder was used to score the swimming activity.]

was about 9 rads per day with dose rates on the order of 6 rads per day during testing. The 0.01 $\mu\text{Ci/g}$ group averaged 135 μCi ^{65}Zn ingested, resulting in a dose of 25 rads, with maximum dose rate of about 0.2 rad per day and dose rate at time of testing of 0.1 rad per day.

Analysis of the data showed no statistically significant differences among groups, although mean values indicated up to a 50% lower swimming activity in the 1.0 μCi group. There was a large variance within and between individuals of the same treatment group.

Conclusions

Because of the relatively small sample size and large variability between individuals of the same group, the tests were not sensitive to small differences. Graphic analysis of means suggest that there may be a lowering of swimming activity in the 1.0 $\mu\text{Ci/g}$ group. Further tests using greater numbers of fish and testing periodically from the time of first feeding of ^{65}Zn may show differences that are statistically valid.

RADIONUCLIDE CYCLING BY PERIPHYTON

Investigations of ^{65}Zn uptake rates by periphyton in a closed system reveal a general pattern characterized by a brief but rapid initial uptake rate, followed by a prolonged slow uptake and eventual equilibrium.

Investigator:
C. E. Cushing

Technical Assistance:
G. W. Johnson

Interpretation of the factors influencing ^{65}Zn concentrations in natural periphyton populations can be aided by controlled laboratory investigations. Studies of ^{65}Zn cycling by periphyton communities were continued this year using the apparatus previously described (Annual Report, 1965). Most of the work was done in a "closed" system, i.e., with continuous recirculation of the same water. Studies were conducted in continuous light (about 1500 foot candles), in total darkness, and with killed communities.

Observations

The closed system contained approximately 330 ml of water with ^{65}Zn present at an initial ambient concentration of about 3 nCi/ml. Temperature was maintained within a degree or two of river temperatures in which the communities were cultured. Results from typical experiments are shown in Figure 1.

The general response of living, lighted communities was one of rapid initial uptake at a rate of about 20 cpm for the first 20 to 30

FIGURE 1. Uptake of ^{65}Zn by Periphyton Communities

min, followed by a rate of about 5 cpm until equilibrium was attained. The pattern suggests an initial, rapid adsorptive uptake followed by a longer, slower period of metabolic absorption. A single, inconclusive test on uptake in total darkness suggests a briefer (10 min) period of initial uptake, and a shorter time to equilibrium. The killed communities also completed the initial rapid uptake stage in about 10 min, and exhibited very little subsequent uptake. The equilibrium level attained was substantially lower than that attained by identical living communities. Approximately 20 to 30% of the ^{65}Zn was removed from solution at equilibrium.

A single test was conducted with a "one-pass" system with continuous injection of ^{65}Zn to maintain an approximately constant ambient concentration of 3 nCi/ml.

The results were surprisingly unlike those reported above, with accumulation of ^{65}Zn at a rate of about 40 cpm throughout the 16-hr test, and no evidence of leveling off.

Conclusions

The limited studies described here show that much work remains to be done on both the effects of photoperiod and state of the community. An unresolved question is that concerned with the effect of new algal growth during an experiment, i.e., can the continuing slower uptake rates be partially attributed to growth? Such an effect might be particularly significant in tests extending over long periods using the one-pass system. In the instance described here, the physical appearance of the community changed noticeably, due largely to accumulation of plankton and debris.

Work also needs to be done on refining the apparatus. The closed system has recently been changed to include an open 6-liter reservoir to provide both aeration and more water to help buffer any physicochemical changes during the experiment. We are also considering the use of photomultiplier tubes and NaI crystals in place of GM tubes in order to obtain better performance from the detection system.

REACTOR EFFLUENT MONITORING: COMPARISON OF PUGET SOUND AND PRIEST RAPIDS
CHINOOK SALMON*

Chinook salmon of Puget Sound and Priest Rapids stock, reared in 0, 2, 4, and 6% reactor effluent, showed differences in mortality, growth, and radionuclide incorporation, attributable only to temperature effects and to the stage of development of the fish.

Investigator:

P. A. Olson

Technical Assistance:

C. O'Malley

R. G. Genoway

Reactor effluent monitoring is most realistically carried out with the local race of chinook salmon (Priest Rapids stock), but it is of biological interest to compare the response of different races. The chinook salmon of the Columbia River include many races which may encounter Hanford's effluent, both as adults and juveniles, to varying degrees, on their migration routes. The tests reported here compared University of Washington College of Fisheries stock from Puget Sound and Priest Rapids stock from the Hanford area. Mortality, growth, and radionuclide uptake were parameters used in evaluating effluent effects.

Observations

Fish were maintained in river water with additions of uncooled reactor effluent to levels of 2, 4, or 6%. Local chinooks were reared for nearly 5 months from newly fertilized eggs (December 8, 1965) to migrant-sized fingerlings (April 29, 1966). The Puget Sound race was reared from newly feeding fingerlings (average weight,

0.5 g) for 3 months (January 24 to April 29). Thus, the results, shown in Table I, are not strictly comparable because of age differences. Nevertheless, in both races, the mortality data showed no significant difference due to effluent concentrations. Mortality differences between races were undoubtedly due predominantly to the earlier stage of development at which the local race was started on experiment. Both races showed significantly greater growth ($P < 0.05$) with increase in effluent concentration attributable to temperature effects.

The concentrations of three major gamma emitters, ^{24}Na , ^{51}Cr , and ^{65}Zn , were approximately proportional to effluent concentration. The isotopes are accumulated directly from the water, the mode of entry being primarily through the gills. Because reactors are located upstream from the hatchery water intake, some radioactivity was observed in the zero percent effluent group.

Conclusions

In this test, local chinook tolerated effluent levels up to 6% without adverse effects during the entire early

* This study supported by funds from the U.S. Atomic Energy Commission's Division of Production.

freshwater development from fertilization of egg to time of migration. Except for areas immediately adjacent to the outfalls, the concentrations of reactor effluent in the Columbia

River range far below this level. No marked racial differences between the Puget Sound and local chinook stock were determined in terms of mortality, growth, or accumulation of radionuclides.

TABLE I. Mortality, Growth, and Radionuclide Uptake of Chinook Salmon Reared under Various Effluent Conditions

Race	Treatment groups(a) (% effluent)	Percent mortality	Mean wt, g	Mean length, mm	Radionuclide uptake (pCi/g wet wt)		
					²⁴ Na	⁵¹ Cr	⁶⁵ Zn
Local (Priest Rapids)	0	18	0.70	39	77	19	6.8
	2	10	0.82	41	750	38	20
	4	13	1.05	45	1390	53	36
	6	13	1.2	46	2210	65	45
Puget Sound	0	2.0	3.0	61	73	26	7.7
	2	1.2	3.8	66	720	43	15
	4	1.0	4.2	69	1370	48	19
	6	1.1	4.2	68	1950	77	33

(a) Each group started with at least 1000 fish.

CHINOOK SALMON SPAWNING NEAR HANFORD - 1966

Number of fall chinook salmon nests was 3100, a 20-year high, which exceeded last year's record count by about 70%. This local increase did not correspond to any general increase in the overall salmon run in the Columbia River.

Investigator:
D. G. Watson

For the twentieth consecutive year, the section of the Columbia River between Richland and Priest Rapids was surveyed from the air to enumerate fall chinook salmon nests. The major purpose of these surveys is to serve as an index in the evaluation of possible effects of discharged reactor effluents on the local stock of salmon.

Observations

Six surveys were made between October 6 and November 23. Spawning had not begun on October 6, was well underway by October 26, and reached a peak on November 2. A total of 3100 salmon nests were observed, greatly exceeding the previous high of 1770

counted in 1965. The 20-year average is 780. Spawning between river mile 371-372 (opposite and just upstream from 100-H Area) and at mile 393 (approximately 4 miles downstream from Priest Rapids Dam) was approximately double that of last year, while the other major spawning areas near Hanford were about the same as last year. The total number of fall chinook ascending McNary and Bonneville Dams, downstream from Hanford, was similar to those of 1965.

In Figure 1, the chinook salmon passing upstream over Ice Harbor and Priest Rapids Dams are expressed as percent of those ascending McNary Dam.

Priest Rapids Dam, on the Columbia River, and Ice Harbor Dam, on the Snake River, are the first dams upstream from McNary Dam. The difference between the sums of the Priest Rapids and Ice Harbor counts and the McNary counts can be attributed to mortality and/or fish using the intervening area for spawning. There is a difference of approximately 30% between McNary and the upstream dams between April and August when no spawning occurs. The apparent greater total over the upstream dams in August is probably due to the inability to maintain the identity of specific groups of fish passing upstream and to seasonally varying travel times. The

FIGURE 1. Weekly Counts of Chinook Salmon Ascending Dams in the Vicinity of Hanford

difference between the McNary counts and the sum of the upstream dams is greatest from September through November, the period just prior to and during spawning of the local fish. If the rate of loss between McNary and the upstream dams is constant throughout the year, the drop in percentage of salmon passing the upstream dams may serve as a rough measure of the portion of fish spawning between these dams. Nearly all the spawning in this area occurs near Hanford.

Conclusions

The number of salmon nests observed in 1966 exceeded the previous high by approximately 70%, indicating no observable detrimental effect of reactor operation on the locally spawning salmon. This increase in local salmon is not the result of a corresponding increase in the total fall run in the Columbia River. Approximately 30% of the fall chinook passing McNary Dam utilized spawning areas near Hanford.

IMMUNE RESPONSE OF SALMONIDS AND EXPOSURE OF RIVER FISHES TO CHONDROCOCCUS COLUMNARIS*

Immunity to subcutaneous challenge, but not to intraperitoneal challenge, was developed in yearling rainbow trout by repeated injections of attenuated columnaris organisms. Ladder facilities at Columbia River dams were shown to be important sites for repetitive columnaris exposure and infection of salmonids.

Investigator:
M. P. Fujihara

Technical Assistance:
R. L. Tramel

In previous studies, columnaris agglutinating titers were developed in yearling rainbow trout by injection of attenuated Chondrococcus columnaris plus Freund's complete adjuvant (Annual Report, 1964). These studies were repeated to determine the degree of active and/or passive immunity to the pathogen afforded by the immunization procedure.

Since migration of salmon is dependent upon passage through ladder facilities, studies were conducted to evaluate the columnaris disease problem at Bonneville, McNary, Priest Rapids, and Rocky Reach Dam sites.

Observations

Yearling rainbow trout from the Natches hatchery, free of columnaris agglutinating titers, were immunized against C. columnaris. Two groups of

* This study supported by funds from the U. S. Atomic Energy Commission's Division of Production.

40 fish were injected either intra-peritoneally or subcutaneously with approximately 1×10^6 attenuated columnaris cells suspended in 0.1 ml of 25% Freund's complete adjuvant. The fish were injected at weekly intervals during the first 4 weeks, and at bi-weekly intervals until the last injections at 14 weeks. Low titers of 1:10 and 1:20 were observed in some fish after 2 weeks. Maximal titers of 1:1280 for intraperitoneally immunized fish and 1:2560 for subcutaneously immunized fish were obtained within 15 weeks. Differences in agglutinating titers between the two experimental groups are of doubtful significance.

Immunized and nonimmunized fish were challenged with approximately 1×10^7 virulent columnaris organisms. The results are summarized in Table I. Active immunity to C. columnaris was observed in fish challenged subcutaneously, but no immunity was observed in fish receiving intraperitoneal challenges. Two weeks after challenge,

columnaris agglutinating titers of 1:640 to 1:5120 were observed in sera of actively immune fish. Preliminary passive immunity tests using pooled sera from immunized fish have been negative.

Since fish ladders are known to harbor large numbers of columnaris infected fish, monthly water samples were taken from Bonneville, McNary, Priest Rapids, and Rocky Reach ladders to determine the release of C. columnaris by river and salmonid fishes into ladder water. Results are shown in Figure 1. Columnaris population estimates were based on 15 individual 1 ml samples of water from each of which 0.25 ml aliquots were surface plated on growth media. Samples were taken near the top of the ladder (inlet) and near the bottom of the ladder (outlet). These columnaris counts represent organisms released to the water by resident fish and fish passing through the ladder rather than organisms entering the ladder from the river. This is indicated both by the large increase in organisms within the ladder, and by the fact that water samples taken 1 mile upstream from all ladders were consistently negative. No organisms were isolated from ladder facilities during winter months when temperatures were low and population density of fish was known to be small.

Further indication of the importance of columnaris exposure and infection in fish ladders was obtained by comparison of columnaris agglutinin titers of ladder fish, and fish gill-netted several miles downstream from Bonneville Dam. Average agglutinin titers for largescale suckers, taken

TABLE I. Susceptibility of Immunized Trout to C. Columnaris Challenge

Subcutaneous challenge	Mortality, 2 wk post- challenge
Nonimmunized trout	16/18
Intraperitoneally immunized trout	7/19
Subcutaneously immunized trout	5/18
Unchallenged controls	2/18
<u>Intraperitoneal challenge</u>	
Nonimmunized trout	8/9
Intraperitoneally immunized trout	9/9
Subcutaneously immunized trout	9/9
Unchallenged controls	2/9

FIGURE 1. *Columnaris* Population and Water Temperature of Fish Ladders from April to November, 1966 (Open bar represents sample near inlet; shaded bar, sample near outlet. Curve shows temperature.)

from the ladder during 2 consecutive months, was 1:500 as compared to 1:200 for suckers gill-netted a few miles downstream from the dam. Eight of 20 ladder fish were infected whereas only 2 of 40 gill-netted fish were infected.

Forty-five silver salmon were sampled during passage through the Bonneville and Priest Rapids ladders. The Priest Rapids sampling was timed to obtain samples of the similar group of salmon which were sampled at Bonneville. Increase in average columnaris agglutinating titers between Bonneville and Priest Rapids was from 1:12 to 1:61. Increase in the incidence of culturable organisms on the fish was from 40% to 83%. Incidence of grossly infected fish increased from 7% to 17%.

Conclusions

This study points up the probable critical significance of fish ladders in the etiology of columnaris disease in the Columbia River. Maximum contamination of fish ladders with columnaris organisms occurs during the period of fish crowding, and highest temperature, when passage of anadromous salmon is at its peak. Not only are these fish repeatedly subjected to columnaris exposure at each dam site, but they may constitute a major vector for spreading the disease throughout the river system. Studies on downstream and upstream migrant salmon for antibody production and possible immunity to *C. columnaris* are planned.

UPSTREAM DISPERSION OF ADULT CADDIS FLIES

Adult river caddis flies (Trichoptera: Hydropsychidae) are dispersed upstream from river reaches occupied by the larvae. The maximum extent of this dispersal would appear to be about 16 km.

Investigator:
C. C. Coutant

This study was undertaken to determine whether adult Columbia River caddis flies (Trichoptera: Hydropsychidae) are dispersed upstream from the river reaches they occupied as larvae. Downstream drift of immature forms, a characteristic common to nearly all aquatic insects, poses the theoretical problem of recolonization of upstream areas. A compensatory upstream migration by adults, particularly females before oviposition, has been hypothesized as a universal feature of these organisms, but there is little evidence for such migration on large rivers.

Organisms growing in the Columbia River downstream from the Hanford reactors are labeled by the presence of radioactive nuclides originating in the reactor cooling water discharges. The most upstream reactor (100-B) provides a boundary above which no Hanford-derived radionuclides should be found in relatively sessile river organisms.

Observations

Adult caddis flies of mixed species composition (largely Hydropsyche cockerelli, Cheumatopsyche enomis and C. campyla) and of both sexes (often predominantly males) were collected upstream and downstream from the 100-B

reactor. Figure 1 summarizes concentrations of ^{65}Zn . It is readily apparent that values were well above background levels (about 1 pCi/g) for several km upstream from 100-B. If a line is visualized representing the general slope of all the data, it intersects the 1 pCi/g detection limit near 16 km above 100-B. These data indicate that there is upriver dispersion of some adult caddis flies.

Data for a particular collecting day show wide variance and do not exhibit consistent decrease as one progresses upstream. These variances may be due largely to sampling error and wind effects. The possible magnitude of sampling error can be estimated from several multiple samplings shown in Figure 1. This error may indicate that the ^{65}Zn activity recorded resides in a few highly radioactive insects mixed with a large population of unlabeled ones. Caddis flies can at times be observed to be carried by the wind.

Conclusions

The study thus far has provided no evidence for directed upstream migration, despite clear evidence for some mode of dispersal. Upstream dispersal may be matched by similar dispersion in all directions. The important

point thus far established is that upstream movement of adults does occur, thereby replenishing depleted populations. For radiological considera-

tions, this movement represents only a small dispersal from a source of environmental contamination.

FIGURE 1. Zinc-65 Concentration in Adult Caddis Flies (mean $\pm$ standard error)

FAIRY SHRIMP IN GRAVEL PIT PONDS

The fairy shrimp, *Streptocephalus seali* Ryder, was observed in temporary ponds created by an elevated water table associated with high Columbia River flows. Appearance of these ponds during warm weather in late spring causes hatching of embryos at high temperature and survival of individuals into late summer.

Investigator:
C. C. Coutant

This study followed the 1966 seasonal appearance of the fairy shrimp, *Streptocephalus seali* Ryder, in seepage ponds on the Hanford Reservation. Casual observations made at the study location in 1965 indicated that individuals of this species persisted well into summer. At other locations they are usually found only during early spring months.

The ponds studied are within a large unused gravel pit, which forms a depression about 10 m deep and about 300 m in diameter and lies within 250 m of the Columbia River. During winter months there is only one small permanent body of water; in midsummer, most of the bottom of the pit is covered with a large contiguous pond.

Observations

Figure 1 illustrates the time sequence of water level changes within the gravel pit. The lowest of the previously dry areas of the gravel pit began to contain standing water in mid-May. Maximum levels occurred about the first of August, followed by a steady decline through August, September, and October. The water never rose to sufficient height to merge the temporary pond area with the permanent pond.

The stratum which forms both river bed and pond area is permeable glacio-fluviatile gravel. Figure 1 also shows daily minimum Columbia River elevation below Priest Rapids Dam, which can be taken as an index of relative river elevations near the gravel pit. The similarity in time-course of the seasonal peaks of Columbia River flow and of water level in the gravel pit is apparent, though change in the gravel pit level lagged somewhat behind change in river level.

Figure 2 indicates daily ranges of water temperatures for surface and bottom layers for representative days

FIGURE 1. Depth of Gravel Pit Pond and Daily Minimum Elevation of the Columbia River below Priest Rapids Dam

FIGURE 2. Range of Water Temperatures in Gravel Pit Pond

in May, June, July, and August. Very high initial temperatures dropped, generally, until mid-June when they began to rise. Diurnal range was least near the end of June. Bottom temperatures were within or slightly below the ranges of surface values.

Fairy shrimp were observed along with the first water in the newly flooded basins on May 20. Nauplius and metanauplius stages were present in very large numbers, even where the volume of water was only a few milliliters. These immature forms were thriving at daytime temperatures (32 °C) generally considered to be at the upper tolerance range for the species. Gravid females were first seen on June 22. Newly hatched nauplii and metanauplii were also found on that date in peripheral basins that had

only recently received water. Most fairy shrimp were grouped in schools during June and July, making accurate quantitative sampling of the ponds impossible. While individuals ranged from very small to large in the pit as a whole, each school seemed to be composed of a restricted size class. On July 15 an apparent fungus infection was first seen in some large shrimp. By August 3 a decided decline in numbers had occurred, with large males predominating in some basins. The fungus infection might have affected decline although this was not investigated. All individuals were scattered; no dense schools were found at this time. Predatory aquatic insects capable of feeding on Anostraca were abundant, probably affecting decline. On August 31, a search of all remaining temporary ponds yielded only one large male.

Conclusions

The hydrography of the gravel pit ponds is controlled by seasonal fluctuations in water level of the Columbia River. Late seasonal survival of fairy shrimp is probably due to the relatively late date at which these ponds receive temporary water, and to successive hatches of embryos in peripheral basins which coalesce as the maximum water elevation is reached. The apparent hatching and survival of embryos of *S. seali* at the high temperature of 32 °C is extraordinary and merits further investigation.

OBSERVATIONS OF THE LIFE HISTORY OF THE LIMPET SNAIL FISHEROLA NUTALLI HALDEMAN

A one-year life span is indicated for this limpet snail, with reproduction occurring during high river flows in June and July and death following shortly thereafter. Dispersal of newly hatched snails to bottom areas free of reproducing adults was observed.

Investigators:

C. C. Coutant
D. G. Watson
C. E. Cushing
W. L. Templeton

Technical Assistance:

G. W. Johnson

The limpet snail, Fisherola nutalli Haldeman, is an important contributor to the biomass of invertebrate organisms in the bottom community of the Columbia River at the Hanford Reservation. Life history data were obtained in the course of periodic sampling of river bottom organisms for radionuclide analysis at the 100-F area sampling site.

Observations

Average weight of limpet snails collected during 1966 is presented in Figure 1. No data are available for the high-water period when the bottom is inaccessible to sampling. These data would seem to represent two year classes, the first including all individuals collected before the high-water period and some collected immediately after return to normal river flows, and the second group of much smaller snails appearing at the end of the high-water period. The absence of large snails from October through December strongly indicates negligible carryover of mature limpets from year to year.

Observations of limpet egg masses were made during high water in June and July. Artificial substrates were

placed in the river prior to rise of water level and these were removed periodically when the bottom was inaccessible. Egg masses containing developing limpets were abundant on these substrates.

Areas of river bottom that had been exposed to drying and were free of limpets prior to the high-water period were abundantly colonized with small limpets after high-water in August. Sampling of these tiny specimens could have been attempted

FIGURE 1. Weight of Limpet Snails Collected from Columbia River Bottom

earlier than noted in Figure 1 except for their exceedingly small size. No large limpets of the preceding year class were found in these previously dry areas. A dispersal mechanism for newly hatched snails, perhaps via water currents, is suggested.

Conclusions

Limpets found in the Columbia River at the Hanford Reservation appear to

have a one-year life cycle. Two discrete year classes are found immediately following return to normal river flows in August and September. At most other times the population is probably a single year class.

Limpets lay eggs during high water in June and July. A dispersal mechanism for newly hatched limpets is suggested which merits further study.

FRESHWATER MUSSELS WITH PEDUNCULATED ADENOMAS

Of 40 freshwater mussels examined, four were found to possess a total of 14 tumors, all arising from the foot. The neoplasms closely resemble the pedunculated adenomas or polyps commonly observed in the human colon.

Investigator:
G. B. Pauley

Technical Assistance:
J. D. Maulsby

Reports of tumors in bivalve molluscs are rare. Those which have been noted in freshwater mussels (Anodonta cygnea⁽¹⁾ and A. implicata),⁽²⁾ soft-shell clams (Mya arenaria),⁽³⁾ and oysters (Crassostrea virginica and C. gigas),⁽⁴⁾ have usually interfered seriously with the physiology of the afflicted animals. Pedunculated tumors have been reported in the gastropods, Limax flavus⁽⁵⁾ and Pleurobranchus plumula.⁽⁶⁾

Observations

Of 40 freshwater mussels (Anodonta californiensis) sampled from the

Columbia River, four animals were observed with multiple tumors located on the foot (Figure 1). The papilliform neoplasms were elevated from the surface of the foot by a firm, but flexible, stalk. The abnormal growths varied in size from 1 x 2 x 2 mm to 4 x 4 x 2 mm, and the number of tumors ranged from two to six per animal.

The tumors were all similar in microscopic appearance, being extensions of the normal foot tissue (Figure 2). The tumors were covered by tall, columnar epithelial cells. This epithelial covering was highly convoluted and possessed deep crypts, giving the neoplasms a papilliform

FIGURE 1. *Pedunculated Adenoma or Polyp (T) on the Posterior Part of the Foot of a Freshwater Mussel (Approximately one and one-half times normal size)*

appearance. Highly compacted muscle fibers formed the stalks and the central portions of the papilliform tumors. Between the epithelial covering and the muscular core there was an abnormally thick and dense layer of basophilic glandular cells. All of the cells comprising these abnormal growths were normal in appearance. Neither mitotic figures nor an inflammatory reaction was present in any of the tumors. The growths appeared to be well defined, noninvasive extensions of the foot. On the basis of these characteristics, these neoplasms were considered benign tumors of glandular and muscle origin, resembling benign polyps of pedunculated adenomas as found in the human colon. The cause of these neoplasms is not known.

FIGURE 2. *Small Polyp Arising from the Foot of a Freshwater Mussel. [Note the separation of the muscular core and basophilic cells (Hematoxylin and eosin stain)]*

Conclusions

Williams reported an adenomyoma on *A. cygnea*, composed of glandular cells just beneath the epithelium and entirely separated from the muscular stalk, and stated it had the appearance of a human polyp.⁽¹⁾ Although the locations are different, the neoplasms of *A. californiensis* observed in the

present study and those observed on A. cygnea by Williams appear to be of the same type.

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INTEROCEANIC CANAL STUDY - FRESHWATER ECOLOGY*

Preliminary studies were conducted to examine the potential radiation hazards to human populations from the use of the freshwater environment in the regions of proposed nuclear excavations in Panama and Colombia.

Investigators:
W. L. Templeton
D. G. Watson

The purpose of this program is to plan and conduct studies in freshwater ecology needed to evaluate and predict the potential radiation hazards to human populations from use of the freshwater environment in the regions of proposed nuclear excavations in Panama and Colombia.

Specifically, the data to be acquired will include those needed:
(1) to describe the general features of the aquatic ecosystems utilized by

the human populations in the regions of reference, (2) to evaluate the natural distribution of certain selected stable and radioactive elements in representative components of the aquatic ecosystems, and (3) to predict the maximum concentrations and/or specific activities, redistribution rate, and persistence of potentially critical radionuclides following fallout deposition and introduction from the excavated canal into the freshwater aquatic ecosystem.

Present studies have been confined to literature review, identification of potentially critical radionuclides, and the preparation of a detailed field program for 1967.

* This study, performed under sub-contract for Battelle Memorial Institute, Columbus, Ohio, is included because ultimate funding derives from U. S. Atomic Energy Commission.

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INDEX OF INVESTIGATORS

Bair, W. J.	57, 61, 63	McClanahan, Beatrice J.	101
Beamer, J. L.	23, 88, 91	McKenney, J. R.	7
Beasley, T. M.	61	Nakatani, R. E.	159, 174, 176
Berlin, J. D.	12, 43, 81, 157	O'Farrell, T. P.	132
Buhler, D. R.	172	Olson, P. A.	180
Clarke, W. J.	23, 38, 57	Palotay, J. L.	23, 38
Cline, J. F.	140, 141, 143	Park, J. F.	57, 63
Conte, F. P.	159	Pauley, G. B.	174, 191
Cooper, P. H.	29	Peakas, J. C.	35
Cosgrove, G. E.	132	Ragan, H. A.	23, 28, 32, 38, 88, 101, 105
Coutant, C. C.	164, 170, 186, 188, 190	Rickard, W. H.	135, 137, 138
Cushing, C. E.	164, 178, 190	Sanders, C. L.	103
Dean, J. M.	155, 162	Seymour, A. H.	176
Dilley, J. V.	73, 74, 76, 81	Sikov, M. R.	40, 42, 47, 48 50, 52, 93
Eberhardt, L. L.	129	Smith, Linda G.	35
Erdman, H. E.	16, 17, 18	Smith, V. H.	88, 91, 105
Erickson, R. C.	176	Stuart, B. O.	66, 67, 68, 70, 80
Fujihara, M. P.	183	Sullivan, M. F.	3, 11
Gillis, M. F.	23	Templeton, W. L.	164, 190, 193
Hackett, Patricia, L.	23, 32	Tombropoulos, E. G.	77, 80
Hanson, W. C.	129, 130	Turner, J. R.	121, 125
Howard, E. B.	23, 57	Uhler, R. L.	137, 145, 150
Hsieh, J. J. C.	96, 147	Vogt, Glenda, S.	23
Johnson, T. S.	159	Watson, C. R.	23, 28
Magula, Mary W.	143	Watson, D. G.	164, 181, 190, 193
Mahlum, D. D.	40, 42, 43, 47, 48, 50, 93	Willard, D. H.	87
Matchett, W. H.	109, 113, 121	Wiley, W. R.	115, 118

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