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PACIFIC NORTHWEST LABORATORY ANNUAL REPORT FOR 1965 IN THE BIOLOGICAL SCIENCES

to the

USAEC DIVISION OF BIOLOGY AND MEDICINE

by

The Staff of the
Biology Department

Edited by

R. C. Thompson and E. G. Swezea

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January, 1966

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FOREWORD

This is the first Annual Report to cover work performed under our new contractual status as the Biology Department of the Pacific Northwest Laboratory operated by the Battelle Memorial Institute. Previously operated by the General Electric Company as a component of the Hanford Laboratories, the change-over took place on January 4, 1965.

The change of contractors involved no personnel changes or immediate organizational changes. In mid-summer of 1965, however, there occurred a general reorganization of the Biology Department. In addition to numerous shifts of personnel between previously existing Sections, two additional Sections were created and a new position established of Senior Research Associate. These changes are reflected in the listing of Biology Department Staff which follows this Foreword.

In view of these changes it seemed an appropriate time to also make rather extensive alterations in our Annual Report. Most immediately obvious will be the changes in format and typography, which, within the limitations of economical publication, should do much to improve readability. These changes only partially account for the decreased bulk of the document, for we have also made a concerted effort to shorten the individual reports and to standardize their plan of presentation.

Individual reports are grouped to correspond approximately with the USAEC's Division of Biology and Medicine budget categories. Each of these categories is introduced by a brief general discussion of our program in that category. Major research accomplishments of the year are highlighted in these introductions. It seems appropriate, however, to add emphasis at this point to a few of the year's significant events.

A major effort was involved, early in the year, in constructing temporary facilities for much of our environmental studies program to replace those destroyed in the fire of November 1964. This was accomplished in as rapid and inexpensive a manner as possible, in view of our anticipated move to new laboratory quarters in 1968 or 1969. With assistance from the Division of Biology and Medicine and the AEC's Richland Operation Office, and the cooperation of many people on the local scene, most of this work was completed before summer.

Two major symposia of international scope were held during the year—the Hanford Symposium on Radiation and Terrestrial Ecosystems under the general chairmanship of Dr. F. P. Hungate, May 3-5; and the Symposium on Swine in Biomedical

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Research, July 19-21, under the joint general chairmanship of Dr. L. K. Bustad and Dr. R. O. McClellan. Scheduled for September 25-28, 1966, is a Symposium on Gastrointestinal Radiation Injury under the general chairmanship of Dr. M. F. Sullivan. Planned for 1967, May 15-17, is a Symposium on Diagnosis and Treatment of Deposited Radionuclides, to be cosponsored with the Hanford Occupational Health Foundation under the joint general chairmanship of Dr. H. A. Kornberg and Dr. W. D. Norwood.

Late in 1965, a rather thorough review of the Biology Department's objectives resulted in the following listing of "priority areas" in which we plan to expand our activities at the expense of other less critical or less productive areas:

- Inhalation studies
- Chronic ^{90}Sr toxicity studies in swine
- Columbia River ecology studies
- Arid lands ecology studies
- Internal emitter metabolism and toxicity studies
- Gastrointestinal radiation damage studies.

These six major areas are not all inclusive. There is, for example, fundamental work at the cellular level in support of nearly all areas listed. We feel that no life sciences laboratory can maintain a healthy research program without studies in progress at this basic level.

If a single major research accomplishment were to be singled out for emphasis, it would probably have to be the growing body of data on hematopoietic neoplasms in our miniature swine chronically exposed to ^{90}Sr. In addition to their obvious bearing on problems of hazard evaluation and permissible exposure limits, these results suggest fascinating possibilities for basic studies on leukemogenesis.

The major new area of research endeavor during the past year has been that associated with the evaluation of reentry hazards of radionuclide power sources and nuclear propulsion systems in space. These studies, undertaken at the request of the Division of Biology and Medicine are still preliminary, but are represented by four reports in this document.

The most traumatic event of the year occurred when Dr. Leo K. Bustad, our chief "sheepherder" for 16 years, left Hanford to become Professor and Director of the Radiobiology Laboratory, University of California, Davis, California. He has our esteem and best wishes for continued success.

H. A. Kornberg, Manager
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ACKNOWLEDGEMENTS

With this Annual Report we are instituting the custom of identify only the investigators involved with each research problem reported, b technical assistance directly involved in the conduct of the experimen still leaves the essential services of many people unacknowledged. Cr most of our studies are the services of the "counting room" supervise A. C. Case, and radiochemical and other analytical services provided b R. F. Keough, J. P. Herring, and their assistants. Cooperative resea of the Pathology Section are vital to many programs, in particular the laboratory analyses supervised by Glenda S. Vogt. The vital function care, involving many people, is coordinated by M. E. Kerr for swine, sheep; by M. G. Brown for the beagles; and by R. F. Howard for the ro Special acknowledgement is due R. L. Buschbom and J. M. Thomas of the Department who have worked closely with many of our investigators in sion of statistical and computer services. Finally, tribute must be librarian, Betty Groff, and to our secretaries and clerks who labor l insure that reports such as this do, eventually, reach the hands of t

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SOMATIC EFFECTS OF RADIATION
RADIATION EFFECTS - GENERAL

Of the ten reports included in this category, five are concerned with the mechanism and treatment of gastrointestinal radiation effects. Long an area of special interest in our laboratory, this effort has now expanded to include more basic studies, as evidenced by the reports on "transport and potential measurements" and on "ultrastructural" effects. This effort will be stimulated in 1966 by the holding at Richland, September 25-28, of an International Symposium on Gastrointestinal Radiation Injury.

Included in this category are three other areas of investigastion. The program on skin irradiation effects has for several years been principally concerned with the development of the Hanford Miniature Swine, whose skin is particularly well adapted to skin studies, but which has an obviously much wider applicability as an experimental animal. This latter point was attested to by the Symposium on Swine in Biomedical Research, held at Richland, July 19-21, 1965, which started out to be a small gathering of investigators using swine as experimental animals and ended up with an attendance of over 150 off-site participants. The proceedings of this symposium, including 72 papers, will be available in book form by mid-summer, 1966.

Studies on the combined effects of chemical carcinogens and radiation, pursued on a limited scale for several years, will probably be terminated in 1966 when the planned preliminary survey is completed. Our continuing program of radiation effect studies on the flour beetle is represented this year by a single report on the combined effects of radiation and DDT.

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SODIUM AND WATER TRANSPORT IN THE IRRADIATED INTESTINE

In vivo perfusion studies show that water and sodium absorption from the small and large intestine of the rat are decreased to about half normal values three days after 1500 R. Movement of these constituents from blood stream to intestinal lumen is similarly depressed in the small bowel but not in the large intestine. Decreased absorption is the principal factor responsible for excessive fluid and electrolyte losses associated with the intestinal radiation syndrome.

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

The excessive losses of water and sodium from the gastrointestinal tract have been tacitly accepted as the proximate cause of death in the radiation syndrome termed "intestinal death." There have been conflicting views, however, concerning the mechanism by which these losses occur. These conflicts have arisen because of the differing methods employed in studying the phenomena and because only part of the functions involved in maintaining homeostasis have been studied in any single investigation.

Observations

Rats were exposed to either 1000 or 1500 R of X-irradiation to the abdominal region, the remainder of the body being protected by a lead shield. Three days after irradiation, when fluid loss is most apparent, the animals were anesthetized and their small or large intestines perfused in vivo with isotonic phosphate buffer. Tritium oxide (^3H_2O) and ^{22}Na tracers were either contained in the perfused buffer or injected intravenously and collected in the perfusate. A minimum of five animals were studied in each treatment group.

The disappearance of ^3H_2O and ^{22}Na from the perfusate, measured at 15 or 30 min intervals for 2 hr, is shown in Figure 1. The data demonstrate the parallelism between the absorption of sodium and water from the small and large intestine and the dose dependent inhibition of absorption after irradiation.

Movement of intravenously injected ^{22}Na into the small intestine (Figure 2) was decreased following irradiation to about half the normal value, but ^3H_2O exsorption was reduced by only about 20%. In the large bowel, the excretion of neither ^3H_2O nor ^{22}Na was appreciably affected by prior irradiation.

Conclusions

These in vivo perfusion studies demonstrate a severe depression of the

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absorptive function for water and sodium in both the small and large intestine following irradiation of the abdominal region. The outflux of sodium and water from the blood into the lumen of the intestine is in no case increased--it is substantially reduced in the small intestine and maintained at approximately normal levels in the large intestine. These results indicate that the loss of water and sodium which characterizes the intestinal radiation syndrome must be primarily attributed to a decreased reabsorption of fluids and electrolytes normally reaching the intestine by way of the bile and other secretions.

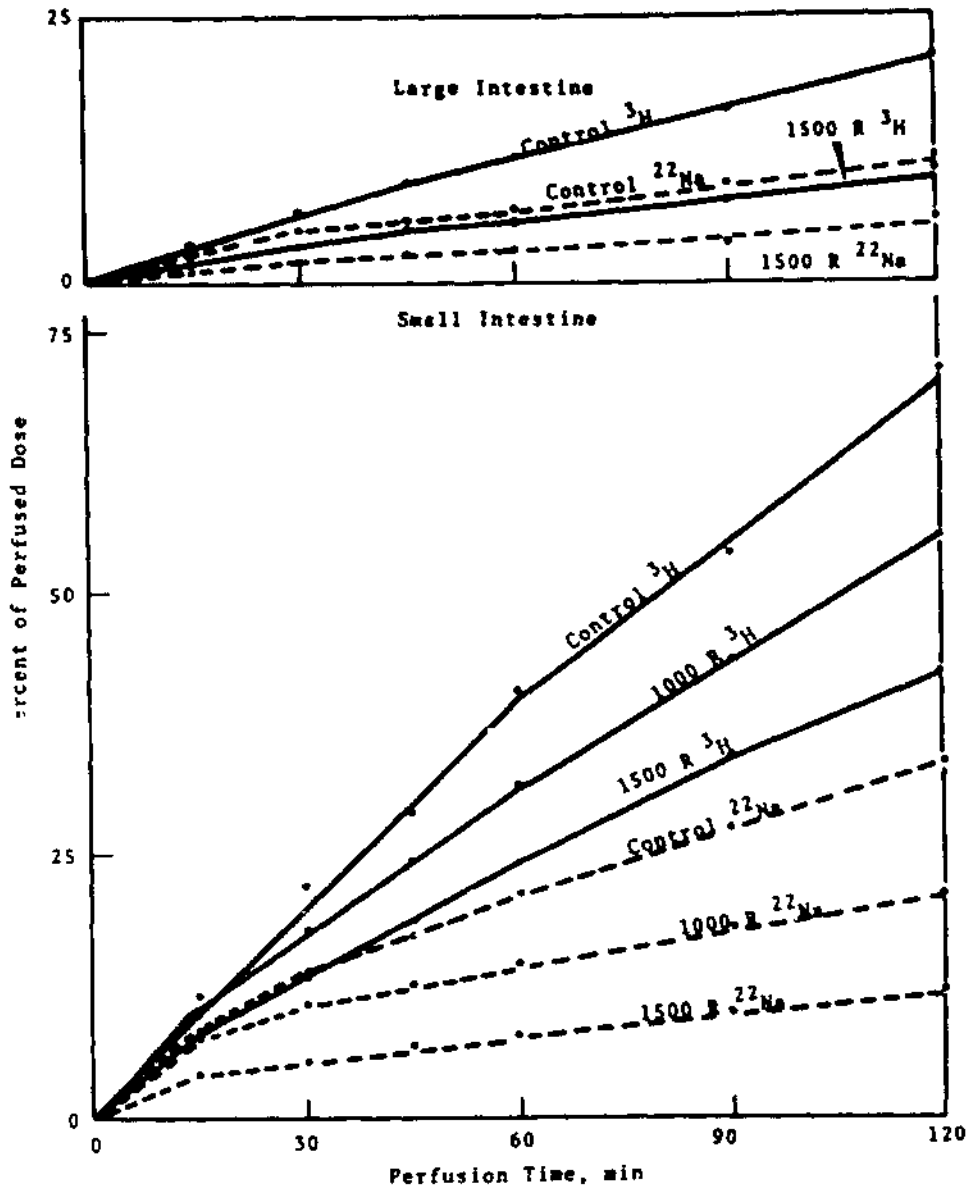

FIGURE 1. ^{22}Na and ^{3}H Insorption from the Perfused Intestine 3 Days After Irradiation

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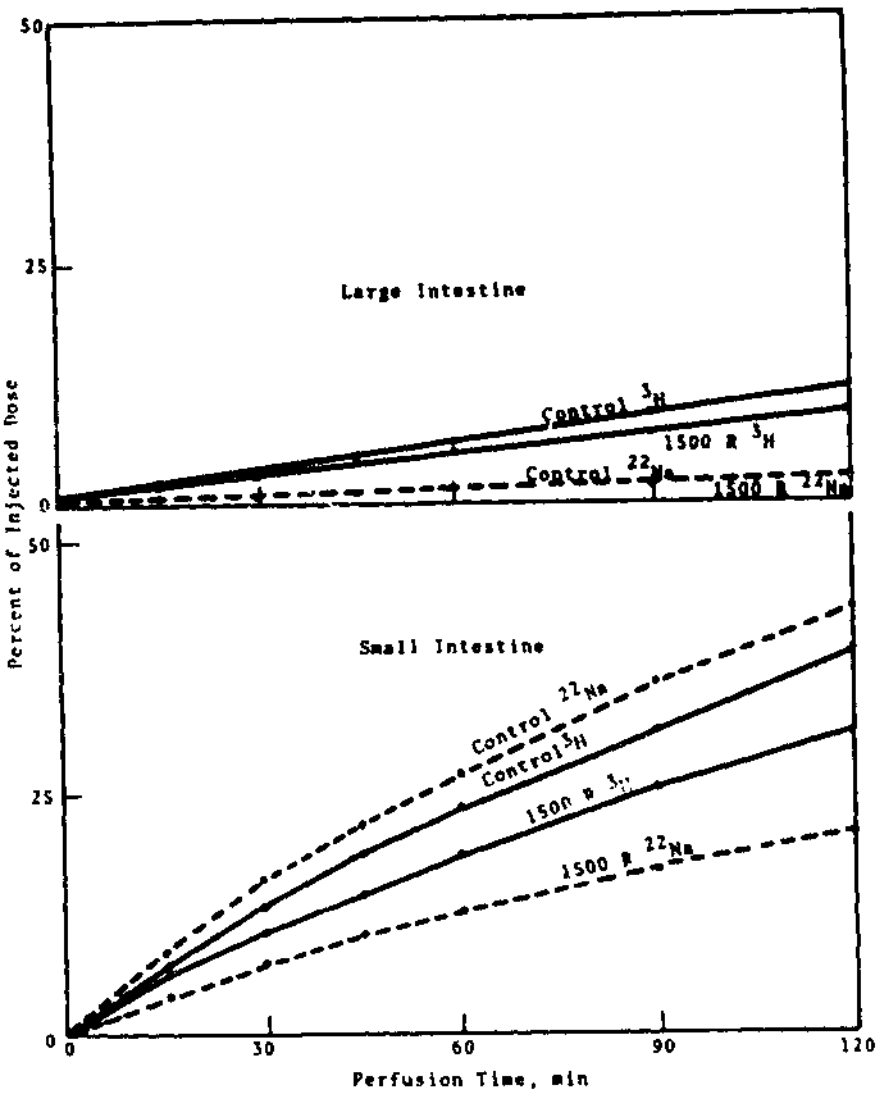

FIGURE 8. ^{22}Na *and* ^{3}H *Exsorption into the Perfused Intestine 3 Days After Irradiation*

TRANSPORT AND POTENTIAL MEASUREMENTS IN ISOLATED RAT INTESTINE

Sodium, potassium, chloride, cesium, and water transport were studied in vitro. Results obtained suggested that there was active chloride absorption and potassium secretion by the ileum. Cesium transport and accumulation resembled that of potassium. Preliminary results demonstrated deleterious effects of X-irradiation on the bioelectric and transport characteristics of the ileum.

Investigator:
J. R. McKenney
Technical Assistance:
B. P. Neal

In vitro techniques were used to study electrolyte absorption by the jejunum and ileum of young adult rats to obtain control data for studies of

the effects of ionizing radiation on intestinal function. The electrolytes studied were sodium, potassium, cesium, and chloride. Measurements were also made of net water and tritiated water transport and the bioelectric potential generated across the intestinal wall. The results of the experiments on chloride transport were of particular interest because of the absence of definitive data in the literature regarding the mechanism of chloride absorption. Preliminary data are reported on the effects of X-irradiation on these transport and bioelectric properties of isolated ileum.

Observations

Nine intestinal segments were usually taken from each rat, everted, and mounted in apparatus for bioelectric potential and radioactive flux measurements.(1) ^{24}Na, ^{42}K, ^{137}Cs, and ^{36}Cl, and ^{3}HOH were used as radioactive labels for their respective stable isotopes and water. Repeated, direct measurements were made of the "absorption permeability" (K_{ms}, unidirectional mucosal to serosal flux of radioactive label/concentration of radioactive label in mucosal solution) together with a single measurement of the net transport and steady state ratio of the serosal and mucosal concentrations (S/M) for each intestinal segment.

During the experiments, the segments were immersed either in inorganic phosphate-buffered solution oxygenated with 100% O_2 at 37 °C or in a bicarbonate-buffered solution aerated with 95% O_2 and 5% CO_2. The composition of the normal media was 1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KCl, 5 mM Na_2HPO_4/NaH_2PO_4, and 15 mM glucose, plus 25 mM $NaHCO_3$ for bicarbonate buffered media, and NaCl to 310 milliosmolar. The pH was 7.4 to 7.7. Choline chloride, mannitol, and Na_2SO_4 were substituted for NaCl to obtain the altered concentrations to be described.

Bioelectric Measurements

Complex changes occurred in the bioelectric potential across intestinal segments both upon altering the sodium concentration and following X-irradiation. Data for segments from portions of the ileum that were shielded with lead during the exposure as well as that for nonshielded ileum exposed to 1500 R are shown in Figure 1. When intestinal segments were in normal media, their serosal surface was positive with respect to their mucosal surface. However, following either choline chloride or mannitol substitution for NaCl, when sodium concentration was reduced to 50 mM in both the serosal and mucosal media, the polarity of the voltage difference often reversed so that the serosal surface was negative. This bioelectric response occurred with the shielded ileal segments, but, as shown in Figure 1, was less evident with the irradiated ileal segments.

During more extensive studies with nonirradiated rats, results similar to those described above were obtained with either jejunal or ileal segments in bicarbonate-buffered media. Using an inorganic phosphate-buffered media, a similar but reduced negative voltage response occurred with ileal segments. In jejunal segments, how-

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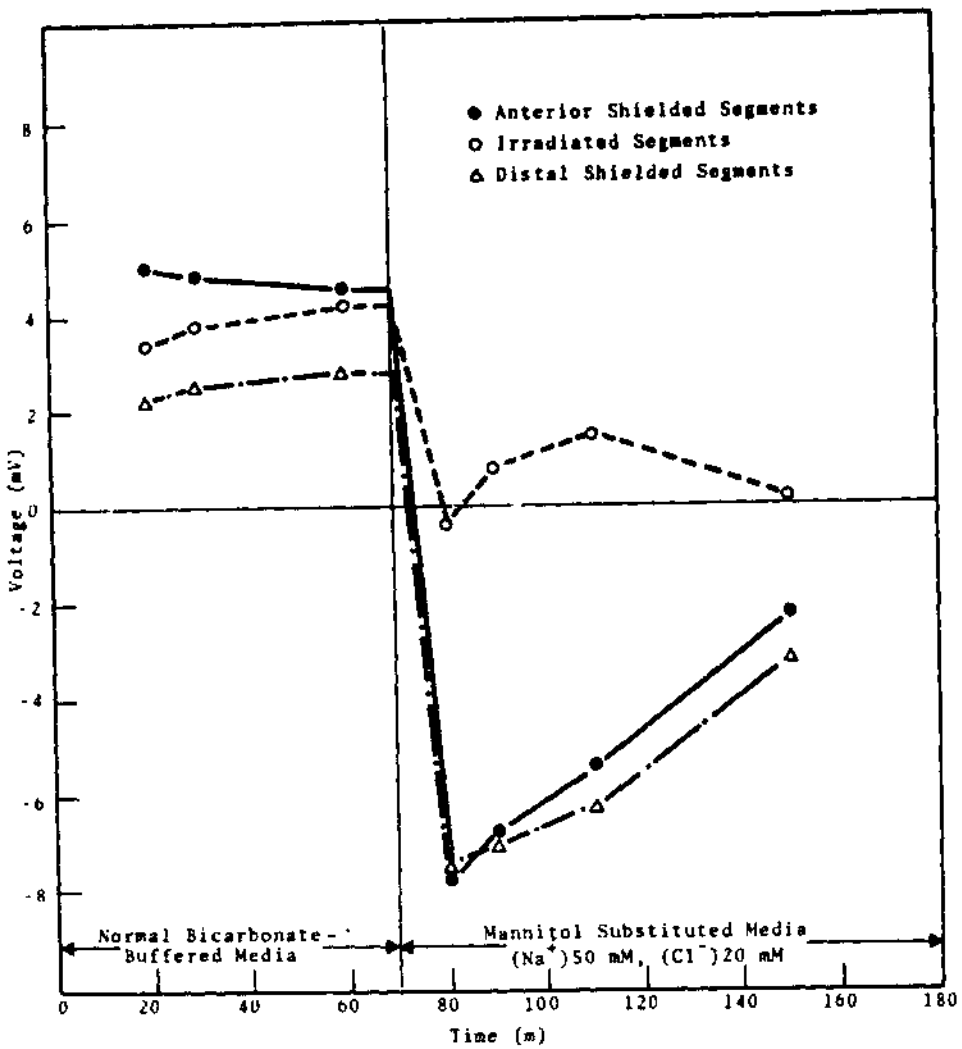

FIGURE 1. Voltage Differences Across Irradiated and Shielded Ileal Segments from Rats 3 Days Following 1500 R X-Irradiation (Each plot represents the average of data for adjacent irradiated or shielded segments from three rats.)

ever, there was an initial negative transient followed by a return to values only slightly lower than those observed in normal media.

Further studies demonstrated that elimination of glucose from the mucosal media caused appreciably larger negative voltages. This effect was observed upon lowering the sodium concentration, with either jejunal or ileal segments, and with either bicarbonate or inorganic phosphate-buffered media.

Chloride and Sodium Transport and X-Irradiation Effects

A series of experiments were performed to evaluate the possibility of a chloride transport mechanism. Chloride-36, ^{24}Na, ^{42}K and ^{3}HOH transport were studied simultaneously.

employing intestinal segments in normal bicarbonate or inorganic phosphate-buffered media; following selective reduction of the chloride concentration to 20 mM (a condition that did not appreciably alter the voltage differences); and following reduction of both the sodium (50 mM) and chloride (20 mM) concentrations so that the bioelectric potentials were either altered in polarity or greatly depressed.

The jejunum and ileum had very different transport characteristics for chloride. In normal media the values of K_{ms} for chloride were about 1/2 those for tritiated water, for both jejunal and ileal segments, and S/M ratios were close to 1.0. However, following selective reduction of the chloride concentration, K_{ms} for chloride, with ileal segments, increased in magnitude to values nearly twice those for tritiated water, and S/M ratios approached values of 2.0. In contrast, K_{ms} and S/M values for jejunal segments increased only slightly. Inclusion or elimination of bicarbonate in the media did not markedly affect chloride transport, and similar results were obtained with either normal or reduced sodium concentrations.

These results indicated that chloride is actively transported in the ileum. The possibility that solvent drag was responsible for the large S/M values for chloride is negated by the fact that simultaneous net water absorption was less than 1/5 the K_{ms} values for tritiated water. The existence of a chloride transport mechanism in the rat ileum is further supported by the large values of K_{ms} obtained for chloride relative to those for tritiated water.

For sodium, in normal media, values obtained for K_{ms} were 3/4 those for tritiated water and the values for S/M were near 1.0. Following reduction of the sodium concentration, the values of K_{ms} approached or slightly exceeded those for tritiated water and values for S/M were between 1.1 and 1.6, with the larger values usually obtained for ileal segments. The data from these experiments support those summarized by Curran,[2] indicating that sodium is actively absorbed. However, in the present experiments, the effects on sodium transport, observed upon reducing the sodium concentration or inclusion of bicarbonate in the media, could not be explained independently of corresponding effects on the voltage differences. The marked differences described between jejunal and ileal segments for chloride were not apparent for sodium.

Following X-irradiation, sodium and chloride transport characteristics by ileal segments were altered. Sodium and chloride transport by the shielded segments for which bioelectric potentials are shown in Figure 1 were similar to that described for nonirradiated intestine. However, in agreement with the altered bioelectric response, sodium and chloride transport by the irradiated segments were depressed.

Potassium and Cesium Transport

Potassium and cesium transport by the intestine were nearly identical and S/M values indicated that both of these electrolytes were secreted in the ileum and to a lesser extent in the jejunum. With normal media, potassium absorption

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permeabilities for jejunal and ileal segments were, respectively, only about 2/5 and 1/5 those for tritiated water.

Conclusions

The voltage difference across the intestine wall is clearly a complex function involving the combined transport mechanisms for sodium, chloride, potassium and bicarbonate. Mechanisms were demonstrated for the transport of chloride and potassium in the ileum and they may be present to a lesser degree in the jejunum. Cesium transport and accumulation were nearly identical to that of potassium. Bicarbonate effects upon the bioelectric and transport characteristics of isolated intestine were complex and require further detailed study.

Following irradiation, tric and transport charact the ileum are altered. Fu is required to correlate t with morphological alterat intestine. Such studies m help in elucidating the me irradiation damage, but ma bute to a better understar transport and bioelectric tics of the normal intesti

References

1. *Description of the app cedures used during th is being prepared for For more immediate ref J. R. McKenney. Diese vol. 26, p. 1131. 196.*

2. *P. F. Curran. Fed. Pr pp. 993-999. 1965.*

EFFECT OF BILE AND RADIATION ON INTESTINAL FLORA

Coliform bacterial counts are increased in the small intestine of rats by either bile duct cannulation or exposure to X rays. The influence of these factors on the bacterial flora appear to be additive.

Investigat
T. D. Mah
M. P. Sul

Technical
Alma L. C

The elimination of bile salts from the intestine has been shown to prevent diarrhea in the X-irradiated rat. Since the products of bacterial action on bile salts are particularly damaging to the mucosa, it was anticipated that bile might also influence the bacterial count and location within the lumen of the irradiated intestine.

Observations

At various times after bile duct cannulation and/or exposure of the abdomen of adult female rats to 1000 or 1500 R of X-irradiation, killed and the small inte into three equal parts. ples of the bacterial flo by mechanically shaking t ments in sterile saline. luted and cultured for 2 in McConkey's agar. Res from animals sacrificed bile duct cannulation an are shown in Figure 1. middle segment, which wo termediate between those the end segments, are om simplicity.

These data show that small intestine is almos

*Consultant Pathologist

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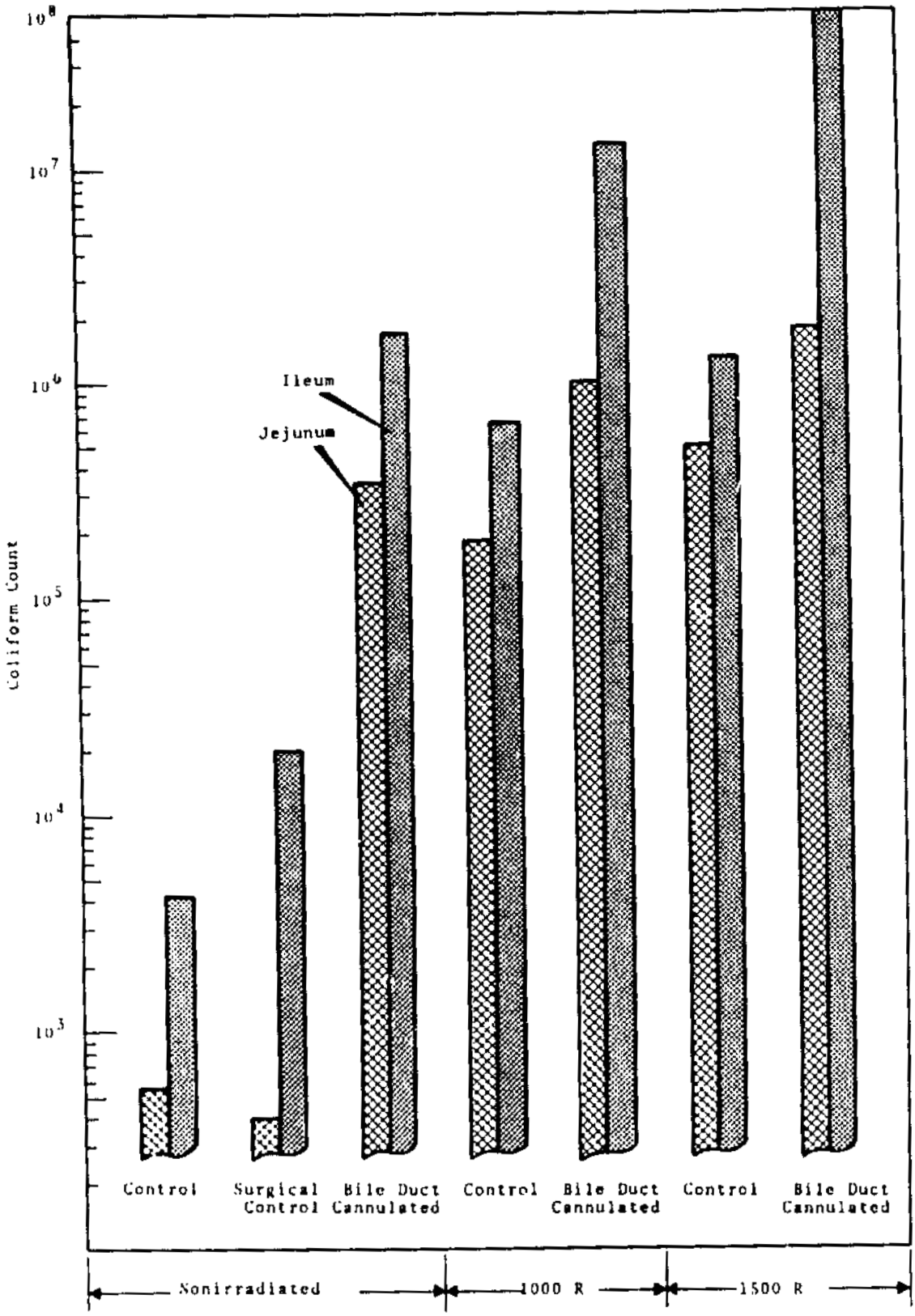

FIGURE 1. Coliform Counts in Rat Small Intestine 4 Days After Bile Duct Cannulation and/or Abdominal X-Irradiation

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form bacteria. Increased counts by orders of magnitude were observed in all parts of the intestine after either irradiation or bile duct cannulation. The highest counts were observed in animals that were both cannulated and irradiated.

Conclusions

It is apparent that the absence of bile promotes the movement of bacteria into the small intestine. Irradiation of the intestine is also followed by a proximal movement of the bacterial flow. It is not known whether this local effect of radiation is caused by decreased intestinal motility or by diminished phagocytic activity resulting from the exposure. The protective action of bile duct cannulation against intestinal radiation injury could be due to the absence of the degradation products of bile salts produced by bacteria which have invaded portions of the tract from which they are normally absent. Drug sterilization of the gut, however, has thus far been shown to have little effect on the intestinal radiation syndrome. Further study is necessary to clarify these inter-related phenomena.

ULTRASTRUCTURAL STUDY OF INTESTINAL RADIATION DAMAGE

The mucosa of bile duct cannulated, irradiated rats was compared with that from rats that were irradiated only. Ultrastructural differences were noted in the columnar cells that might indicate an action of bile and/or radiation on their secretory activity.

Investigators:
*T. D. Mahony**
J. D. Berlin
Technical Assistance:
R. R. Adee

The influence of bile salts on the intestinal radiation syndrome in the rat has been studied in this laboratory and effects on the histological appearance of the small intestine as observed by light microscopy have been reported (Annual Report, 1964). With the hope of obtaining insight as to the mechanism by which bile salts might influence radiation effects, an electronmicroscopic study was initiated. Only preliminary results from this study are available.

Observations

Rats which had received 1000 and 1500 R abdominal X-irradiation were sacrificed at intervals up to 6 days post-irradiation and sections of the intestine were fixed in glutaraldehyde-osmium tetroxide and embedded in Epon. Observations previously reported by others on the effects of intestinal irradiation were confirmed, but some differences were noted, perhaps due to the improved method of preparation. When the intestines of bile duct cannulated, irradiated rats were compared with those from rats that were irradiated only, the large goblet cells on the tips of the villi, noted by light microscopy, were confirmed in the bile fistula group; but additional qualitative differences were observed that will require further study. Changes were seen in the golgi apparatus of the columnar cells that might represent differences in their secretory function. A difference in the thickness of the

*Consultant Pathologist

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mucopolysaccharide layer in the striated border of the two groups was observed which might support this conclusion. This latter finding is illustrated in Figures 1 and 2.

Conclusions

Ultrastructural changes in the mucosa of bile duct cannulated and/or irradiated rats suggest that the bile may exert some influence on the secretory activity of the epithelial cells. These are very limited observations which will require extensive additional study for confirmation.

FIGURE 1. Normal Control Rat Ileum Showing the Striated Border of the Columnar Epithelial Cell

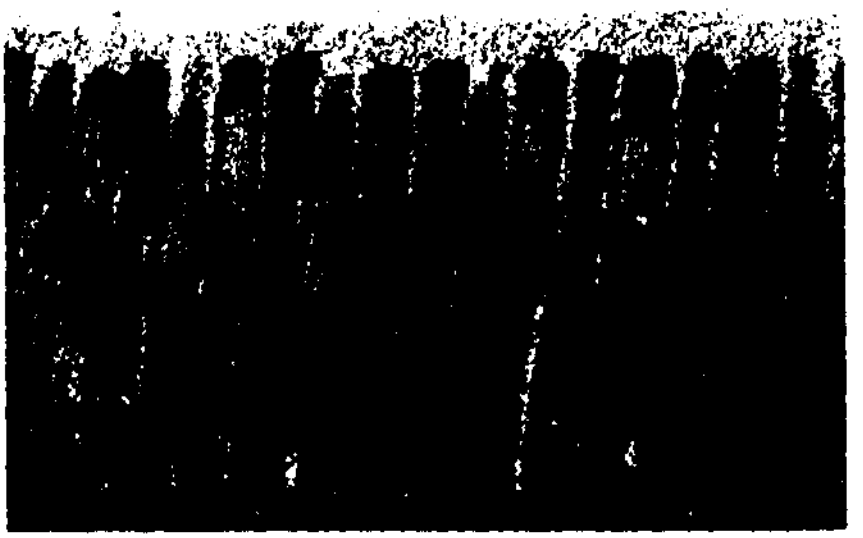

FIGURE 2. Ileum from a Bile Duct Cannulated Rat Showing a More Dense Mucus Layer Covering the Tips of the Villi and Microvilli

ALIMENTARY PASSAGE OF ALLYL INULIN

Iodine-131-labeled allyl inulin was tested as a possible inert, soluble food label and was found to be unstable in inorganic phosphate-buffered saline at pH values in the biological range. Qualitative differences in the passage rates of ^{131}I-allyl inulin and ^{144}Ce may be explained by differences in their physical and biological behavior.

Investigators:
J. R. McKenney
R. F. Keough

Inulin is widely used for glomerular filtration rate measurements and for measurements of the extracellular space in tissues. Because of chemical separation and counting problems with ^{14}C- or ^{3}H-labeled inulin, the potential of ^{131}I-allyl inulin as a nonabsorbed label in studies of gastrointestinal function was studied.

Observations

In preliminary stability tests the ^{131}I-allyl inulin solution was passed through an anion exchange resin (Dowex 1-X4) to remove inorganic iodide, transferred into phosphate buffer, adjusted to pH values of 6.2, 7.2, or 7.7, and stored in a CO_2 free atmosphere to minimize pH drift. Aliquots of the stored solutions were later passed through the resin to determine the degree of disassociation. After 3 hr of storage, 1, 10, and 40% of the ^{131}I had disassociated from the compound at pH values of 6.2, 7.2, and 7.7, respectively; and by 24 hr, the respective degrees of decomposition were 10, 40, and 60%.

Rats preconditioned on 10 g powdered food per day for 3 days were fasted overnight and given 2 g of rat chow that included a mixture of ^{144}Ce (initially chloride) and ^{131}I-allyl inulin. They were killed at intervals and the distribution of ^{144}Ce and ^{131}I determined along the intestinal tract. After 1 hr, 18% of the administered ^{144}Ce was in the ileum, compared to 32% of the ^{131}I. This difference persisted, and at 2 hr about 31% of the administered ^{131}I was in the cecum and colon while only 17% of the ^{144}Ce had reached that segment. After 7 hr appreciable decomposition of the administered ^{131}I-allyl inulin had occurred as indicated by a thyroidal uptake of 2.5% and as further indicated by the relatively large amounts of ^{131}I recovered from the proximal small intestine, presumably due to secretion of absorbed iodine.

Conclusions

It is clear that ^{144}Ce and ^{131}I-labeled allyl inulin do not move along the gastrointestinal tract at the same rate. Cerium probably reacted with the intestinal contents to form an insoluble compound while ^{131}I-allyl inulin, being quite soluble, may have moved in the liquid phase ahead of the undigested intestinal contents. This physical difference, in addition to the instability and biological behavior of the iodine label, must be responsible for the different rates of passage. These studies also showed that decomposition of ^{131}I-allyl inulin occurs at pH values in the biological range and suggest that similar difficulties with this compound may be encountered when it is used as an indicator of glomerular filtration rate.

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LATE EFFECTS OF SKIN IRRADIATION

Skin lesions produced by beta burns in sheep and swine 10 years ago have not changed in gross appearance during the past few years. A metastasising fibrosarcoma and keratoacanthoma developed in a 10-year-old sheep at skin sites exposed to 16,000 rads from a ^{32}P source 9 years ago

Investigators:
H. A. Ragan
W. J. Clarke
*L. K. Bustad**

A comparative study was initiated 10 years ago to determine the variation in the skin reaction of rabbits, swine, and sheep after acute exposure to doses of 2000, 8000, and 16,000 rads of beta irradiation.(1) The animals are being held for lifetime study with annual observations and descriptions made of the gross lesions. George, et al.(2) described a fibrosarcoma which occurred in a rabbit at one skin site exposed to 16,000 rads of ^{32}P.

Observations

With the exception of neoplasms occurring in a sheep at two skin sites the gross lesions of the animals still alive remain unchanged over the past several years. The late response in pig skin appears more severe than that observed in sheep skin. The loss of hair is more complete and with more cicatrix formation. In irradiated areas of sheep skin there may be some regrowth of a slightly finer-than-normal wool and considerable melanin deposition.

Tumor development occurred in a sheep at two sites exposed to 16,000 rads from a ^{32}P plaque applied 9 years previously. The pathologic diagnosis of one site was a fibrosarcoma, and of the second site, a keratoacanthoma. Two years ago both areas contained a densely cornified lesion approximately 3 cm in diameter with no evidence of ulceration. Prior to death, this year, the fibrosarcoma appeared as a massive, cauliflower-like growth approximately 10 cm in diameter with extensive surface necrosis. This was a pedunculated mass extending above the skin surface approximately 4 cm. The gross lesion at the second site was a cornified nodule which had not greatly changed from the appearance observed 2 years ago, and was not grossly suspected of being neoplastic. At necropsy the prescapular lymph node nearest the fibrosarcoma was greatly enlarged and the cut surface had a swirled appearance which resembled portions of the skin lesion. Other lymph nodes and tissues appeared normal with no evidence of metastases.

Histologically the fibrosarcoma consisted of interlaced bundles of moderately cellular fibrous connective tissue, not covered by epidermis and with surface necrosis. The tumor extended through skin layers and into subcutaneous tissue and musculature. There were highly vascular areas which in some cases appeared suggestive of hemangioma formation. In other areas there was a marked increase in the reticular fibers of the dermis. Adjacent to this was another fibrosarcoma which infiltrated along fascial planes. Blood vessels in this region showed a marked proliferation of the intima, media, and adventitial layers. The prescapular lymph node was greatly

*Present Address: Radiobiology Laboratory, University of California, Davis, California.

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enlarged with metastases of fibrosarcomatous tissue which resembled the primary tumor at this skin site. There was also extensive necrosis and hemorrhage present in a few residual lymph vessels having metastatic foci in their lumina. There was a moderate number of cells with mitotic figures visible in both the primary and metastatic tumor.

The keratoacanthoma consisted of hyperkeratosis with a whorled appearance surrounded by nests of infiltrating cells with epithelioid characteristics and also masses of lymphocytes and granulocytes. At the periphery there was a marked down-growth of hyperkeratotic tissue having whorled keratotic centers. These fibroblastic cells infiltrated well into the subcutis and musculature accompanied by masses of inflammatory cells.

The site on this sheep which was exposed to 2000 rads, except for a thinning of the epidermal layer and loss of the rete pegs, appeared relatively normal. The 8000-rad site progressed from normal tissue into areas of marked thinning of epidermal layers, disappearance of the rete pegs and a decrease in adnexal structures. Adjacent to this was an area of keratinized material and melanin pigments protruding above the surface.

Conclusions

Of the three species used in this skin study, tumor development occurred in the two (sheep and rabbit) which appeared the least sensitive to radiation. In swine the greater skin sensitivity to radiation may result in fewer cells being only partially damaged and thus having the potential of tumor development. If this is indeed the case, tumors may develop at sites exposed to 16,000 rads as the pigs become older. The period from exposure to gross neoplasia was approximately 4 years in the rabbit and 9 years in the sheep.

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CONGENITAL NEONATAL ANEMIA IN HANFORD MINIATURE SWINE

An apparent genetically related anemia has occurred in newborn Hanford miniature swine. This appears to be a macrocytic, hyperchromic anemia resulting from maturation arrest in precursors of the erythrocytic series.

Investigators:
B. A. Ragan
J. L. Palotay
W. J. Clarke

Technical Assistance:
J. L. Beamer
P. L. Sheldon

For the past several years this laboratory has been developing a breed of miniature swine (Hanford Miniatures) for use in biomedical research. To accentuate desired characteristics a fairly rigid inbreeding program has been followed. The only apparent genetically related problem thus far observed has been a severe anemia causing death of newborn pigs shortly after birth. The occurrence of this anemia has been unpredictable with sporadic incidence both within litters and among litters of various sows.

Observations

The affected pigs are extremely pale at birth with severely blanched mucous membranes. They are ambulatory but weak and usually die within 24 hours after birth. Ingestion of milk does not seem to be a factor since some anemic piglets have milk in the alimentary tract at death while others do not. The dams have shown no signs of illness and have had normal blood values. Their ration is complete for all nutrients known to be required by swine. The sows which have farrowed anemic litters have received the same pelleted ration as our other pigs and numerous different batches of feed have been used. This virtually eliminates the feed as a primary source of the problem, either from a deficiency or toxic contaminant basis. Clinical findings have not resembled isohemolytic anemia, Eperythrozoonosis, iron deficiency, or other described anemias of neonatal swine. This appears to be a macrocytic-hyperchromic anemia. Stained smears of peripheral blood from anemic pigs reveal some anisocytosis and polychromatophilic erythrocytes. The mean corpuscular volume and mean corpuscular hemoglobin are greater in anemic than control piglets. A comparison of the peripheral hemogram of anemic and normal animals shortly after birth is shown in Table I.

The usual findings at necropsy are a normal sized, pale piglet with severely blanched mucous membranes, very watery blood, and extremely pale musculature. The most constant finding has been a diffusely amber-colored liver without the mosaic pattern seen in fatty degeneration. The kidneys have been pale and often have fine, scattered petechiae in the subcapsular cortex. The bone marrow grossly has usually appeared hypoplastic although in some cases it is hyperplastic. Other tissues are generally normal appearing except for varying degrees of paleness. There has been no gross evidence of hemolysis or icterus.

Histopathologic examination confirms in most cases the necropsy findings. The liver has various changes from cloudy swelling to necrosis. The

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TABLE I. Comparison of Some Erythrocytic Values of Anemic and Control Hanford Miniature Swine (mean ± standard deviation)

Component	Anemic	Control
Packed cell volume, %	12.5 ± 5.5	35.1 ± 4.1
Hemoglobin, g/100 ml	4.1 ± 1.7	12 ± 1
Erythrocytes, $10^6/mm^3$	2 ± 0.8	6 ± 0.6
Cell volume index[a]	1.09	1.00
Color index[b]	1.05	1.00
Mean corpuscular volume μ^3	63.1[c]	57.8[c]
Mean corpuscular hemoglobin, $\mu\mu g$	20.6[d]	19.7[d]
Mean corpuscular hemoglobin concentration, %	32.6	34.1

(a) *Cell volume index* = $\frac{PCV/normal\ PCV}{RBC/normal\ RBC}$.

(b) *Color index* = $\frac{Hb/normal\ Hb}{RBC/normal\ RBC}$.

(c) *Significant difference in values* ($P < 0.01$).

(d) *Significant difference in values* ($P < 0.05$).

same pathologic conditions are seen in the kidney but have been less severe. The splenic lesions usually consist of a deficiency of both red and white pulp with the red pulp more severely involved. In none of the soft tissues has excess hemosiderin been present, confirming the necropsy observation that this is not a hemolytic anemia. The most striking histopathologic findings have involved the bone marrow. Biopsy specimens reveal normal structural development of bone. Both biopsy and impression smears of the marrow indicate a normal progression of activity and quantity of the lymphocytic and granulocytic series. In the erythrocytic series, however, there is an apparent maturation arrest as evidenced by a severe paucity of erythrocytes and a predominance of erythrocyte precursors. Many cells in the megaloblastic series show abnormal forms.

Conclusions

These preliminary observations seem to indicate a genetic origin of the disease. The time of death and the bone marrow dyscrasia indicate a disorder originating near term. This could well be an inherited deletion or inability to utilize some essential erythrocyte maturing factor. In type, this condition appears to most closely resemble megaloblastic anemia seen in human infants, which has familial tendencies, although the maturation arrest may be occurring at a more primitive stem cell level.

Our current breeding program is designed to effect matings with the highest probability of producing this anemia. We hope to be able to farrow anemic pigs on a relatively predictable basis. Future studies will include karyotyping for abnormal chromosome types, electrophoretic and immuno-

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phoretic patterns, radioisotope uptake and distribution of trace elements concerned with erythropoiesis, and intensive examination of bone marrow and peripheral blood components.

COCARCINOGENIC EFFECTS OF CHEMICALS AND RADIATION

Female Charles River CD rats were more susceptible to induction of liver tumors by DAB than were males. The injection of ^{144}Ce-^{144}Pr appeared to increase the susceptibility of the female to DAB carcinogenesis.

Investigator:
D. D. Mahlum
Technical Assistance:
Joan O. Hess

Rumsfeld et al.[1] reported that male rats were more susceptible to the induction of liver tumors by azo dyes than were females. Experiments in our laboratory on the effects on the internal emitter, ^{144}Ce-^{144}Pr, on tumor production by dimethylaminoazobenzene (DAB) suggested that males were less susceptible than females. Consequently, an experiment was designed to determine the sex response and the effect of ^{144}Ce-^{144}Pr on liver tumor induction by DAB.

Observations

Both sexes of Charles River CD rats were fed a purified casein-sucrose diet containing 0.09% DAB. Subgroups of each sex were injected with 20 μCi ^{144}Ce-^{144}Pr either 2 or 4 weeks following initiation of DAB feeding. All females and about one-half of the males were killed after 6 months of DAB feeding. The remaining males were killed at 9 months. Livers were examined grossly for the presence of tumors. Tissue samples of full-blown tumors and suspect areas were fixed for histologic examination.

Liver tumor incidence was very low in the males killed after 6 months on the DAB diet (Table I). Complete data for the 9-month males are not yet available. On the other hand, females showed a 39% incidence of liver tumors after 6 months on DAB. Females that received ^{144}Ce-^{144}Pr after 2 weeks on DAB feeding showed a 62% incidence and those receiving the radionuclide at 4 weeks had a 46% incidence. No tumors were found in rats that were injected with 20 μCi ^{144}Ce-^{144}Pr but fed control diet that did not contain DAB.

TABLE I. Influence of Sex and ^{144}Ce-^{144}Pr on Induction of Liver Tumors by DAB

Treatment	Time of ^{144}Ce Injection, Weeks	Incidence After 6 Months DAB Feeding	
		Male	Female
DAB	- -	0/15	12/31
DAB + ^{144}Ce-^{144}Pr	2	1/15	18/29
DAB + ^{144}Ce-^{144}Pr	4	0/15	13/28

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Conclusions

It is apparent that Charles River CD males exhibit l .s susceptibility to DAB carcinogenesis than females. Under the conditions of this experiment, ^{144}Ce-^{144}Pr acted synergestically with DAB to increase liver tumor incidence in the females.

References

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HEPATOCARCINOGENS AND RADIONUCLIDE METABOLISM

Dietary administration of certain hepatocarcinogens to rats altered the metabolism of injected ^{144}Ce, ^{239}Pu, and ^{59}Fe. The changes produced varied with carcinogen and radionuclide used.

Investigator:
D. D. Mahlum

Technical Assistance:
Joan O. Hess

Dietary administration of dimethylaminoazobenzene (DAB), N-2-fluorenyldiacetamide (DAF), and ethionine to rats resulted in decreased clearance of cerium and plutonium from the liver (Annual Report, 1964). In the experiments reported here, carcinogens were fed for varying periods of time before ^{144}Ce, ^{239}Pu, or ^{59}Fe were injected intravenously. Tissues were analyzed for radioactivity 24 hr postinjection to determine the effect of chemical carcinogens on the distribution of these radionuclides.

Observations

DAB was fed at a level of 0.06% for 270 days prior to radionuclide injection. Ethionine was fed at a level of 0.3% and DAF at a level of 0.025% for 21 days prior to radionuclide injection. Each treatment group consisted of from three to nine male rats.

Liver uptake of ^{144}Ce was inhibited by both DAF and ethionine (Figure 1), while kidney, spleen, and femur levels increased. DAB had relatively little effect on the tissue distribution of ^{144}Ce.

Plutonium-239 in ethionine-fed rats showed an increased uptake in liver, kidney, and spleen, but a slightly decreased femur level (Figure 2). A similar effect was observed when ^{59}Fe was

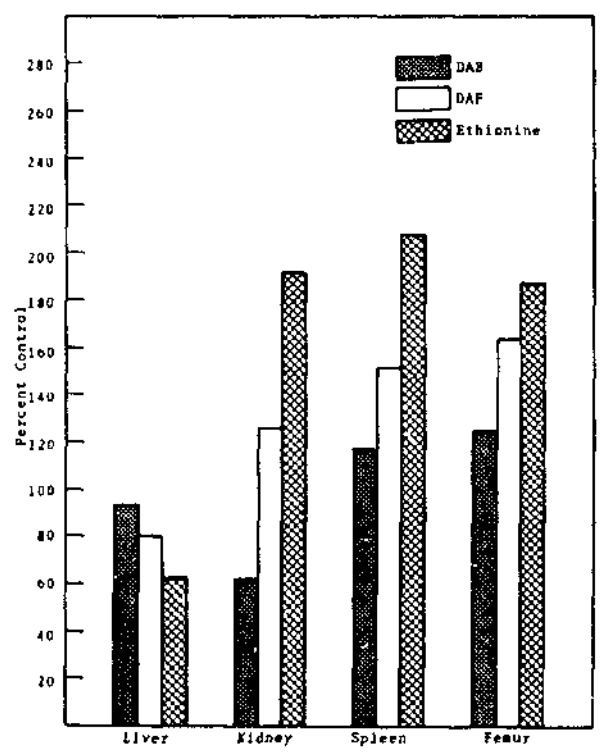

FIGURE 1. Effect of Dietary Carcinogens on Tissue Uptake of ^{144}Ce

given to ethionine-fed animals (Figure 3). DAF, on the other hand, had little effect on ^{59}Fe levels in the liver but kidney and spleen values were decreased and femur levels increased.

Conclusions

The distribution of an element may be markedly altered by dietary administration of certain well-known hepatocarcinogens, particularly, ethionine and DAF. This may be due to a decreased uptake by an organ normally responsible for its removal from the blood. This decrease could result in an increased retention time by the blood allowing distribution to other tissues. Clearance studies have shown that DAF and ethionine both increased blood retention time of ^{144}Ce, which is in keeping with this hypothesis.

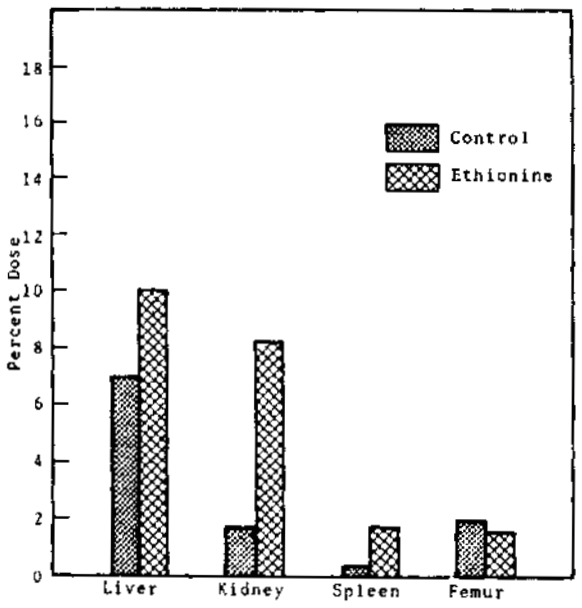

FIGURE 2. Effect of Dietary Ethionine on Tissue Uptake of ^{239}Pu

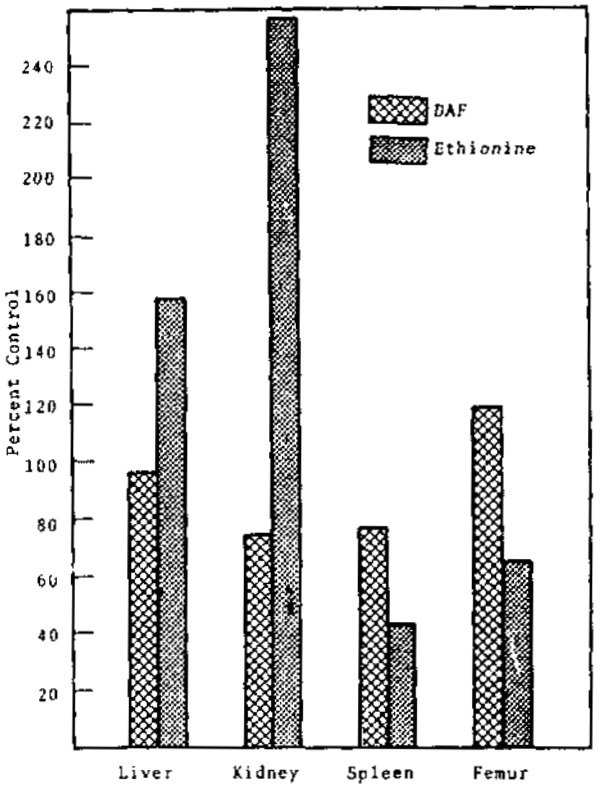

FIGURE 3. Effect of Dietary Carcinogens on Tissue Uptake of ^{59}Fe

RADIATION AND DDT EFFECTS IN FLOUR BEETLES

Modification of fitness parameters of homogenic populations singly or doubly stressed with X-ray and DDT were specific for species and strains of flour beetles.

Investigator:
H. E. Erdman

Technical Assistance:
Ethel H. Jaschek

Insecticides and ionizing radiations can induce population changes which under selection are beneficial or harmful. Productivity modifications due to radiation plus insecticides have important applications in economic entomology, evolution, and radiation ecology. Such changes are known in some

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cases to have a genetic basis. Studies were designed to determine the early effects of DDT and X-radiation, singly and in combination, on mortality and productivity of flour beetle species and strains (Tribolium confusum Duval "Chicago Standard" and T. castaneum Herbst "Brazil cl" and "Sooty").

Observations

Five replicate populations, each containing ten pairs of virgin beetles X-rayed with 0, 1, 2, and 4 kR of X-rays (250 kVp, 30 mA, 0.25 mm Cu + 1.0 mm Al filtration, 0.86 mm Cu HVL and 2.5 in. between target and subjects produced 1 kR/min), were established in food containing 0, 5, 10, 20, and 50 ppm DDT. Incubation was at 32 °C and 70 to 75% relative humidity. Every 2 weeks for 6 weeks parental mortality was recorded and living adults were placed on fresh comparable food. The old food was reincubated and F_1 adult biomass and numbers were the measures of productivity. Viability calculated as proportions of F_1 controls were considered as genetic recessives; whereas, lethality proportions (one minus viability value) were considered genetic dominants. Expected lethality, for example, the sum of homozygous and heterozygous lethal combinations from a cross in which one population was given 2 kR and the other population was given 10 ppm DDT in their food, was compared with the observed lethality in populations given 2 kR, then cultured in 10 ppm DDT spiked food.

Parental mortality of Chicago Standard or Sooty was not altered by 0 to 4 kR X-ray or 0 to 50 ppm DDT levels. The 50 ppm of DDT increased numbers of

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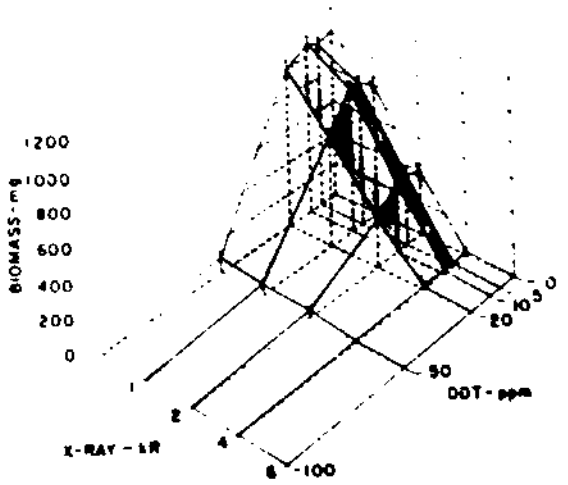
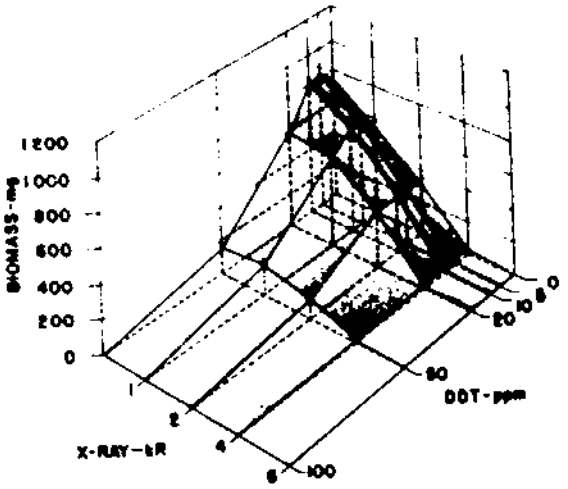
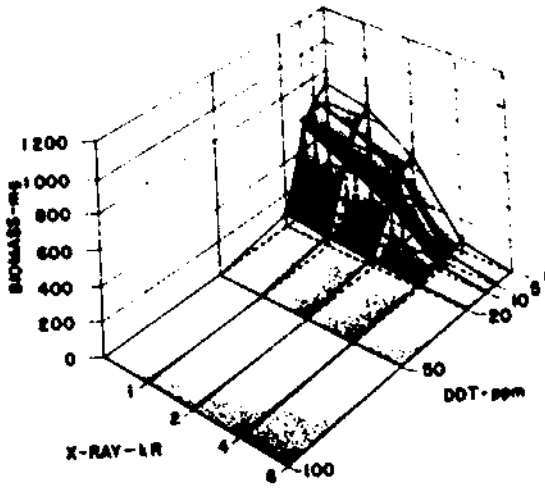


T. CONFUSUM DUVAL
"CHICAGO STANDARD"



T. CASTANEUM HERBST
"SOOTY"



T. CASTANEUM HERBST
"BRAZIL cI"

FIGURE 1. Effect of X Ray and/or DDT on F_1 Biomass

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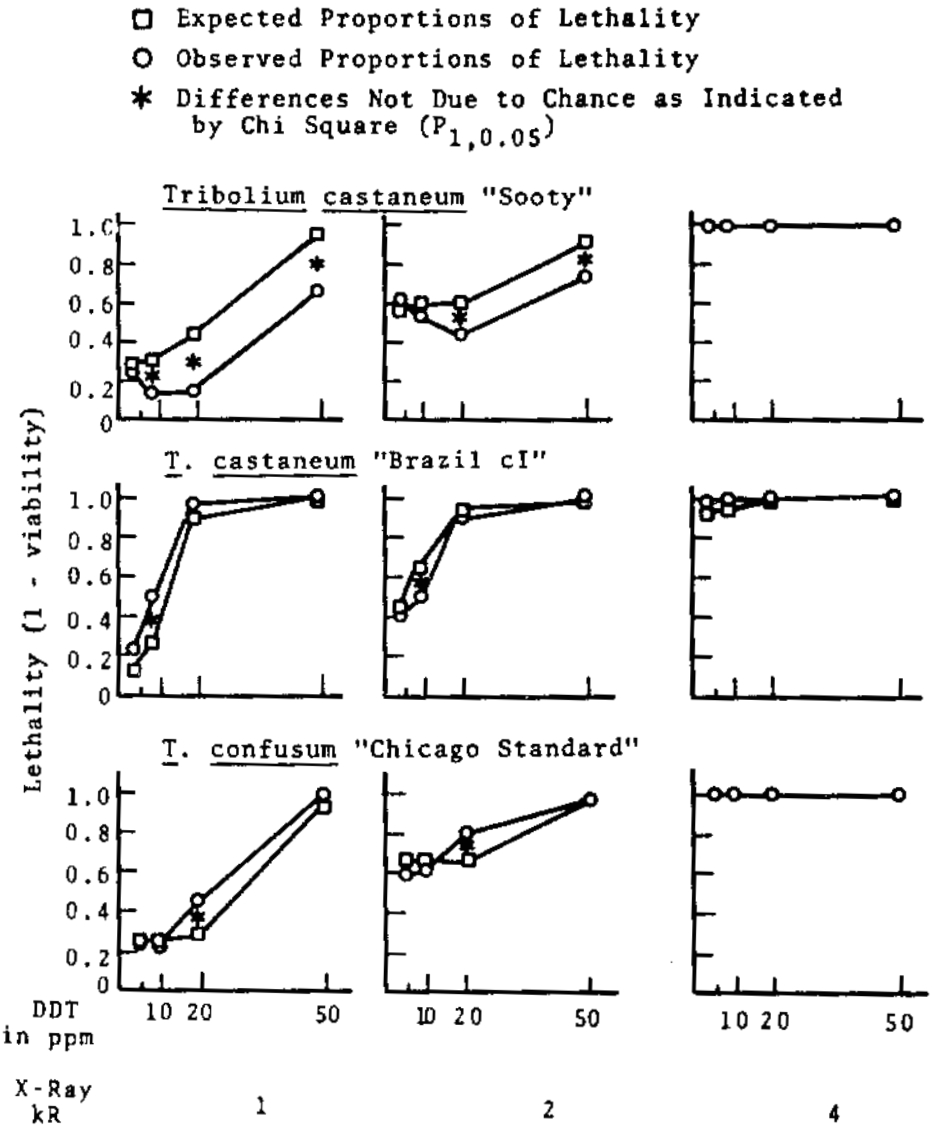

FIGURE 2. Expected and Observed Effect of Combined X Ray and DDT Treatment on F_1 Lethality

Morphological, physiological, or behavioral adaptations might explain the responses of these flour beetles to stress from insecticide. Future work will determine the responses of similarly treated hybrids between Sooty and Brazil cI. Perhaps this will suggest a genetic basis for the physiological detoxification mechanism(s) resulting in greater productivity of Sooty compared to that of Brazil cI.

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TOXICITY OF RADIOELEMENTS

This budget category includes the majority of all research conducted in the Biology Department. For this reason we are grouping for separate attention the reports concerned with inhalation of radioelements. Several reports on the toxicity and metabolism of radioelements in aquatic organisms are separately grouped with related environmental radiation studies.

Of the programs remaining for consideration in this section, the largest is that concerned with the effects of chronically fed radiostrontium in miniature swine. This long-term study, initiated in 1958, has reached the point where many effects, some of them unexpected, are now being observed. The high incidence of leukemia in some of these animals offers exciting opportunities for basic studies on leukemogenesis. The next several years should be the "pay-off" years in this program into which so much has been invested.

As new programs take the center of the stage others must retire. This year, for the first time in many years, there is no report on chronic feeding of radioiodine to sheep. A few animals remain in this life-time study but they now constitute a very minor aspect of our large animal effort.

A number of reports are concerned with the metabolism and toxicity of a variety of radionuclides in both large and small animals. Noteworthy among these are three reports relating to the evaluation of the hazards from nuclear reactors and radionuclide power sources (SNAP devices) in space. These are all concerned with the biological fate and effect of relatively large and insoluble radioactive particles. A fourth report, on the inhalation of large particles, is grouped with other inhalation studies.

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

Studies continue into the biological effects of daily ingestion of ^{90}Sr by miniature swine fed 1 to 3100 μCi/Day. Currently 281 animals are alive and being maintained for lifetime observation and study. In animals over 2 years of age, two cases of neoplasia of hematopoietic tissue have been observed in the 25 μCi/Day group, and 15 in the 125 μCi/Day group. Fifteen cases of hematopoietic neoplasms or extramedullary myelopoiesis have been diagnosed in F_1 generation of offspring less than 6 months old in the 625 μCi/Day group.

Investigators:
J. L. Palotay
H. A. Ragan
W. J. Clarke
Glenda S. Vogt
J. L. Beamer
Technical Assistance:
P. L. Sheldon

Previous progress reports have described the experimental design and the biological effects of daily ingestion of ^{90}Sr fed to miniature swine at levels of 0, 1, 5, 25, 125, 625, or 3100 μCi/day (Annual Reports 1959 et seq.). To summarize briefly, at 3100 and 625 μCi/day, the animals developed a severe neutropenia and thrombocytopenia and died after about 3 or 9 months, respectively, on ^{90}Sr feeding. At 125 μCi/day a moderate neutropenia and thrombocytopenia developed until near death at 3 to 4 years when the decline in erythroid elements became precipitous. At 25, 5 and 1 μCi ^{90}Sr/day no definitive changes were seen in the peripheral blood, with the exception of a possible slight reduction in platelet count in the 25 μCi/day groups after several years of ingesting ^{90}Sr. Some of the original animals fed ^{90}Sr starting at 9 months of age are now 8 years old, and their offspring, exposed to ^{90}Sr from conception, are over 6 years old.

Observations

Table I summarizes the number of animals at each of the ^{90}Sr feeding levels. This report will be largely devoted to a description of the hematopoietic tissue neoplasms and myeloproliferative lesions. Table II summarizes the cases of hematopoietic

TABLE I. Number of Animals at Each ^{90}Sr Feeding Level (Number alive and on experiment, 12/31/65, shown in parentheses)

Feeding level, μCi/day	Original generation	Offspring (F_1 and F_2)	Total
3100	5 (0)	-- --	5 (0)
625	6 (0)	38 (3)[a]	44 (3)
125	12 (9)	72 (14)	84 (23)
25	19 (11)	165 (31)	184 (42)
5	10 (5)[a]	110 (24)	120 (29)
1	24 (20)[a]	277 (51)	301 (71)
0	55 (45)	307 (68)	360 (113)
		Total	1098 (281)

[a] Alive but removed from ^{90}Sr feeding.

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TABLE II. Leukoproliferative Disorders in Miniature Swine Chronically Ingesting ^{90}Sr

Animal[a]	^{90}Sr, uCi/day	Age at death, months	Maximum WBC, $10^3/mm^3$	Evidence of neoplasia at necropsy	Pathologic diagnosis
1	1	72	8	+	Reticulum cell sarcoma
2	25(b)	52	33	+	Granulocytic leukemia
3	25(b)	50	48	+	Granulocytic leukemia
4	125(b)	73	51	+	Granulocytic leukemia
5	125	37	2	+	Reticulum cell sarcoma
6	125	36	10	+	Lymphosarcoma
7	125	48	70	+	Granulocytic leukemia
8	125	28	4	±	Leukosarcoma, localized
9	125	31	274	+	Granulocytic leukemia
10	125	37	172	+	Granulocytic leukemia
11	125	37	80	+	Lymphocytic leukemia
12	125	35	249	+	Granulocytic leukemia
13	125	29	---	±	Reticulum cell sarcoma
14	125	31	76	+	Granulocytic leukemia
15	125	26	52	-	Lymphocytic leukemia
16	125	28	42	+	Lymphocytic leukemia
17	125	33	3	+	Leukosarcoma, localized
18	125	28	39	+	Granulocytic leukemia
19	625	28	107	+	Granulocytic leukemia
20	625(b)	17	1	±	Leukosarcoma, localized
21	625	3.5	5	±	Leukosarcoma, localized
22	625	3.2	5	±	Leukosarcoma, localized
23	625	3.2	2	±	Leukosarcoma, localized
24	625	3.5	6	±	Leukosarcoma, localized
25	625	3.3	3	±	Reticulum cell sarcoma
26	625	3.5	4	±	Leukosarcoma, localized
27	625	3.5	8	±	Leukosarcoma, localized
28-35	625	3.5	<10	±	Ectopic myelopoiesis

(a) *Estimated average total radiation dose to hematopoietic tissue:*

Animals 2- 3 = 2,000 rad.
Animals 4-18 = 12-15,000 rad.
Animals 21-35 = 5,000 rad.

(b) *Started ingesting ^{90}Sr at 9 months of age--for all others exposure started in utero.*

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tissue neoplasms or ectopic myelopoiesis observed at the 1, 25, 125, or 625 μCi ^{90}Sr/day levels.

Animal number 1 (Table II) was exposed to ^{90}Sr in utero via milk during the suckling period, and then received 1 Ci ^{90}Sr daily for her lifetime. Since other animals at the 1 μCi/day level have exhibited no aberrations in the peripheral blood, nor lesions in other tissues, this case probably represents a naturally occurring neoplasm unconnected with ^{90}Sr exposure. At necropsy, milliary, discrete foci of neoplastic cells were spread throughout most organs. These foci were raised, white, and usually 1 to 3 cm in diameter as opposed to the foci seen in the higher dose level lesions which were usually not raised above the surface, were reddish-grey, and usually 0.1 to 1 cm in diameter.

The clinical course in all animals over 2 years of age has been relatively acute, with an average survival time from diagnosis until death of about 2 weeks. In a few cases the first indication of impending leukemia was obtained from the routine quarterly blood sampling, and consisted of a slight increase in the white blood cell count (WBC) with some abnormal leukocytes, and a drop in erythrocyte and platelet numbers. Usually, however, lethargy and partial anorexia were the first signs of illness. In cases of granulocytic or lymphocytic leukemia examination of peripheral blood during the initial illness revealed a leukocytosis with immature and abnormal leukocytes present. As the WBC increased, anemia and thrombocytopenia became more severe. In two cases, during the initial leukocytosis, there was the usual platelet drop. As the WBC increase continued there occurred a concomitant rise in platelet count above normal level. This has been observed at times in human cases of chronic granulocytic leukemia where platelet counts may increase to more than 10 times the normal value. In most of our animals the radiation-induced thrombocytopenia probably overshadows any tendency for thrombocytosis.

When the packed red cell volume approached 12%, or other signs of impending death were evident, the animal was euthanatized. The most common gross lesions observed in animals with granulocytic or lymphocytic leukemia were enlargement of the lymph nodes and spleen. Extravasation of blood was not a consistent lesion, but was often present in varying degrees. The splenic enlargement and appearance was a particularly striking and pathognomonic lesion, and was consistent in all cases of granulocytic but not lymphocytic leukemia. Spleen weights ranged from 190 to 790 g compared to control weights of 100 to 150 g. The cut surface bulged and the normal architecture was completely disrupted, having a mushy appearance from an apparent overgrowth of parenchymal tissue and poorly defined trabeculation. Greyish foci varying from 1 to 10 mm in diameter were commonly observed in the kidney and liver and frequently were found in other organs. Bone marrow was hypoplastic in appearance, due in most cases to radiation damage but also influenced by an infiltration of neoplastic tissue which was observed in

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about one-third of the cases. A severe, progressive icterus developed in one animal and was found at necropsy to be caused by biliary stasis from greatly enlarged hepatic lymph nodes. This node chain has been more severely invaded by neoplastic cells than any other lymphatic tissue. The interlobular septa of the liver often were very prominent, enlarged, and infiltrated with cells, giving a distinct mosaic appearance to the organ.

Neither the peripheral blood changes nor the gross lesions at necropsy were as distinct nor constant in cases of localized leukosarcoma or reticulum cell sarcoma. Lesions were usually confined to a few small greyish or reddish-grey foci in the various organs, and to lymph nodes which had a greyish cast and apparent neoplastic overgrowth of cells.

Deaths in the animals less than 6 months of age in the 625 μCi/day group were usually not suspected as being due to a neoplastic process but were attributed to radiation-induced anemia and thrombocytopenia. However, at necropsy there commonly were observed some discrete foci as described for the older animals. There was no instance of splenic enlargement in this group.

The histologic appearance confirmed the gross necropsy findings. Accumulations of specific neoplastic cells were found in the lymph nodes, spleen, and interlobular connective tissue of the liver, as well as in the discrete foci often present in other organs. The architecture of the spleen was difficult to discern in most cases, with marked infiltration of the splenic pulp with neoplastic cells. The leukemic cell overgrowth became so severe in some cases that practically no normal structure remained. The vasculature of many major organs was sometimes filled with the invading cells.

Classification of these neoplastic leukoproliferative lesions was based on the peripheral blood and on histologic examination of the tissue. When the WBC was elevated, the classification was made as granulocytic or lymphocytic leukemia depending on the predominant cell type. If the WBC was normal or depressed, the disorder was classed as leukosarcoma localized, or reticulum cell sarcoma depending on the cell type histologically.

Classification of the myeloproliferative lesions observed in the young F_1 animals from the 625 μCi/day group was particularly difficult and perplexing. A classification of neoplasia was made on the basis of distinct immaturity of the predominant cell and the tendency to invade surrounding tissue. However, the non-neoplastic foci of ectopic myelopoiesis did not occur in areas usually observed as extramedullary hematopoietic centers in young animals (liver or spleen), but in kidney, cardiac muscle, testes, adrenal, thyroid, and other unusual sites. The reasons for the shift to these locations is not understood. It may be that these foci are pre-neoplastic lesions and the animals die from radiation damage before the true neoplastic character of the cells has become obvious. Additional histochemical stains may help resolve this problem.

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Conclusions

The continued failure to observe bone tumors after up to 8 years chronic exposure to ^{90}Sr remains a striking feature of this experiment, as contrasted to other large animal studies in progress elsewhere. This feature was extensively discussed in our previous progress report (Annual Report, 1964). The markedly increased incidence of hematopoietic tissue neoplasms observed during the past year would seem to offer exciting possibilities for basic studies on leukemogenesis in a large animal. These possibilities we hope to exploit in parallel with the continuing lifetime observation of the swine presently on experiment.

STRONTIUM AND CALCIUM EXCRETION IN THE RAT

Investigators:
Patricia L. Hackett
R. C. Thompson

Following the intraperitoneal injection of ^{45}Ca and ^{85}Sr to mature rats on a high calcium diet, the ratio of urinary/fecal excretion of both radionuclides decreased with time postinjection. This effect was shown to be due to an increase in excretory clearance via the intestine.

Several years ago we studied the urinary and fecal excretion of ^{90}Sr and ^{45}Ca in the rat, following a single injection, as a function of age and dietary calcium level (Annual Report, 1959). The results were in general agreement with conclusions previously reached from retention studies involving serial sacrifice and femur analysis. One peculiarity in the data, however, attracted our attention. The ratio of radionuclide excretion in the urine as compared to

diet containing 2% calcium and 0.5% phosphorus. After 5 days on this diet, half of the animals (Group I) were injected, intraperitoneally, with ^{85}Sr (30 μCi) and ^{45}Ca (60 μCi). The remaining 14 animals (Group II) received an injection of saline. Forty days later the Group II animals were injected with the radionuclides and Group I animals were sham-injected. Seven days after the second injection all animals were sacrificed and plasma samples obtained. Following radio-

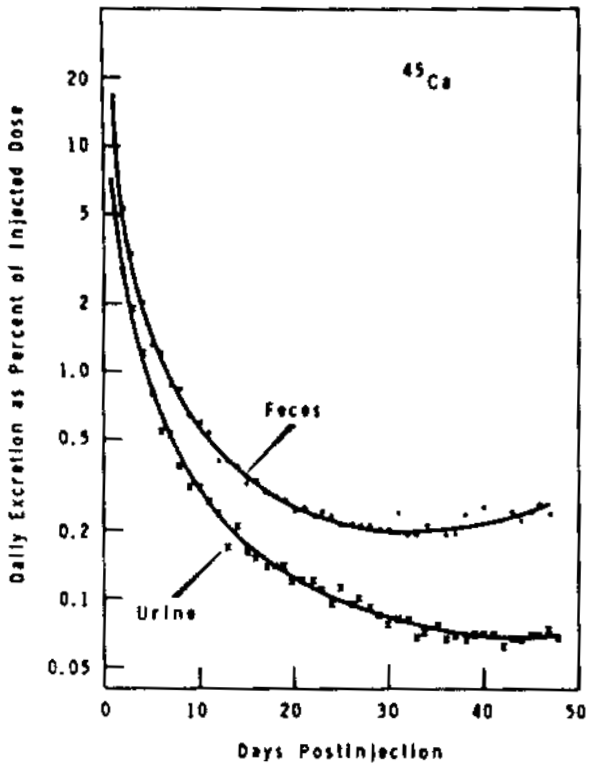

FIGURE 1. Excretion of ^{45}Ca in Urine and Feces

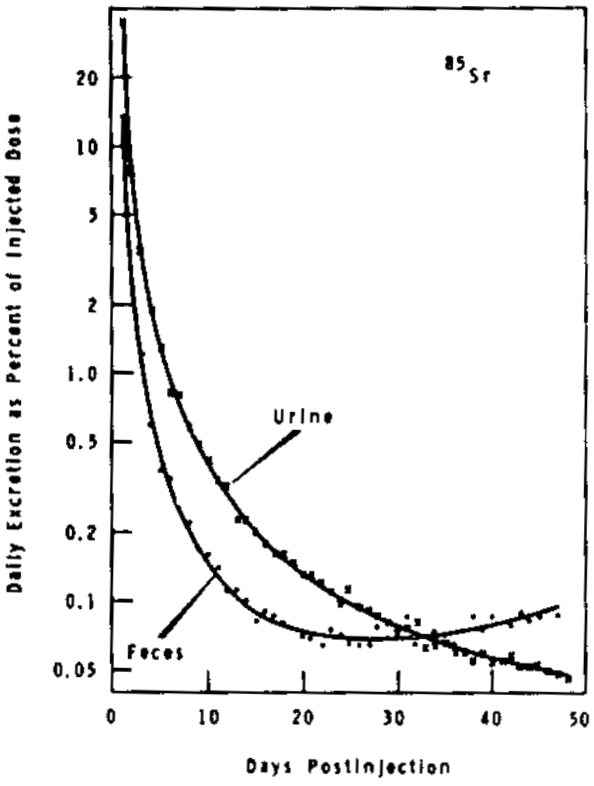

FIGURE 2. Excretion of ^{85}Sr in Urine and Feces

The ^{85}Sr excretion curves are shown in Figure 2. Again there was a statistically significant and sustained increase in fecal excretion beyond about the 25th day after injection. By the end of the experiment fecal excretion of ^{85}Sr substantially exceeded urinary excretion.

The ratio of urinary to fecal excretion is plotted in Figure 3 as a function of time postinjection. Over the 47-day period there is an approximately sixfold decrease in this ratio for ^{85}Sr, and an approximately twofold decrease for ^{45}Ca. Also shown in Figure 3 are the urinary/fecal excretion ratios for the Group II animals which received radionuclide injections 40 days after the Group I animals. These ratios correspond

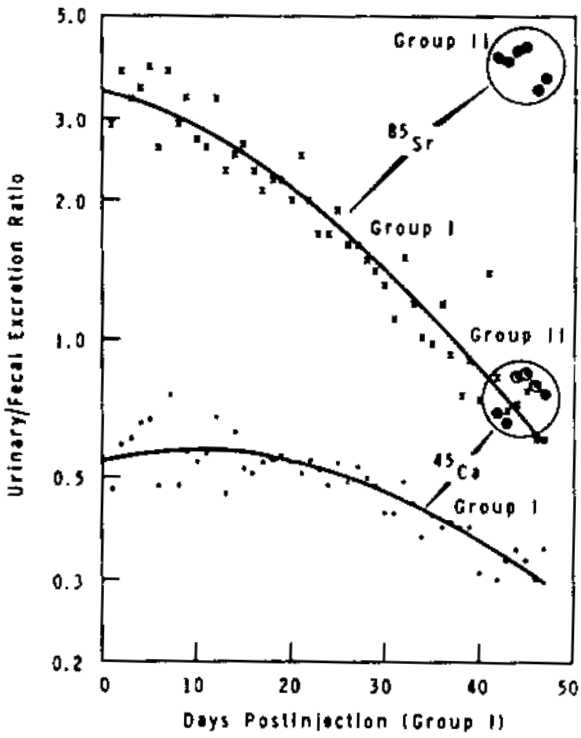

FIGURE 3. Urinary/Fecal Excretion Ratios for ^{45}Ca and ^{85}Sr

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quite closely between the two groups when compared at comparable times postinjection but are very different when compared at the time of measurement. This indicates that the change in urinary/fecal excretion ratio is a function of residence time of the radionuclide in the animal and not related to age, time on diet, or other factors common to both groups.

The plasma clearance data summarized in Table 1 sheds additional light on the situation. For both ^{45}Ca and ^{85}Sr, renal clearance values did not change appreciably between 7 and 47 days postinjection. Clearance via the intestine, however, increases approximately twofold for ^{45}Ca, and approximately fivefold for ^{85}Sr. It seems evident, therefore, that the anomolous behavior of the urinary/fecal excretion ratio must be attributed to changes in the denominator of this fraction. Both the changing intestinal clearance and the peculiar upturn to the fecal excretion curves point in this direction.

Conclusions

Two possible explanations might be offered for these observations. Either (1) the form of calcium or strontium released to the blood from bone may change, with time following deposition, to one more readily excreted via the intestine (or less readily reabsorbed from the intestine), or (2) the mechanism of intestinal excretion may be altered as a result of the deposition of ^{85}Sr and ^{45}Ca in bone. The latter alternative would involve the assumption of radiation damage; and, although the radionuclide levels employed might conceivably have caused significant damage to the bone, it is difficult to see how such damage could affect the processes of intestinal excretion of calcium and strontium. The possibility of a

TABLE 1. Clearance Data

		After 7 days deposition (Group II)	After 47 days deposition (Group I)
Calcium-45			
Circulating in plasma	Expressed as % of current body burden ± standard deviation	0.51 ± 0.11	0.084 ± 0.022
Excreted/day in urine		1.1 ± 0.4	0.17 ± 0.07
Excreted/day in feces		2.2 ± 0.4	0.74 ± 0.28
Renal clearance	Expressed as plasma vols/day ± standard deviation	2.3 ± 0.9	2.2 ± 1.1
Intestinal clearance		4.3 ± 1.1	9.1 ± 3.4
Strontium-85			
Circulating in plasma	Expressed as % of current body burden ± standard deviation	0.20 ± 0.04	0.018 ± 0.007
Excreted/day in urine		3.2 ± 0.5	0.22 ± 0.05
Excreted/day in feces		1.3 ± 0.4	0.49 ± 0.19
Renal clearance	Expressed as plasma vols/day ± standard deviation	15 ± 3	13 ± 6
Intestinal clearance		6.6 ± 1.2	30 ± 19

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changing form of calcium and strontium in the blood seems more attractive. Early after injection blood ^{45}Ca and ^{85}Sr is derived principally from freely exchangeable sites on bone surfaces. With the passage of time, however, it is, to an increasing extent, derived from firm binding sites in bone that are unearthed by irreversible bone resorption processes. It is conceivable that calcium and strontium released from such firm binding sites may exist in a different chemical form, more subject to excretion via the intestinal route.

RADIOSELENIUM STUDIES IN SHEEP

Selenious acid, labeled with ^{75}Se, in water or oil suspension was subcutaneously injected into sheep fed either high or low selenium rations. The high selenium ration favored early rapid elimination. Injection as an oil suspension doubled the retention half time of the long-term component.

Investigators:
C. R. Watson
H. A. Ragan
Beatrice J. McClanahan

The trace element, selenium, is essential for proper nutrition and growth of certain animals. Lambs born to selenium-deficient ewes may exhibit a muscle atrophy syndrome known as white muscle disease. While this condition may be avoided by supplemental feeding or intravenous injection of selenium, a simple treatment administered by the farmer is desirable. As part of a cooperative project with Drs. O. H. Muth and J. E. Oldfield of Oregon State University, the retention and distribution of selenium in sheep was studied by externally monitoring the gamma-emitting radionuclide ^{75}Se.

Observations

Twelve Corriedale ewes were maintained on either 0.01 or 0.26 ppm selenium forage for 37 weeks prior to and throughout this 4-month experiment. Five milligrams of selenium as selenious acid labeled with 150 μCi of ^{75}Se were injected subcutaneously in the right axillary region. The injection solution was diluted to 5 ml with either isotonic saline or a peanut oil-beeswax emulsion. The four groups of three animals each were routinely monitored in a large animal total body counter equipped with a 4 x 9 in. NaI (Tl) crystal and a 200 channel pulse-height analyzer.

The retention curves (Figure 1) were well represented as a sum of two exponentials. The rapid component in all cases exhibited a biological half-life of less than 1 day. On a high selenium diet this was the major component, accounting for about 65% of the injected ^{75}Se. On a low selenium diet the rapid component was smaller and its magnitude was influenced by the nature of the material injected. An approximately 85-day half-time was observed for the long-term component when aqueous selenious acid was injected and was independent of dietary selenium. When selenious acid was injected in an oil and beeswax suspension, significantly ($p < 0.05$) longer half-times of 135 and 166 days were observed for the second component. This may be due to a delayed release of the ^{75}Se from the

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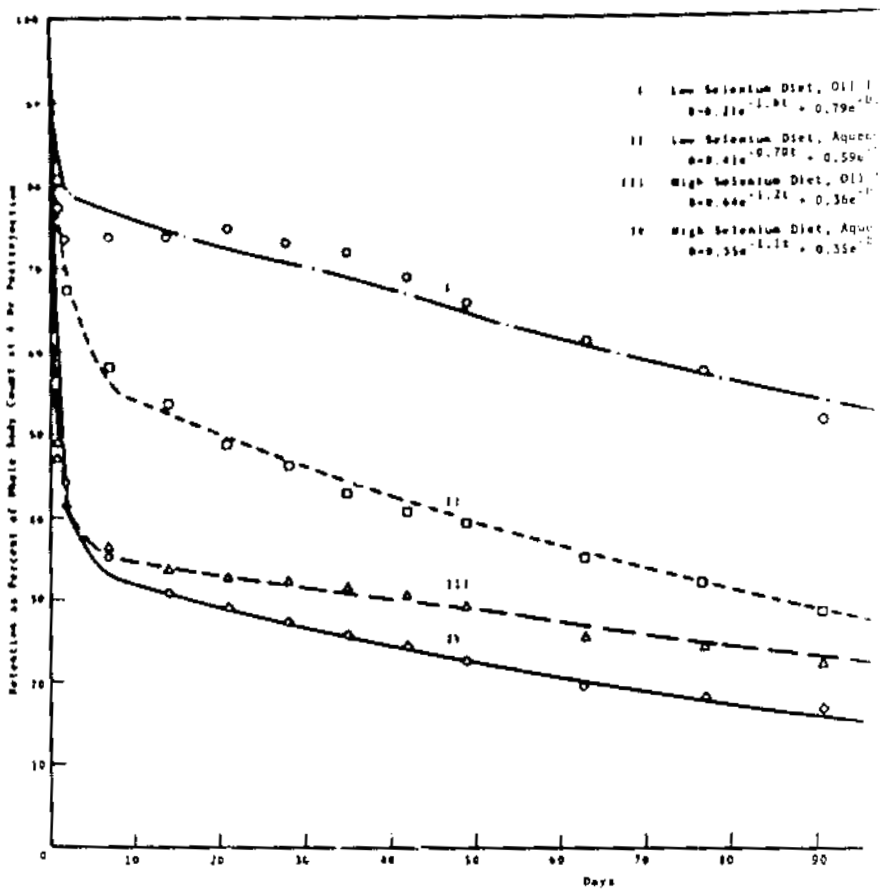

FIGURE 1. ^{75}Se Retention in Sheep Fed Either a High (0.26 ppm) or Low (0.01 ppm) Selenium Diet

injection mixture, in which case, another component should be included in the model.

Conclusions

Selenious acid in a peanut oil-beeswax vehicle shows promise as a prophylac ic for white muscle disease in sheep. Selenium i hicle was retained ous solution and a tivity remained 4 jection. Addition obtained on the tr ewe to lamb.

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APPLICATIONS OF THERMOLUMINESCENCE DOSIMETRY

Thermoluminescence dosimetry was used for in vivo gamma-ray dosimetry of ^{137}Cs in sheep. The sheep data substantiate the use of the whole body as the critical organ for ^{137}Cs ingestion.

Investigator:
C. R. Watson

Radiation dosimetry is often a time-consuming, cumbersome appendage of radiobiological experiments. Conventional dosimeters are limited in range, size, energy dependence, and evaluation time. To overcome some of these problems we have employed a thermoluminescence dosimetry (TLD) system (Madison Research and Development, Inc.) using lithium fluoride powder (TLD-100, Harshaw Chemical Co.) as the phosphor.

Observations

The in vivo ^{137}Cs gamma dose distribution measurements described in the 1964 Annual Report were confirmed and extended. Rams were fed 50 μCi of ^{137}Cs daily and implanted with dosimeters after equilibrium body burdens were established; others were implanted prior to radionuclide feeding. Thick-walled (4 mm) Teflon dosimeter capsules were used, as before, to shield the LiF from the beta particles. Dose measurements in the equilibrium animals, including both 1964 and 1965 data, are shown in Table I. Using prior-implanted dosimeters, the dose distribution pattern remained constant

TABLE I. ^{137}Cs Gamma Radiation Dose at Various Locations in Male Sheep (Expressed as mrad/mCi-day of feed)

	Sheep 1	Sheep 2	Sheep 3	Sheep 4
Viscera				
Diaphragm	103	130	---	---
Spleen	---	103	111	78
Rumen wall	102	95	---	---
Left kidney	148	115	133	142
Abdominal wall	---	110	---	84
Small intestine	135	123	147	136
Mesentery	142	160	178	129
Scrotum				
Adj. right testis	88	75	62	73
Adj. left testis	94	70	---	---
Exterior	---	---	89	---
Muscle				
Supraspinatus	85[a]	140	---	---
Longissimus dorsi	---	135	122	109
Right gluteus	78[a]	---	149	129
Left gluteus	83[a]	155	167	149
Exterior				
Mid-cervical	55	77	67	---
Anterior lumbar	52	60	---	---
Flank	67	75	---	---
Calculated γ Dose[b]				
At center	225	225	200	200

[a] *Dosimeters implanted superficially, all other dosimeters in muscle were approximately 5 cm under the skin.*

[b] *Bertinchamps and Cotzias, Science, vol. 128, pp. 888-890, 1958. (Based on the assumption that the radionuclide is uniformly distributed in an aqueous cylinder.)*

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whether the sheep were killed 11, 19, or 27 days after the start of ^{137}Cs feeding (Table II). In all cases the gamma dose to the gonads was less than that to the whole body. Doses calculated by assuming that the body is an aqueous cylinder with a homogenous distribution of radionuclide were in rough agreement with the measured values, indicating the validity of the TLD technique.

In other applications we have successfully employed the TLD technique to estimate cumulative radiation dose in 0, 2, or 4% reactor effluent water after a month, for rapid calibration of our ^{60}Co irradiation facility, and to evaluate the dose to various organs of a swine during routine roentgenographic skeletal surveys.

Conclusions

These applications of LiF TLD indicate the potential usefulness of the system in measuring radiation doses in biological studies. The ^{137}Cs gamma-ray studies in sheep support the NCRP recommendations which assume that the use of the whole body as critical organ will adequately consider the gamma radiation dose to the gonads. Because the beta dose from ^{137}Cs to the gonads may be as great as the gamma dose, further attempts to measure both beta and gamma radiation in vivo are indicated.

TABLE II. Accumulated Gamma Radiation Dose Distribution in Male Sheep Ingesting 50 uCi ^{137}Cs/Day

	Day 4	Day 7	Day 11	Day 14	Day 19	Day 21	Day 27
Average measured body burden, uCi	101	158	229	261	232	250	332
Calculated accumulated gamma radiation dose at center of 60 kg cylinder, mR	50	130	290	490	640	710	1120
Measured accumulated gamma radiation dose, mR							
Viscera							
Kidney			310		570		
Rumen wall			220		330		
Abdomen wall			190		310		910
Mesentery			360		620		1120
Scrotum			130		250		400
Musculature							
Longissimus dorsi			160		370		770
Gluteus			200		340		880

AGE DEPENDENCE OF RADIOIODINE RETENTION IN RATS

Rats, from prenatal through 1 year postnatal, were injected with radioiodine and sacrificed at intervals thereafter to obtain comparison of retention and distribution as a function of age and iodine content of the diet. Although the data have not been completely evaluated, it is clear that significant age differences will be defined.

Investigator:
N. R. Sikov
Technical Assistance:
Barbara J. Schulz

There are many indications that the distribution and retention of various radionuclides vary with animal age. These variations have seldom been subjected to systematic study. Iodine-131 was selected as the first material for

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such a study since substantial data were available upon which to base the experimental design.

Observations

Rats of five ages were studied: 19-day fetuses, newborn animals, weanlings (21 days), young adults (3 to 4 months), and older adults (10 to 12 months). Half of the animals were maintained on a normal stock diet; the other half were placed on a low-iodine diet 1 week prior to injection and fed this diet until sacrifice. All of the rats were intraperitoneally injected with 0.01 μCi of carrier-free ^{131}I/g of body weight, and randomly selected animals were sacrificed at 2 hr, 1 day, 3 days, 7 days, 13 days, or 20 days later. To evaluate the transmission of radioiodine from the lactating mother to the newborn animals, these were further subdivided into three groups: (1) the newborn animals were allowed to remain with their mothers, (2) newborn rats from injected mothers were cross-fostered to uninjected mothers, and (3) newborn rats from control litters were cross-fostered to lactating females which had been injected after 19 days gestation. At sacrifice, the thyroid glands, gonads, and an aliquot of blood, stomach, duodenum, gastric contents, and duodenal contents were removed from each rat for gamma counting; the thyroids were subsequently used for autoradiography. The ^{131}I content of the carcasses after removal of these specimens was also determined. Each age-diet group consisted of approximately five rats of each sex at each time of sacrifice.

At the time of this writing most of the experimental animals have been sacrificed but only qualitative impressions of the data can be given. As anticipated, the retention of radioiodine was uniformly higher in the rats on a low-iodine diet than in those on a normal stock diet. The ratio of the total thyroid ^{131}I retention of the animals on the low iodine diet to the retention of those on a normal diet appeared to vary relative to age. Retention per milligram of thyroid gland was inversely related to age.

Conclusions

At this point it seems that within-group variability is sufficiently low and between-group differences sufficiently large that the final data should produce a definitive picture of the relations between age and iodine metabolism in the rat. This investigation will be extended to include the determination of age-related differences in sensitivity.

NEPTUNIUM TOXICITY AND PLASMA LIPIDS IN THE RAT

Neptunium-237 increased plasma free fatty acids in female but not in male rats. Cholesterol levels were decreased and Triton-induced hypertriglyceridemia was inhibited in both sexes by prior ^{237}Np treatment.

Investigator:
D. D. Mahlum
Technical Assistance:
Joan O. Hess

The toxic effect of ^{237}Np includes the induction of fatty livers in female but not in male rats. An evaluation of mechanisms that may be involved in fatty liver development requires a knowledge of changes in the plasma

lipid constituents: triglyceride, free fatty acid, phospholipid, and cholesterol. Some of the treatments used in these experiments indicated a correlation between plasma lipid levels and ^{237}Np-induced liver fat deposition.

Observations

Rats were pretreated for varying lengths of time with 6 or 12 mg/kg of ^{237}Np. Blood samples were withdrawn and analyzed for cholesterol-free fatty acids. A nonionic detergent, Triton WR 1339, was administered to block movement of triglyceride from the blood to tissues and thus to accentuate defects in the mechanism for secretion of triglyceride from the liver. Triton produces a dramatic hypertriglyceridemia but this effect is inhibited when the liver fails to secrete at a normal rate.

Experiments summarized in Figure 1 showed that plasma cholesterol values were decreased in normal and hypercholesteremic female as well as in normal male rats one day after ^{237}Np injection. Although ^{237}Np decreased plasma cholesterol in both sexes, plasma free fatty acids were increased only in the female (Figure 2). This increase in plasma free fatty acids and the increase in liver fat were prevented by treatments such as adrenalectomy, hypophysectomy, or nicotinic acid injection (Figure 2).

The action of Triton in elevating blood triglyceride and the effect of ^{237}Np in eliminating this response (Figure 3) suggests that neptunium reduces triglyceride movement into the blood. This response was not sex dependent.

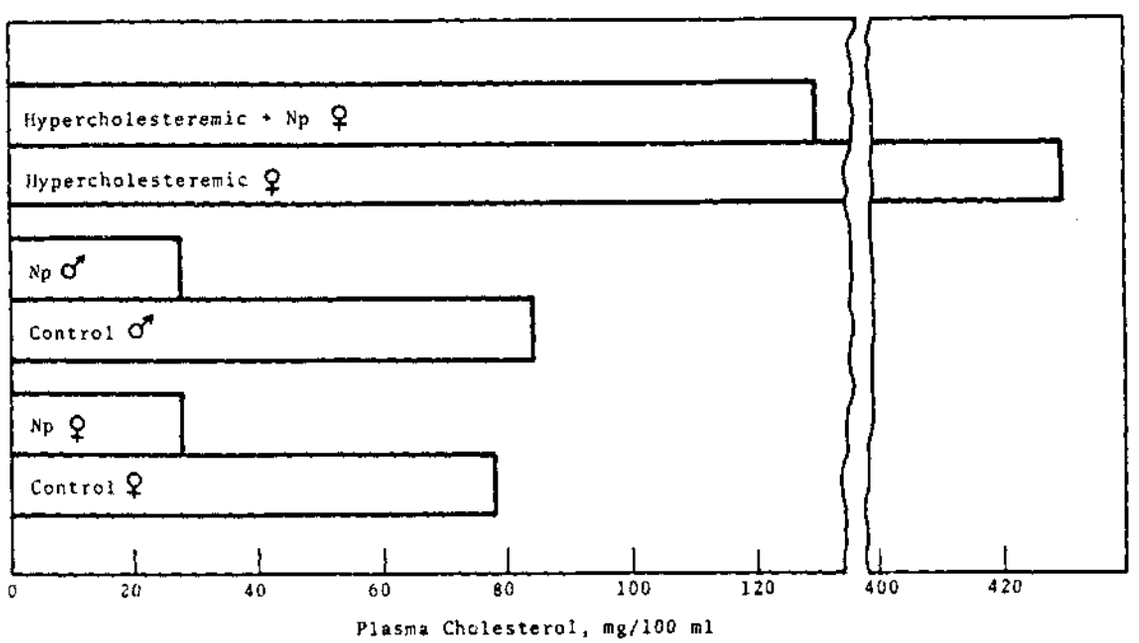

FIGURE 1. Effects of ^{237}Np on Plasma Cholesterol Levels in Normal and Hypercholesteremic Rats

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Conclusions

The alterations in plasma lipids observed after ^{237}Np treatment indicate defects in the lipid transport system. The faulty transport of lipid from the liver is probably not in itself sufficient to cause fatty liver development since both males and females show similar responses. It is more likely that the increased mobilization of free fatty acids combined with decreased fat movement from the liver is necessary to cause the observed accumulation in the liver.

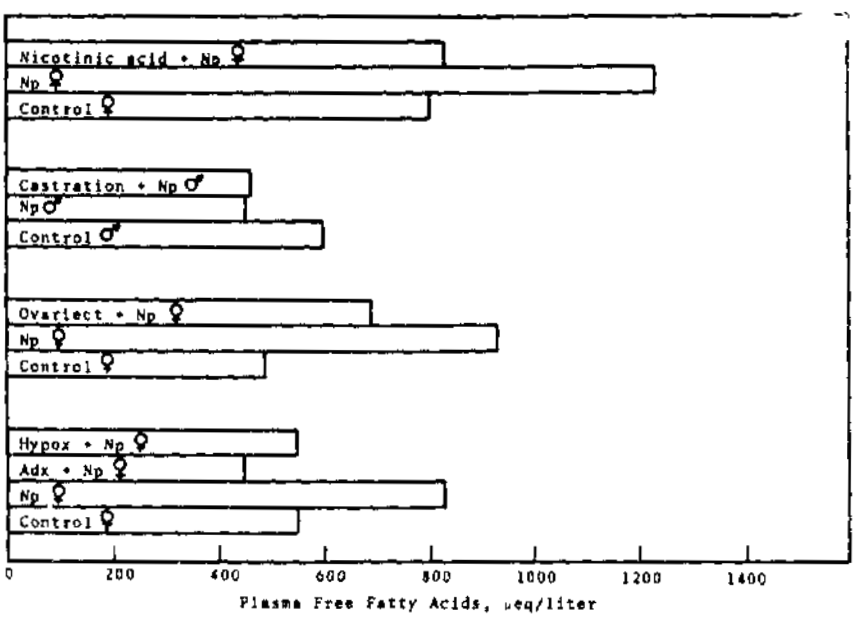

FIGURE 2. Effect of Various Treatments on Plasma Free Fatty Acid Levels Following ^{237}Np Injection

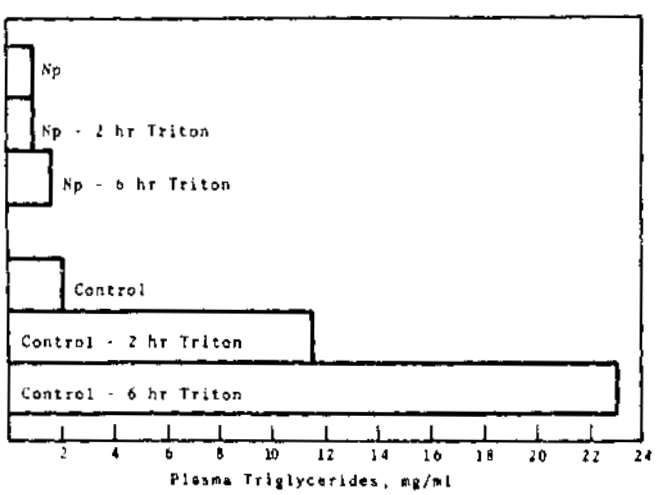

FIGURE 3. Inhibition of Triton-Induced Hypertriglyceridemia in the Rat

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EFFECT OF INGESTED RADIOACTIVE PARTICLES IN THE RAT

Insoluble particles of 1 mm diameter, containing about 1 mCi ^{89}Sr, produced discrete focal lesions during their passage through the gastrointestinal tract of the rat.

Investigators:
M. F. Sullivan
*T. D. Mahony**
Technical Assistance:
Alma L. Crosby

The effect of ingested, nonabsorbed radionuclides on the gastrointestinal tract will depend upon the quality of radiation, as determined by the radionuclide involved, and on the kinetics of movement of the radionuclide through the intestine. Calculations of radiation dose to the gastrointestinal tract have usually been based on the assumption that the radionuclide was uniformly distributed throughout the intestinal contents and spending a certain prescribed length of time in each segment of the tract. Such assumptions are clearly not applicable to the behavior of discrete large particles such as have been proposed for use in SNAP (Systems for Nuclear Auxiliary Power) isotopic power packages or as fuel forms for use in nuclear reactors for space applications.

Observations

These studies employed simulated reactor fuel particles obtained from the Health Physics Division, Oak Ridge National Laboratory. The particles were about 1 mm in diameter and consisted of depleted uranium in a graphite matrix. Each particle contained about 800 μCi ^{89}Sr plus 40 μCi ^{90}Sr-90Y. Dose rates were 13,000 rad/hr at the surface and 4000 rad/hr at a tissue depth of 50 mg/cm^2. Single particles or small numbers of particles were administered to rats by stomach tube. Daily collections of the excreta were monitored for passage of the "hot" particles. Of 215 particles administered, 37 were excreted on the first day, 118 on day 2, 41 on day 3, 11 on day 4, and 8 on days 5 through 8. Since ingested material will normally traverse the rat gastrointestinal tract within 24 hr, or less, it is apparent that these particles show some tendency for delayed passage.

Histologic examination provided evidence of radiation damage to the glandular stomach, lower ileum, cecum, and large bowel. Seven of eight rats given single particles exhibited such lesions; these were sharply localized occupying, on cross section, less than one-third of the intestinal circumference. Figure 1 shows the nature of such a lesion in the cecum. In other rats, given up to eight particles at a

FIGURE 1. Ulcer in the Cecum Produced by Single ^{89}Sr-Labeled Particle with Decreasing Mucosal Damage Toward the Edges of the Illustration

*Consultant Pathologist

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treatment, the lesions were more numerous and, in some cases, diffuse because of the increased radiation dose. The relatively high incidence of these lesions was surprising since one might have expected that the particles would usually be imbedded within, and shielded by, the intestinal contents. Examination of a few of the excreted fecal pellets containing ^{89}Sr-labeled particles disclosed that, in nearly every instance, these particles were located at the surface of the pellet.

Conclusions

The results of these experiments suggest that the holdup of large radioactive particles within the gastrointestinal tract may pose significant problems, and that the usual assumptions employed in calculating radiation doses from ingested internal emitters may not be applicable to such particles. Of particular significance is the indication that the position of a particle within the lumen of the intestine is not determined by chance, but that it may be preferentially located against the wall of the intestine. Observation of the incidence and distribution of focal damage appears to be the most direct approach to the evaluation of detailed particle movement through the gastrointestinal tract.

EFFECTS OF RADIOACTIVE PARTICLES ON THE SKIN AND GASTRIC MUCOSA OF MINIATURE SWINE

Strontium-89-labeled, 1 mm particles were exposed to the gastric mucosa and to the skin of Hanford miniature swine. No appreciable damage was observed in the gastric mucosa after exposures of 1 or 4 hr (13,000 or 52,000 R surface dose). Skin exposures of the same duration resulted in slowly healing lesions. This apparent difference in sensitivity was probably due to technical difficulties encountered in the intragastric exposures.

Investigators:
J. R. McKenney
H. A. Ragan
*R. O. Shannon**

Relatively little information is available concerning the effects of intensely radioactive particles after ingestion or deposition on skin. Such information is of concern in the evaluation of potential hazards of nuclear reactors or radionuclide power sources in space. A limited study was made with Hanford white miniature swine to determine the skin and gastric mucosal reactions following exposure to such particles.

Observations

The particles employed were 1 mm in diameter, contained about 700 μCi ^{89}Sr plus 40 μCi ^{90}Sr-^{90}Y, and had dose rates of about 13,000 rad/hr at the particle surface and 4000 rad/hr at a tissue depth of 50 mg/cm^2. Gastric mucosal exposures were made using inflated gastric balloons to which six of the particles were cemented. Skin exposures were made on the same pigs by taping particles to the lateral cutaneous surface of the abdominal wall. The exposures were for periods of 1 or 4 hr. The particles were obtained from and dosimetry

*USAF, VC, previously assigned to Pacific Northwest Laboratory. Present address: University of Michigan, Ann Arbor, Michigan.

measurements supplied by the Health Physics Technology group at Oak Ridge National Laboratory.

Control studies showed no grossly apparent changes in the mucosa of the stomach following insertion and inflation of the gastric balloons. A patchy congestion occurred in the gastric mucosa of three pigs sacrificed at 2, 3, and 15 days following a 1-hr exposure to ^{89}Sr particles. A similar reaction was seen in two animals killed 1 or 3 days after a 4-hr exposure. Discrete lesions were not observed. Since the precise location of the particles on the gastric mucosa could not be determined, it was impossible to correlate the areas of congestion with the actual sites irradiated. There was no evidence of a gastric mucosal reaction in one animal sacrificed 5 weeks following a 4-hr exposure period, the time interval at which skin reactions were most apparent.

Mild skin effects were evident between 10 and 15 days after a 1-hr exposure to a single particle. A slight redness was apparent immediately following the 4-hr exposure period, and at 2 to 3 weeks an erythema developed that was followed by necrosis of a 3 mm area. This area at 4 weeks gave the appearance of an encrusted ulcer. The ulcer had almost completely healed at 10 weeks.

Conclusions

These are very limited and preliminary observations. The greater severity of the skin lesions might suggest that early reaction of the gastric mucosa to particulate radionuclide sources would be less severe than that of the better understood and more easily studied effects on skin. However, the gastric mucosal exposures were less well controlled than the skin exposures; displacement of the sources along the mucosal surface could well account for the less severe and more diffuse reaction.

INGESTION OF PLUTONIUM OXIDE PARTICLES BY SWINE

The absorption in swine of ingested particulate, refractory $^{238}PuO_2$ is less than 10^{-6} of the dose. No gross pathologic changes were noted from 1 Ci of $^{238}PuO_2$ except in one pig where particles became entrapped in the intestinal wall. Passage time for this material was as long as 14 days.

Investigators:
V. H. Smith
J. L. Palotay
H. A. Ragan
Beatrice J. McClanahan
Technical Assistance:
J. L. Beamer
P. L. Sheldon

Plutonium-238 is one of the more promising fuels used in SNAP devices as a heat source, for conversion to electrical power. A potential hazard in the manufacture and use of this material is the possibility of ingestion with subsequent irradiation of the gastrointestinal tract, absorption into the body and accumulation in organs and tissues. To investigate the extent of this hazard pigs were fed ^{238}Pu as the refractory dioxide, the tissue plutonium content determined, and the gastrointestinal tract examined for damage.

0012362

Observations

The $^{238}PuO_2$ employed was in the form of standard production SNAP fuel particles obtained from Mound Laboratory, and was administered to the pigs in a capsule by stomach tube. Prior to the plutonium administration the female, Hanford miniature swine (weight about 44 kg) were conditioned to metabolism cages. Intravenous catheters were placed in two ear veins and an indwelling catheter in the urethra. After 4 to 5 days the ear vein catheters became inoperable and blood was taken from the anterior vena cava. The urinary catheter functioned perfectly for the 14-day duration of the experiment. The animals were killed by exsanguination after deep, Nembutal-induced, anesthesia. Samples were taken for histopathologic examination and radioanalyses. A scintillation probe sensitive to the plutonium X-rays was used to monitor the gastrointestinal tract to check for entrapped particles. The feces were analyzed by gamma counting, but all other samples required use of TTA extraction procedures.

Radioanalyses are complete for one pig; only fecal data are as yet available for two others. The diversity of the fecal excretion patterns is apparent in Figure 1. All the pigs were still

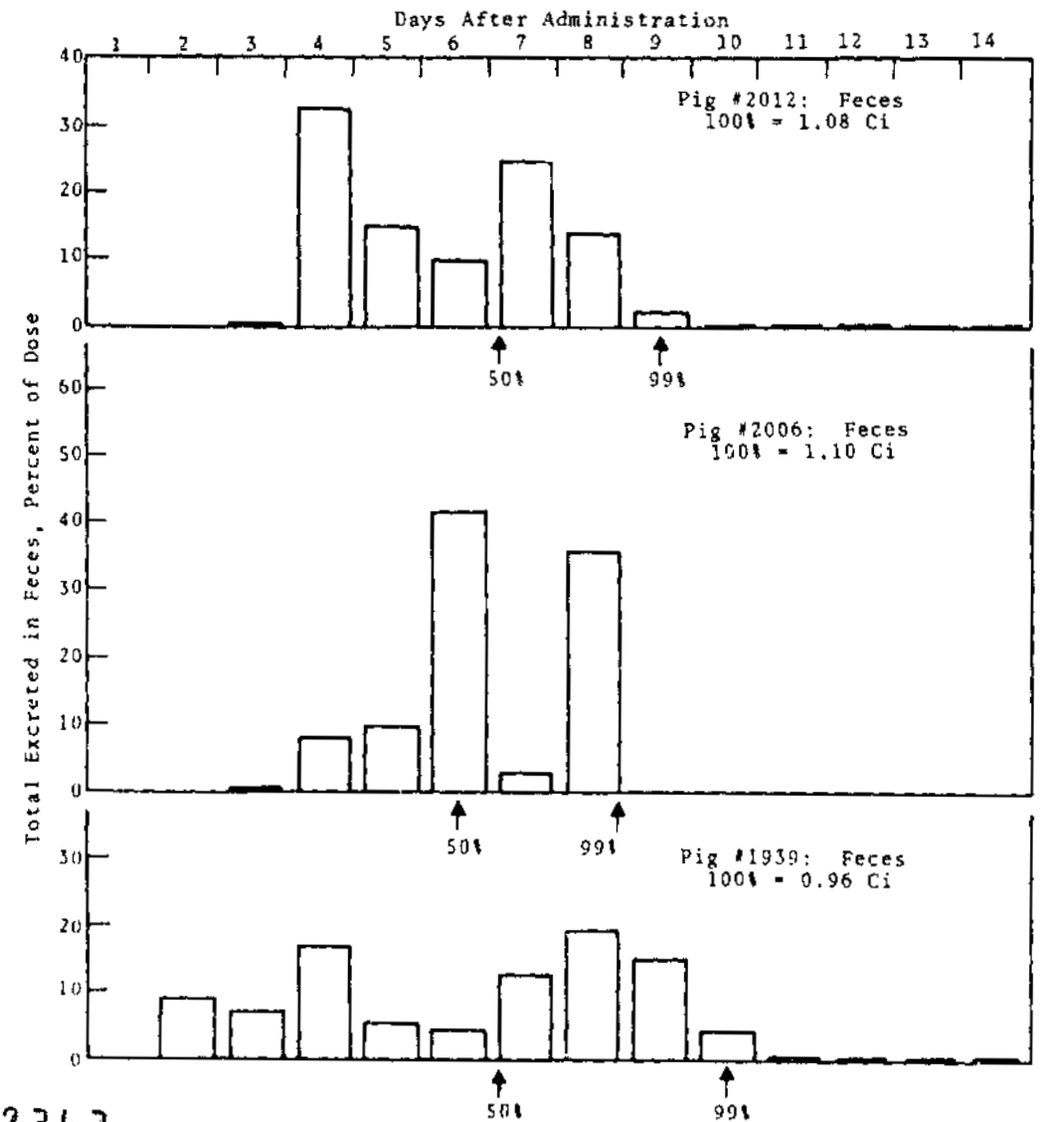

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FIGURE 1. Relative Amounts of Plutonium Present in Feces Versus Time Postingestion

excreting $^{238}PuO_2$ particles on the 14th day. There is a suggestion of a bimodal distribution of activity in the fecal output which may indicate some fractionation by particle size but this has not yet been confirmed.

In one animal, at necropsy, several $^{238}PuO_2$ particles were entrapped within and covered by the mucosa at the ileocecal junction. This had resulted in considerable local inflammation.

Shown in Figure 2 are the blood concentrations and urine output of plutonium for one pig. Such absorption as occurred was essentially limited to the first few days following ingestion, although 50% excretion via the feces did not occur until 6 to 7 days postingestion. At its peak, the blood contained about 200 times the total amount of plutonium excreted in urine.

Table I shows the very low levels of plutonium accumulated in the organs and tissues. The relatively high levels in lymph nodes, particularly in the gastric lymph nodes, are noteworthy, and suggest

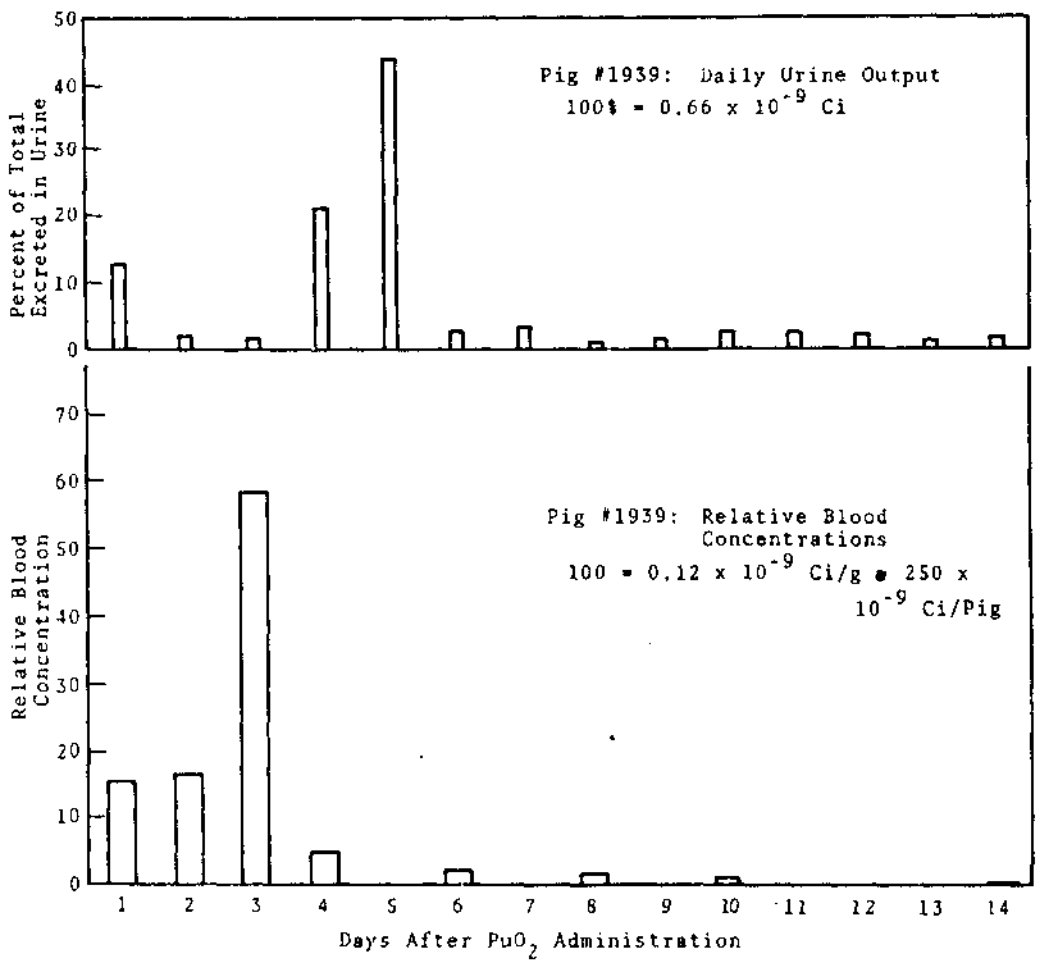

FIGURE 2. Relative Amounts of Plutonium Present in Blood and Urine Versus Time Postingestion

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TABLE I. ^{238}Pu *Content of Tissues of Pig Number 1939 14 Days Postingestion*

Tissue	Content, nCi	Concentration, pCi/g wet wt
Lungs	0.08	0.34
Spleen	0.09	1.2
Muscle[(a)]	1.5	0.71
Kidneys	1.10	1.0
Heart	0.08	0.56
Liver	0.31	0.43
Skeleton	0.25	0.12
Gastric lymph nodes	111.0	
Mesenteric lymph nodes	0.37	
Aortic lymph nodes	0.10	
Bronchial lymph nodes	4.4	
External iliac lymph nodes	3.0	

[(a)] *Assuming 42% of body weight is muscle.*

that a small amount of very fine dust accompanying the particles may have entered the lymph system and collected in the nodes. Alternatively, a plutonium colloid formed from material solubilized in the stomach, may have been filtered by the lymph nodes. The high liver-to-bone ratio suggests that the plutonium in the blood stream was in a very fine particulate state or associated with a filterable carrier. The total plutonium measured in urine and tissues indicated an absorption of about 2×10^{-7}; 98% of this absorbed plutonium was deposited in lymph nodes and may represent the fine dust accompanying the larger particles.

Conclusions

With complete results from only one pig sweeping conclusions are scarcely justified. It would appear that ingestion of these particles affords a negligible hazard so far as internal deposition of plutonium is concerned. The unexpectedly long passage times and the possibility of entrapment of particles in the gastrointestinal tract require further study.

0012365

INHALATION STUDIES

Our largest singly oriented program is that c
studies of the inhalation of radioelements.
this grouping are reports on radioelement ren
the problem of radioactive particles derived
applications of nuclear energy, which are rel
tion and pulmonary retention problems.

Several reports deal with the kinetics of dep
retention of a variety of radionuclides. The
pally experiments in beagles, although some c
done with rats. In some instances animals a:
for observation of long-term effects. Notab
is the long-term study of plutonium oxide ef
which is now showing a substantial incidence
neoplasia, some 4 to 5 years subsequent to t
inhalation exposure.

Another group of reports describes more basi
the biochemical level. These studies are of
directly or indirectly with the development
for accelerating the removal of radioactive
the lung. Of particular interest are severa
gesting an interrelationship between cigaret
cyanide levels, lung clearance rates, and pu
plasia.

Also reported are the results of continuing
subcellular physiology of lung tissue, which
the groundwork for an ultimate better unders
basic processes involved in the deposition,
removal of particulate material from the lun

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CHRONIC EFFECTS OF INHALED PLUTONIUM DIOXIDE IN DOGS

Of 40 dogs exposed to $^{239}PuO_2$ aerosols, 13 died after 29 to 66 months. The body burden at death ranged from 0.5 to 3 μCi with 40 to 75% of the body burden in the lungs, 20 to 50% in the bronchial and mediastinal lymph nodes, 2 to 21% in liver, and 1 to 7% in skeleton. Cause of death was pulmonary insufficiency resulting from the severe pulmonary fibrosis. Seven animals showed bronchiolo-alveolar carcinomas.

Investigators:
J. F. Park
W. J. Bair
W. J. Clarke
Technical Assistance:
L. R. Richardson

This report updates the rather extensive summary of this experiment published last year (Annual Report, 1964). Forty beagle dogs, given single 10- to 30-min exposures to $^{239}PuO_2$ aerosols 3 to 6 years ago, are being held for study of long-term translocation of ^{239}Pu and for observation of biological effects.

Observations

A total of 13 dogs have died or were sacrificed when death was imminent 3 to 5-1/2 years after inhaling $^{239}PuO_2$ (Table I). The body burden at death ranged from 0.5 to 3 μCi. The lungs and bronchial and mediastinal lymph nodes contained 66 to 95% of the body burden, depending upon the time of death after exposure. The maximum skeleton burden, 7%, occurred after 5 years.

The liver contained 2 tc 6% of the body burden in all dogs, except four which exhibited substantially higher liver burdens and which were among those having primary pulmonary tumors. Three of these dogs, Numbers 213, 216, and 85, were the only dogs showing metastases to the bronchial lymph nodes. Since the amounts of ^{239}Pu in the bronchial and mediastinal lymph nodes were low in these dogs, considering the length of time after exposure, it would appear that the metastases may have released plutonium bound in the lymph nodes. This might account for the increased levels in the liver and skeleton of these three dogs. The relationship between lung and lymph node plutonium concentration is illustrated in Figure 1. The relatively low values for lymph nodes with metastatic tumors are evident.

The first indication of pathology in the exposed dogs was lymphopenia. The mean lymphocyte counts, about one-half of control values 33 weeks post-exposure, decreased to one-third before death (Figure 2). There was no associated decrease in eosinophils. Monocytes and basophils were within

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TABLE I. Mortality and Tissue Distribution of ^{239}Pu in Dogs After Inhalation of $^{239}PuO_2$

Dog number	Time of death, months after exposure	Age at death, months	Plutonium, uCi: Deposited in lungs[b]	Plutonium, uCi: Body burden at death	Distribution of plutonium at death, percent of body burden: Lungs	Bronchial lymph nodes	Liver	Bone	Average radiation dose to lungs[c] estimated rads
DIED or sacrificed when death was imminent									
182	29	42	5.8	2.7	74	21	2	1	12,000
184	31	49	5.9	2.5	75	17	5	1	12,000
272	32	46	7.1	2.9	63	17	14	4	12,000
215[a]	38	81	3.9	1.4	50	42	2	4	9,000
93[a]	40	52	5.3	1.8	59	27	6	2	12,000
173[a]	44	58	6.9	2.1	46	48	2	2	19,000
106[a]	45	66	9	2.7	50	37	6	2	23,000
183	45	77	3.1	0.9	52	39	6	3	10,000
180	48	65	5.1	1.4	42	49	5	3	15,000
76	51	73	5.7	1.4	47	45	3	4	15,000
213[a]	56	76	5.7	1.2	49	27	15	5	15,000
216[a]	60	96	2.6	0.5	51	26	15	7	6,000
85[a]	66	84	7	2.1	36	30	21	6	21,000
SACRIFICED for tissue distribution of plutonium									
104	28	58	1.7	0.6	68	26	2	1	5,000
79	30	52	2.1	0.9	34	57	6	1	6,000
158	30	42	0.3	0.1	33	53	10	1	800
64	55	77	0.05	0.01	45	50	1	4	200

[a] Primary pulmonary tumors.

[b] Estimated amount deposited below ciliated epithelium, where clearance processes were not effective in causing rapid removal.

[c] Average radiation dose assumes uniform distribution of absorbed α-energy from plutonium particles in the lung and neglects plutonium translocated from the lung to other tissues.

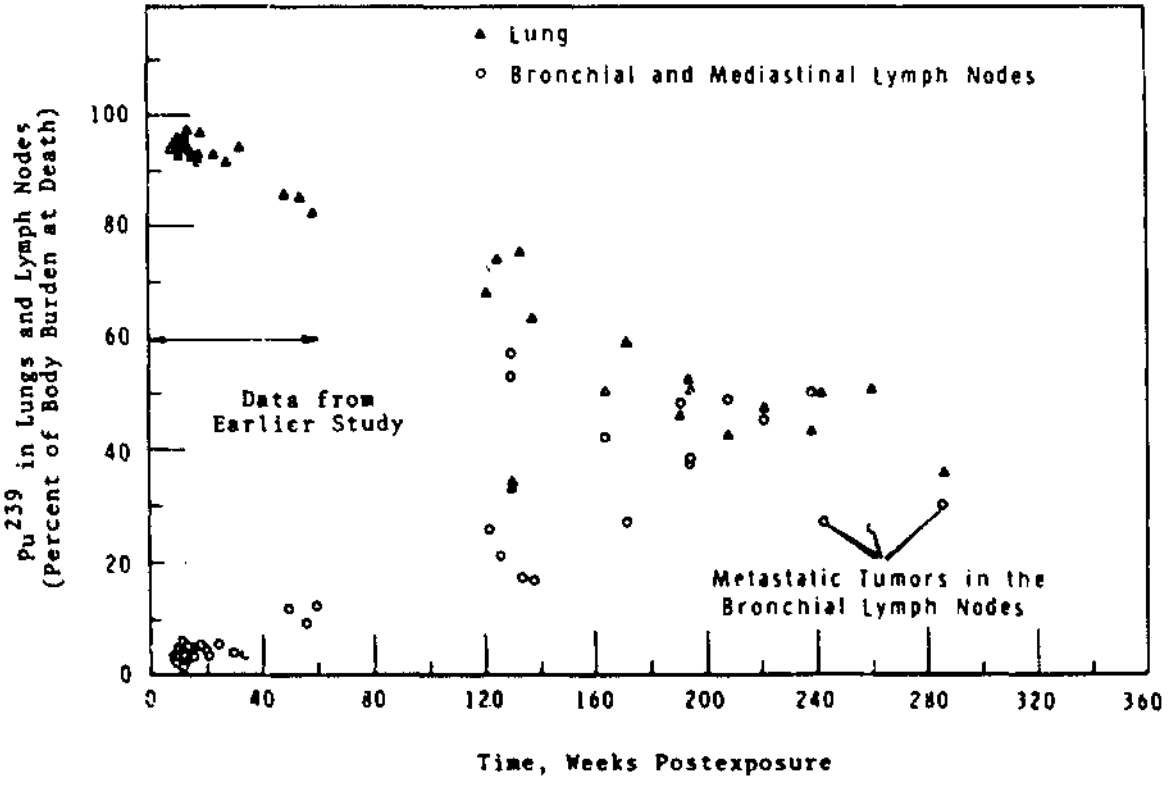

FIGURE 1. ^{239}Pu in Lungs and Bronchial and Mediastinal Lymph Nodes

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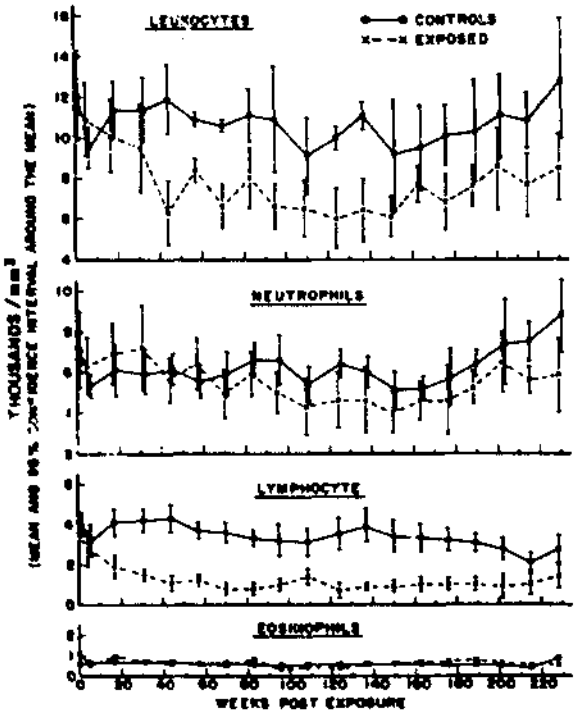

FIGURE 8. Leukocyte Values of Dogs after Plutonium Inhalation

normal range. Total leukocytes reflected the decrease in lymphocytes. Neutrophil levels were not significantly different from the controls.

The histologic changes seen in the lungs and lymph nodes of these dogs were described in the previous report (Annual Report, 1964). The only differences noted in the animals that died subsequent to that report were the metastases found in dogs 213, 216, and 85. In these animals, metastatic foci from the primary lung tumors were seen in the bronchial and mediastinal lymph nodes. Extension to other tissues or organ systems, however, was not apparent. As in those reported earlier, the origin of the bronchiolo-alveolar tumors appeared to be from the bronchiolar and/or alveolar epithelium. In no instance could derivation be associated with bronchial epithelium or bronchial glands.

Conclusions

The plutonium content of the lung, the observed pulmonary pathology, and the clinical evidence of cardiopulmonary insufficiency point to the lung as the critical organ causing death. Although the highest concentration of ^{239}Pu has consistently occurred in the bronchial and mediastinal lymph nodes, there was no indication that the resulting severe pathology in the lymph nodes, including our first evidence of metastases from the lung, was a concomitant cause of death. Seven of the 40 animals exposed to plutonium have shown primary pulmonary tumors. This is a current incidence of about 18%. The lowest body burden found in animals with a tumor was 0.5 μCi, of which half was in the lungs.

TAURINE EXCRETION FOLLOWING PLUTONIUM OXIDE INHALATION

A decrease in circulating lymphocytes is usually correlated with an increased excretion of taurine in the urine of beagle dogs exposed to aerosols of $^{239}PuO_2$. Preliminary studies suggest that the increased level of urinary taurine originates from, and reflects the destruction of, circulating lymphocytes.

Investigator:
J. V. Dilley
Technical Assistance:
R. J. Franke

It has been shown in this laboratory that beagle dogs develop a prolonged and progressive lymphopenia after inhalation of $^{239}PuO_2$. Work from other

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laboratories has suggested that elevated urinary taurine levels following X-irradiation reflect an increased destruction of lymphocytes, although all blood elements are reduced by this type of damage. The purpose of this study was to investigate the relationship between lymphopenia and increased levels of urinary taurine in dogs having lung burdens of ^{239}Pu.

Observations

Twenty-four hour urine samples were collected from a control dog and from each of five lymphopenic dogs that had inhaled $^{239}PuO_2$ 3 years previously. Taurine was separated on a Dowex 50-X8 resin column and further purified by descending paper chromatography in a solvent system of n-butanol-acetic acid-water. The ninhydrin-stained taurine spot was eluted in 71% ethanol and spectrophotometrically determined. The results of these determinations are shown in Table I. In four of the five exposed beagles, taurine excretion was higher than in the control dog.

Cortisone acetate produces a prompt lymphopenia and might be expected to produce rapid elevated urinary taurine levels if taurine is truly arising from destroyed lymphoid cells. To test this hypothesis a beagle dog was given cortisone on 3 successive days and total urine collected for the 24 hr period following each injection. The results of this experiment are shown in Table II. There was a marked increase in taurine excretion for the first 48 hr followed by a marked decrease during the next 24 hr.

Conclusions

There is an evident correlation between the degree of lymphopenia in ^{239}Pu-treated dogs and the increase of taurine excretion. It does not necessarily follow that the source of the taurine is destroyed lymphoid cells; however, such a hypothesis is supported by the results of cortisone treatment which produces a marked lymphopenia and also a large increase in taurine excretion. These results suggest that in dogs with lung burdens of $^{239}PuO_2$, lymphocyte production continues but their destruction is accelerated, perhaps by alpha radiation, resulting in an effective decrease in cell half-life and a

TABLE I. Urinary Taurine Levels in Beagle Dogs After $^{239}PuO_2$ Inhalation

Dog	Preexposure lymphocyte counts,[a] per mm³ blood	Postexposure lymphocyte counts,[b] per mm³ blood	Taurine excreted mg/24 hr
269 (control)	3380 ± 305	3015	81
267	3236 ± 285	1140	161
268			149
278	3430 ± 374	1574	81
281	4090 ± 510	2345	179
283	2490 ± 380	1430	109

[a] *Preexposure counts are the average and standard error of five determinations made during the first 5 months of 1962.*

[b] *Postexposure counts are the average of two determinations made in 1965 before and after the urine samples are taken.*

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TABLE II. Effect of Cortisone on Urinary Taurine Levels

Collection/period, hr	Taurine excreted, mg
0 to 24	263
24 to 48	636
48 to 72	34

lymphopenia. The possibility remains that the increased excretion of taurine results from an accelerated conversion of cystine to taurine, totally unrelated to the lymphopenia. This possibility is being investigated in other experiments.

LARGE PARTICLE INHALATION STUDIES

Ten and 26 μ, ^{59}Fe-labeled ceramic microspheres were deposited by inhalation in the lungs of beagle dogs. From 50 to 70% of the ^{59}Fe was excreted during the first 4 days after exposure, almost exclusively in feces. There was little clearance of the remaining material during the ensuing 2 months.

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

The biological fate of inhaled particles larger than 5 μ in diameter has received little attention. Very small proportions of the larger particles are expected to reach the alveolar lung and those deposited in the upper respiratory tract are cleared rapidly. However, in view of the high dose rates that may be associated with large radioactive particles, the few penetrating to the respiratory bronchioles may constitute a serious hazard.

Observations

Studies were performed in four beagle dogs exposed to aerosols of ceramic microspheres labeled with ^{59}Fe.* Two of the dogs were exposed to particles of 10 μ, nominal diameter, and the other two dogs were exposed to particles of 20 μ, nominal diameter. Particle size analysis of the 20 μ material showed a count median diameter of 26 μ with about 1% of the particles ranging below 16 μ; the smallest size observed was 4 μ.

The fraction of inhaled material which was initially deposited varied between 70 to 90%. Retention of this initially deposited material was followed by whole-body counting, and by analyses of excreta and tissues upon sacrifice of one animal from each group at 2 months postexposure. Figure 1 shows that one-half to three-fourths of the initially deposited material was cleared within a few days postexposure; thereafter excretion was almost negligible. Twenty to 30% of th initially deposited 10-μ particles and 60% of the 26-μ particles were still retained 2 months after inhalation.

Whole-body scanning was accomplished by passing the animal through an array of collimated counters. For both 10-μ and 26-μ particles, the majority of the radioactivity, immediately postexposure, was localized in the area of the lung with smaller amounts extending from the nasc-pharynx throughout the tracheal region. By 4 hr postexposure, radioactivity in the head and throat regions had

*Obtained from Minnesota Mining and Manufacturing Company.

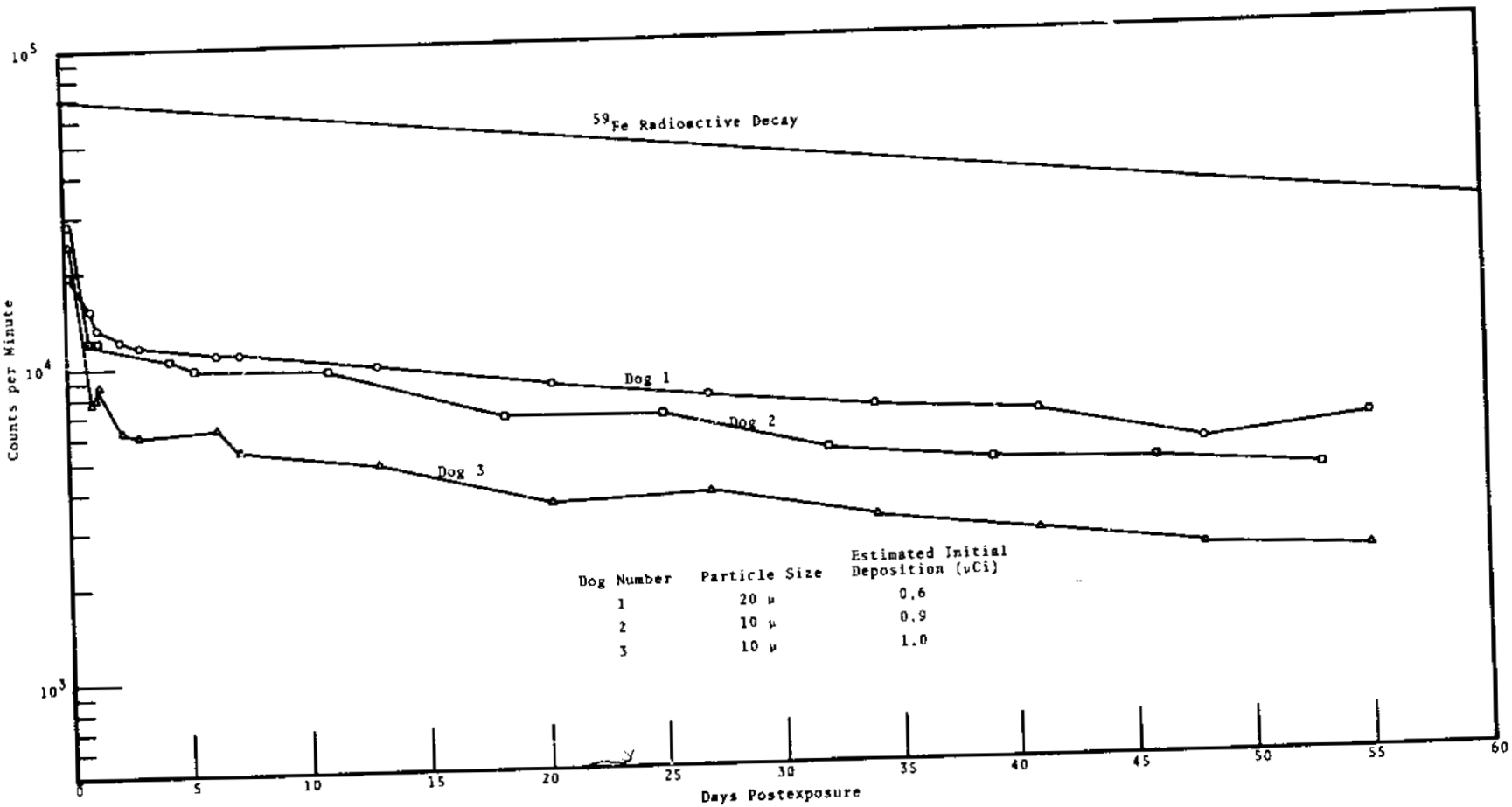

FIGURE 1. Whole-Body Counting Rates of Beagles Exposed to ^{59}Fe-Labeled Microspheres

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largely disappeared; but a spread of the "lung peak" over the abdominal area indicated movement of swallowed material through the gastrointestinal tract. By 24 hr postexposure, the abdominal "peak" had disappeared and radioactivity was concentrated in the lung area where it remained, essentially undiminished, for 2 months.

Of the total activity collected in the excreta, 70 to 80% was eliminated with the first day's feces; the rates of ^{59}Fe elimination via the feces decreased more than a hundredfold during the first 10 days postexposure. Elimination of ^{59}Fe in the urine was initially a hundredfold lower than elimination in the feces, but the rate of elimination in the urine dropped less rapidly than that in the feces; during the period from 10 days to 2 months postexposure about half as much activity appeared in the urine as in the feces. During this period the total daily excretion amounted to no more than a few tenths of a percent of the activity remaining in the lung. Because of the increasingly significant role of urinary excretion with the time postexposure, it seems likely that ^{59}Fe excreted after the first few days represents that which was leached from the deposited particles rather than removal of the particles themselves.

Tissue analyses of two animals sacrificed 2 1/2 months after inhalation of the particles showed essentially all of the ^{59}Fe confined to the lungs. Multiple transverse sections (20 μ thick) were taken of the various lobes of the lung to locate and identify particles by autoradiography (Figure 2). Twenty particles were observed in 64 microscopic sections from the dog that had inhaled 26-μ microspheres, and 65 particles were observed in 78 sections from the dog that had inhaled 10-μ microspheres. There was a tendency, particularly apparent with the 26-μ particles, for preferential deposition in the right lung. In most instances, particles were located in the deep lung, adjacent to alveolar wall. There was also evidence on the autoradiograms of ionic ^{59}Fe representing radioactivity leached from the microspheres.

Conclusions

Particles as large as 30 μ in diameter are apparently respirable, as evidenced by direct measurement in tissue sections. These particles penetrated to the alveoli and were tenaciously retained. Twenty to 60% of initially deposited material in the size range of 5 to 30 μ was retained for 2 months postexposure, and the very slow loss of activity after the first few days seemed due to leaching of ^{59}Fe from the particles rather than removal of intact particles.

Although only a few animals have been studied, the pulmonary deposition and prolonged retention of inhaled 10-μ and 26 μ microspheres focus attention on the potential hazards of inhaled radioactive materials of this size range. In further experiments the influence of particle density and composition upon deposition and retention of inhaled large particles will be studied.

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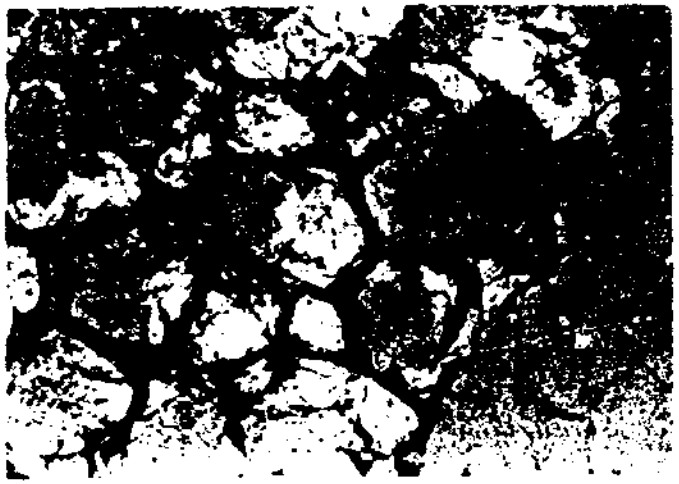
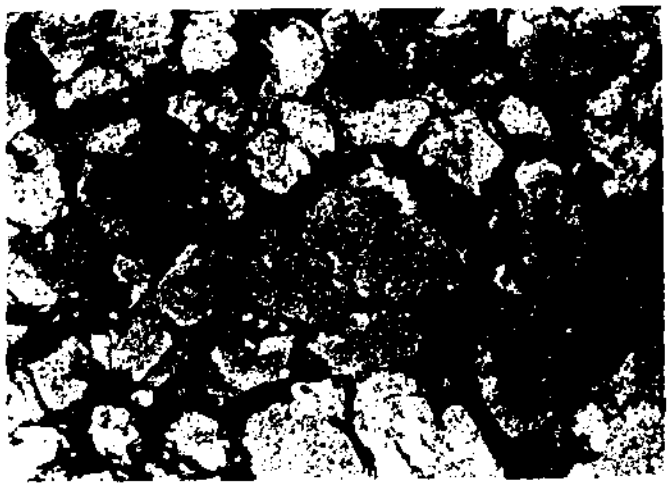
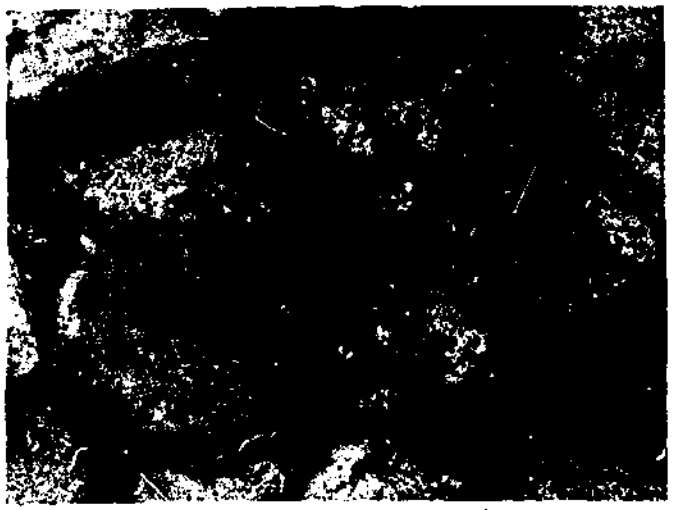


Alveolar Area. 205X. Dog Number 1. 26 μ Experiment.



Alveolar Area. 205X. Dog Number 2. 10 μ Experiment.

Alveolar Duct Area. 205X. Dog Number 1. 26 μ Experiment.



Alveolar Area. 512X. Dog Number 2. 10 μ. Experiment.

FIGURE 2. Autoradiographs of Inhaled ^{59}Fe-Labeled Ceramic Microspheres in the Lungs of Beagles 83 and 81 Days After Exposure

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STRONTIUM INHALATION STUDIES

Rats were exposed to aerosols of ^{85}Sr-labeled strontium oxide, fluoride, carbonate, titanate, or phosphate. Only the titanate was significantly retained in the lungs over a 2-week period. From eighty to 90% of the body burdens of the other ^{85}Sr compounds rapidly accumulated in the skeleton.

Investigator:
D. H. Willard
Technical Ass
M. D. Snyder

The metabolism of strontium has been extensively investigated with regard to the potential hazard of ^{90}Sr in fallout. Strontium-90 is also one of the isotopes used in the Systems for Nuclear Auxil- Power (SNAP) Program. Little is known, however, concerning the metabolism of inhaled ^{90}Sr, especially in respect to the relative fate of different strontium compounds.

Observations

Groups of 24 rats were simultaneously exposed for 30 min to an aerosol of the ^{85}Sr-labeled compound. diately, 2, 5, 7, or 14 days posure, groups of four or fiv killed for tissue analysis. employed were analyzed by X-r fraction procedures to confir chemical species. The stront nate was found to be contamin about 25% strontium oxide.

Data on the total body ret the compounds tested are show ure 1. Two weeks after expos body burdens ranged between 8

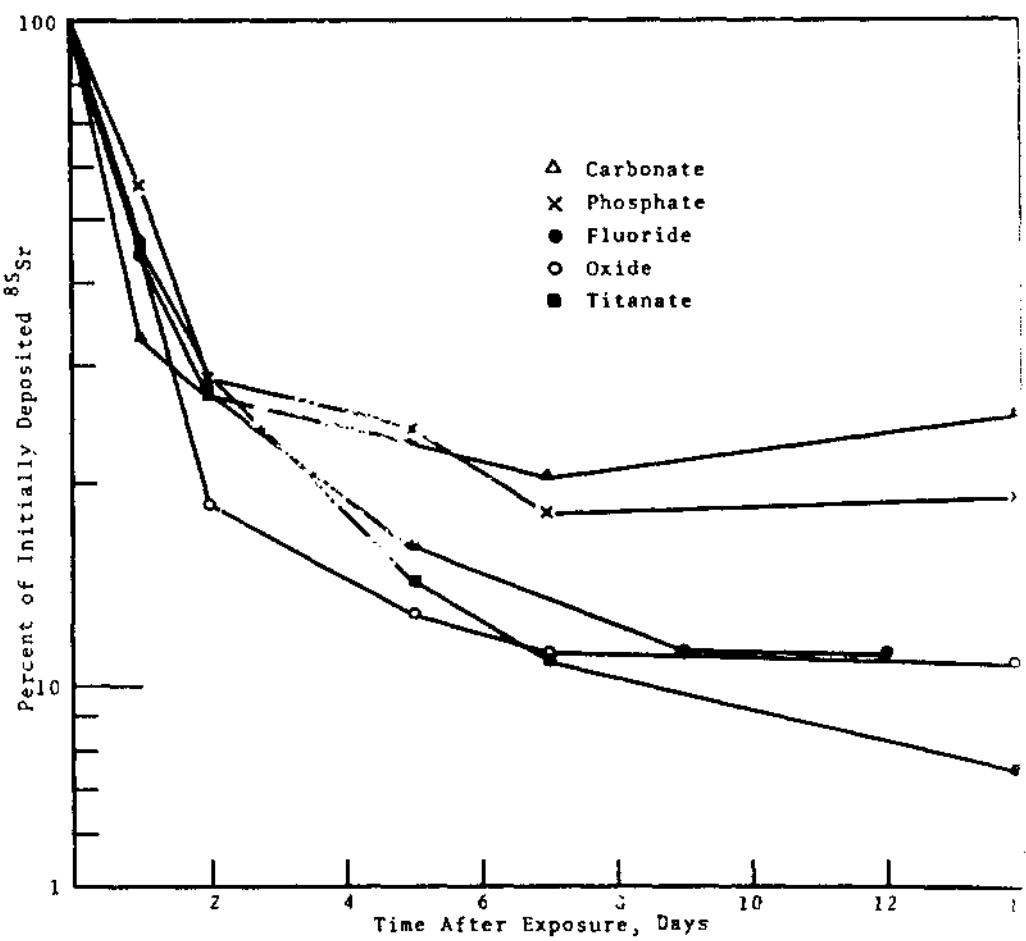

FIGURE 1. Total Body Retention of ^{85}Sr Following Inhalation

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of the initial amount deposited. The curves are closely grouped and the slopes are similar.

Pulmonary retention of the compounds is shown in Figure 2. With the exception of strontium titanate, all were rapidly cleared, less than 1% remaining after 5 days. The behavior of strontium titanate was strikingly different with 40% of the initial amount deposited remaining in the lung 2 weeks after exposure.

The ^{85}Sr lost from the lungs but retained in the body deposited almost exclusively in the skeleton (Figure 3). By 4 days postinhalation, 80 to 90% of the retained body burden was in the skeleton, except in the case of the titanate of which only 25% was in the skeleton. Translocation of the titanate to the skeleton may, in fact, be

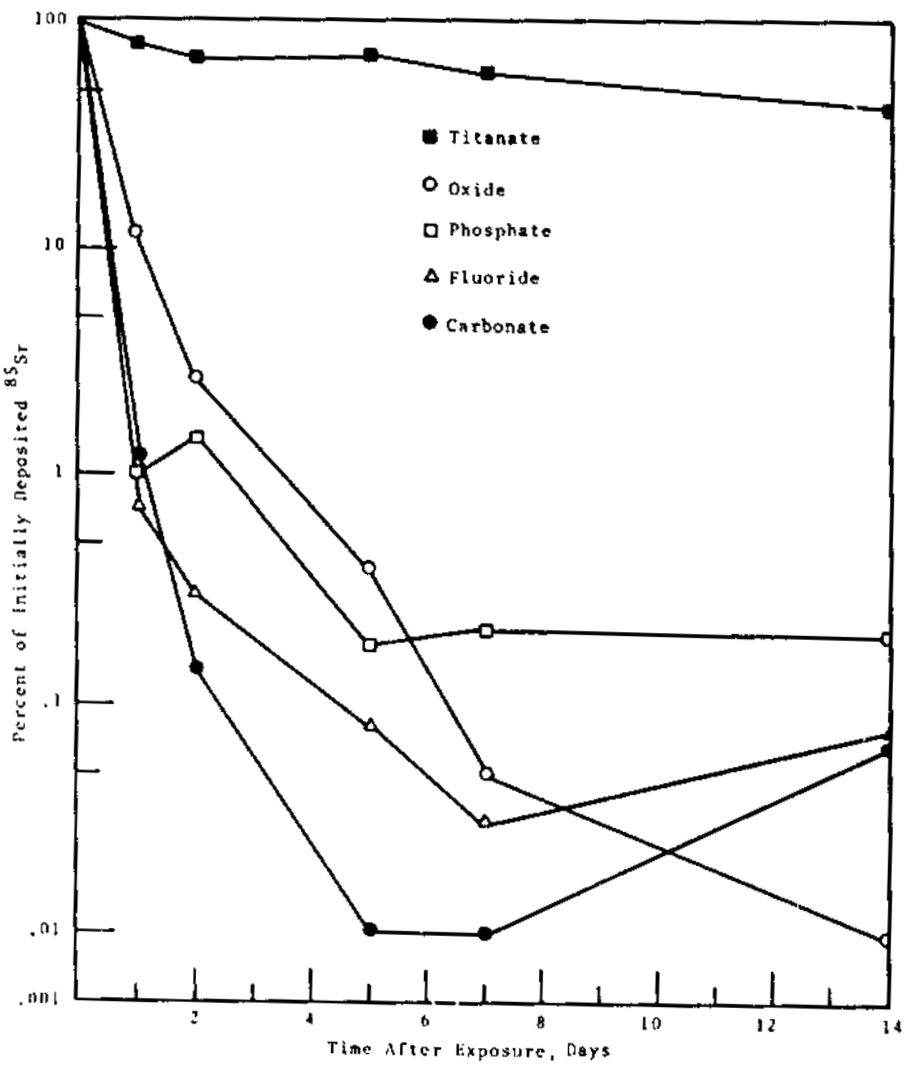

FIGURE 2. Lung Content of ^{85}Sr Following Inhalation

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very much lower than this figure would indicate since the titanate was contaminated with about 25% of the more soluble strontium oxide.

Conclusions

Although the strontium compounds tested have different chemical properties and solubilities, their reactions in the lungs were almost identical with the exception of strontium titanate which, being relatively insoluble, was largely retained in the lung.

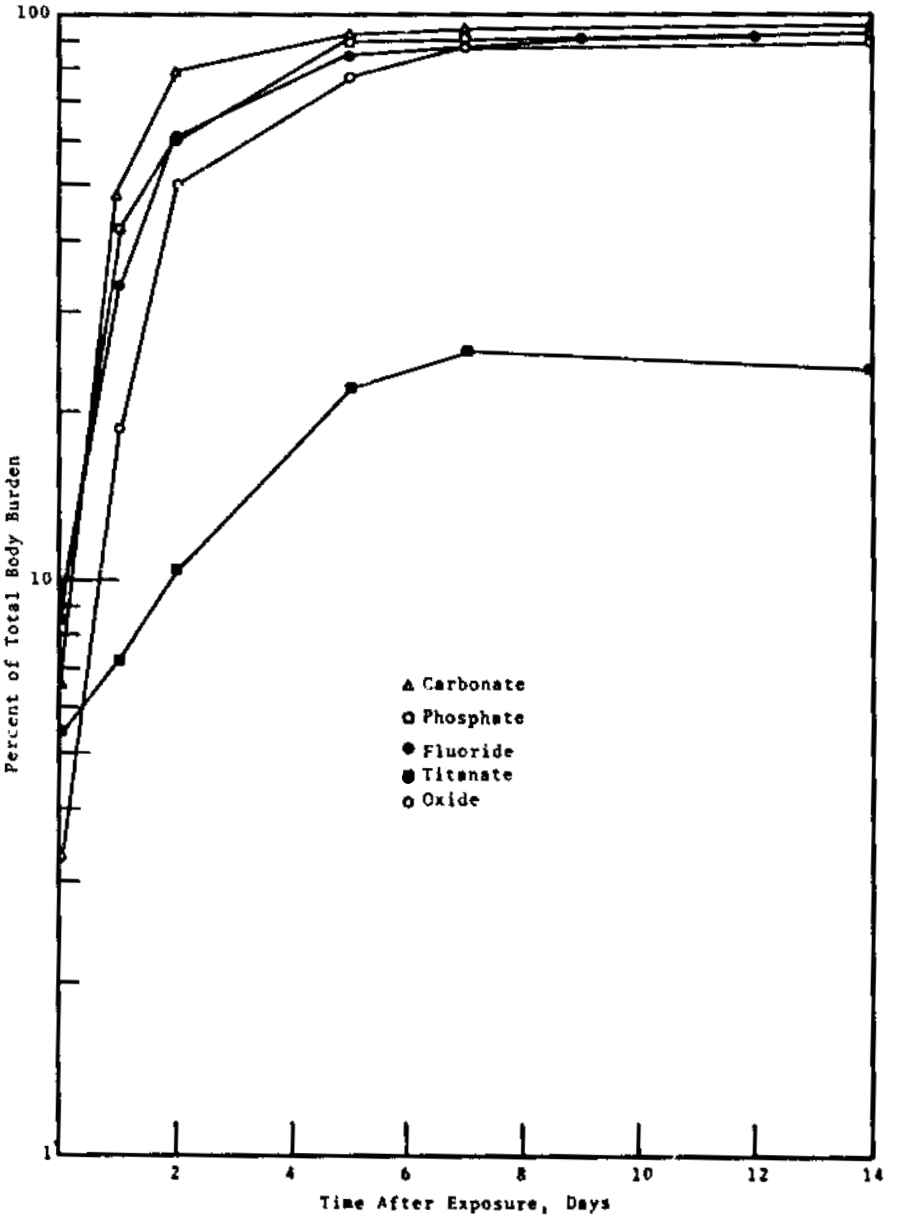

FIGURE 3. Skeleton Content of ^{85}Sr Following Inhalation

0012378

PROMETHIUM OXIDE INHALATION STUDIES

Beagle dogs were exposed to submicron size aerosols of promethium oxide. About half of the material initially deposited in the lungs was lost during the first week; that remaining was retained tenaciously with some evidence of translocation from the thoracic to abdominal regions.

Investigator:
B. O. Stuart
Technical Assistance:
J. C. Gaven
M. D. Snyder

Promethium-147, a weak beta emitter (maximum energy of 0.22 MeV), is a major constituent of fission product radioactivity at one or more years postfission. It is currently being recovered in megacurie quantities for possible use in nuclear auxiliary power devices, presenting a possible inhalation hazard during processing. Previous work in this laboratory determined the distribution, excretion, and biological effects of inhaled and injected ^{147}Pm perchlorate in beagle dogs (Annual Report, 1963). The present studies are concerned with the biological fate and effects of inhaled promethium oxide.

Observations

Due to the absence of hard gamma emissions from ^{147}Pm, previous attempts at whole-body counting of dogs exposed to soluble promethium perchlorate were unsatisfactory. Therefore, high-flux neutron activation of ^{147}Pm was employed in the present study to produce a hard gamma-emitting isotope as a tracer for whole-body counting. Following decay of the shorter-lived ^{148}Pm, ^{149}Pm, and ^{150}Pm, calcined $^{147+148m}Pm_2O_3$ was prepared for aerosol inhalation studies. On the day of exposures, the ratio of ^{147}Pm (half-life, 2.6 yr) to ^{148m}Pm (half-life, 42 days) radioactivities was about 20.1. The ^{148m}Pm gamma peaks of 0.550 and 0.630 MeV were used for whole-body counts and longitudinal scans.

Figure 1 shows the whole-body retention of $^{147+148m}Pm_2O_3$ in six beagles after inhalation of particulate aerosols having a count median diameter (CMD) of 0.06 μ. Dialysis tests of the 0.1% Pluronics suspension of promethium oxide used for aerosol generation showed 3 to 4% of the radioactivity to be ionic. Forty to 50% of the initially deposited material was cleared during the first week postexposure, predominantly by the fecal route. Whole-body counts beyond 5 or 6 days postexposure reflected essentially only radioactive decay.

Figure 2 illustrates representative longitudinal scans of one of these dogs immediately after exposure and at 1 month postexposure. Intermediate scans showed a rapid reduction of the initial head radioactivity peak, with a translocation of some thoracic radioactivity to the abdomen within a few days postexposure. At 1 month after exposure, although overall levels are considerably reduced (note scale difference), more of the remaining $^{147+148m}Pm$ radioactivity had been translocated and localized in other regions of the body.

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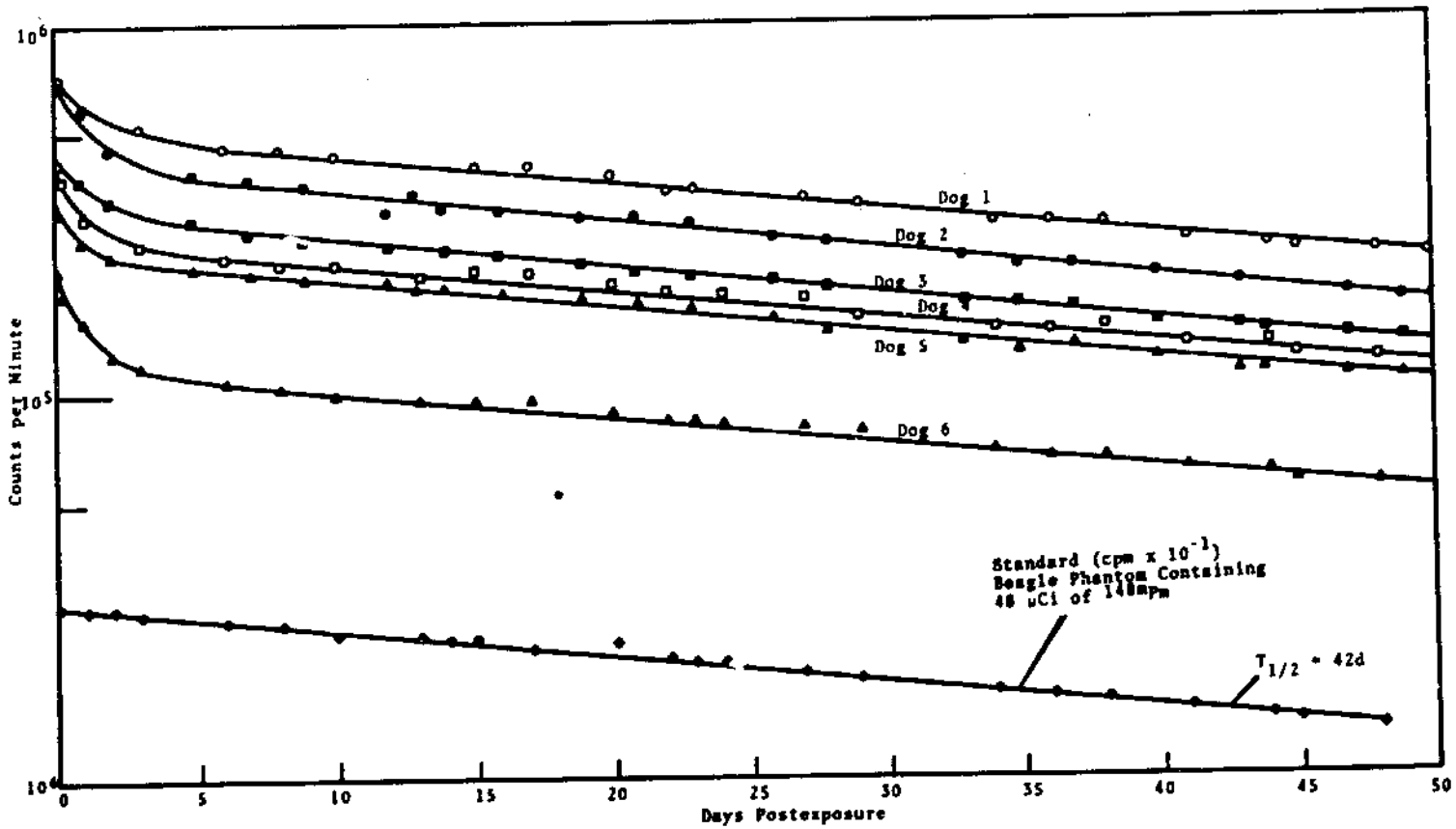

FIGURE 1. Whole-Body Retention of $^{147+148m}Pm$ After Inhalation of Promethium Oxide by Beagle Dogs

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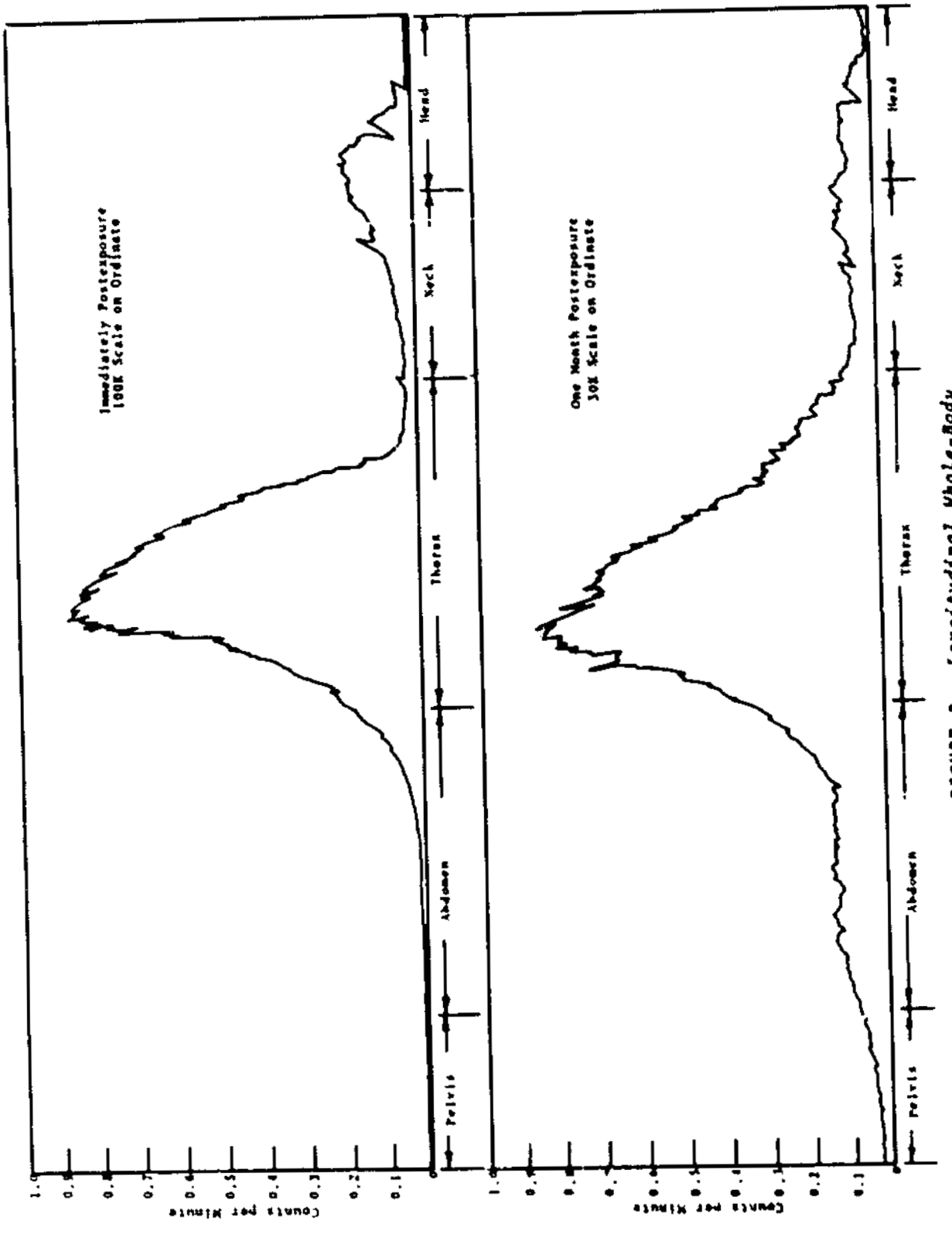

FIGURE 2. Longitudinal Whole-Body Scans of 147+148mPm Radioactivity in a Beagle After Inhalation of Promethium Oxide

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Conclusions

The data obtained to date suggest that about 50% of the initially deposited $^{147+148m}Pm_2O_3$ inhaled as 0.06 CMD particles was eliminated within the first week after exposure. The remaining whole-body burden was reduced essentially only by physical decay, although some radioactivity was translocated to other organs. This is similar to the behavior of inhaled promethium perchlorate which was previously shown to be distributed 40% in the liver and 35% in the skeleton at 1 and 2 months after inhalation. Periodic sacrifices of the animals in the present study and continuing excreta analyses will provide further information on the distribution and excretion of inhaled promethium oxide.

RUTHENIUM OXIDE INHALATION STUDIES

Beagle dogs showed 50 to 80% pulmonary retention of inhaled submicronic ^{106}Ru-$^{106}RhO_2$ up to 1 year after exposure. At 7 months postexposure 88% of the total body burden was in the lungs. Excretion of ^{106}Ru-^{106}Rh was predominantly by the fecal route.

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

The high yield fission product pair ^{106}Ru-^{106}Rh constitutes a potential hazard in fallout and during the processing of spent reactor fuels. Its 1.0 year physical half-life and energetic beta and gamma emissions present a dangerous combination following internal deposition. The present study with beagle dogs extends earlier work from this laboratory on the pulmonary retention of ^{106}Ru-$^{106}Rh_2$ in mice.(1)

Observations

Six beagles were exposed to aerosols of calcined ^{106}Ru-$^{106}RhO_2$ particles having count median diameters ranging from 0.07 to 0.12 μ. Periodic whole-body counts and longitudinal scans for radioactivity have indicated that 50 to 80% of the initially deposited material (20 to 30 μCi, total beta radioactivity) localized predominantly in the lungs of these animals for at least 1 year post-exposure. Figure 1 illustrates the whole-body retentions of ^{106}Ru-^{106}Rh in two dogs for 7 months after a single exposure. The effective half-lives following a rapid initial clearance period of 2 or 3 weeks were 285 and 277 days for Dogs 1 and 2, respectively. Similar results were obtained for the other four dogs.

Table I shows the tissue radioactivity contents as percent of whole-body burdens of these dogs sacrificed 7 months postexposure. In agreement with weekly longitudinal whole-body scans, nearly all of the radioactivity remained in the lungs, with translocation of small amounts to the tracheobronchial lymph nodes. Radioactivity levels in the liver and skeleton were very low.

The logarithmic relationship between the fractions of the body burden excreted daily and time after exposure is shown in Figure 2. Initial radioactivity levels of fecal samples were more than two orders of magnitude greater than those of urine samples. Daily fecal excretion of ^{106}Ru-^{106}Rh dropped rapidly over the first 41 days after exposure, but urine levels remained nearly constant. The almost

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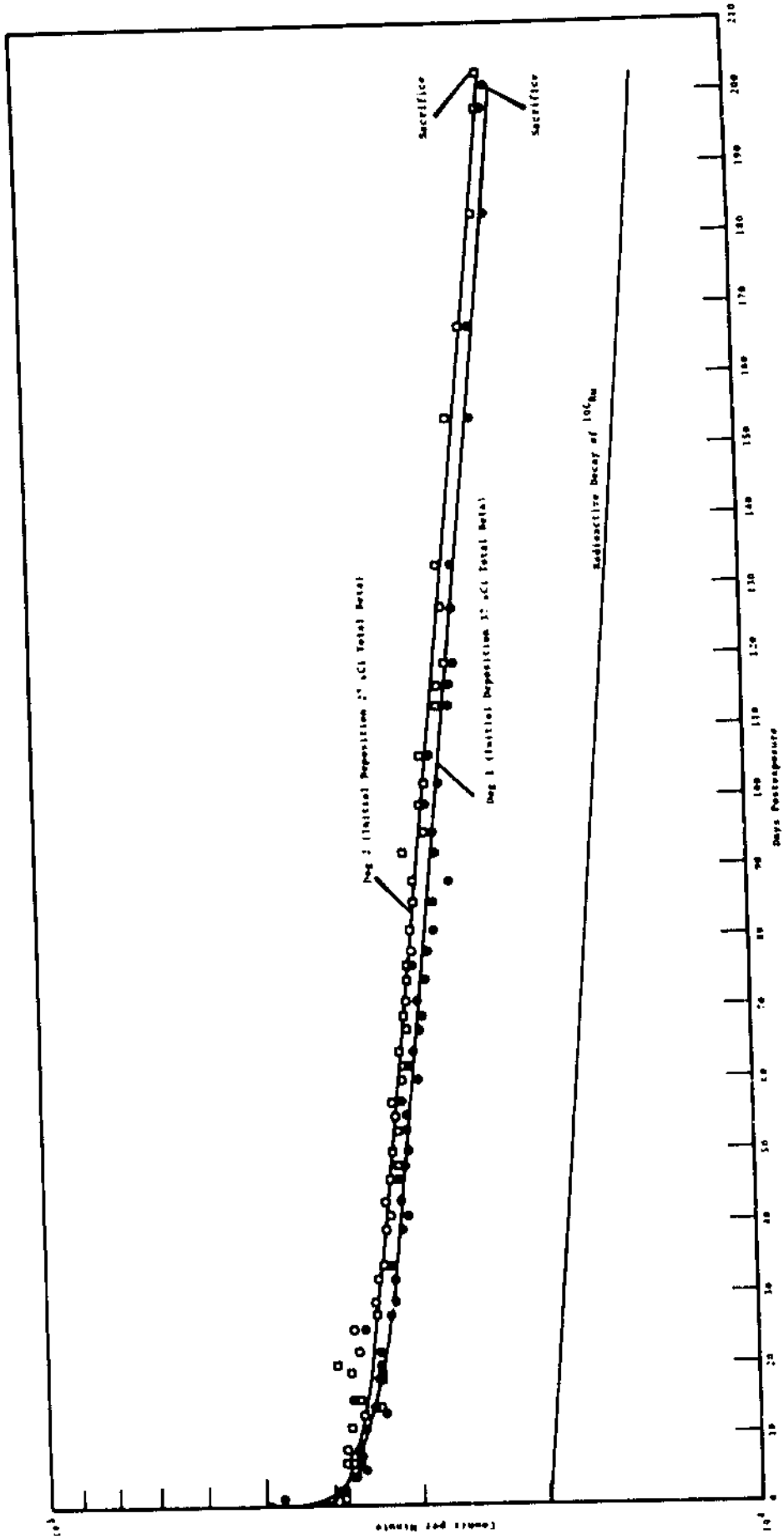

FIGURE 1. Whole-Body Counting Rates in Beagles After Inhalation of Calcined ^{106}Ru-$^{106}RhO_2$

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TABLE I. *Distribution of ^{106}Ru-^{106}Rh in Beagles 7 Months After Inhalation of ^{106}Ru-$^{106}RhO_2$*

Tissue	Percent of total body burden[a]	
	Dog 1	Dog 2
Tracheobronchial lymph nodes	1.9	0.94
Lungs	97.6	98.4
Kidneys	0.006	0.007
Spleen	0.007	0.013
Liver	0.056	0.080
Bone	0.17	0.24

[a] *Total body burdens of Dogs 1 and 2 were 19.2 and 18.6 uCi (total beta) at sacrifice, corrected for physical decay from day of exposure.*

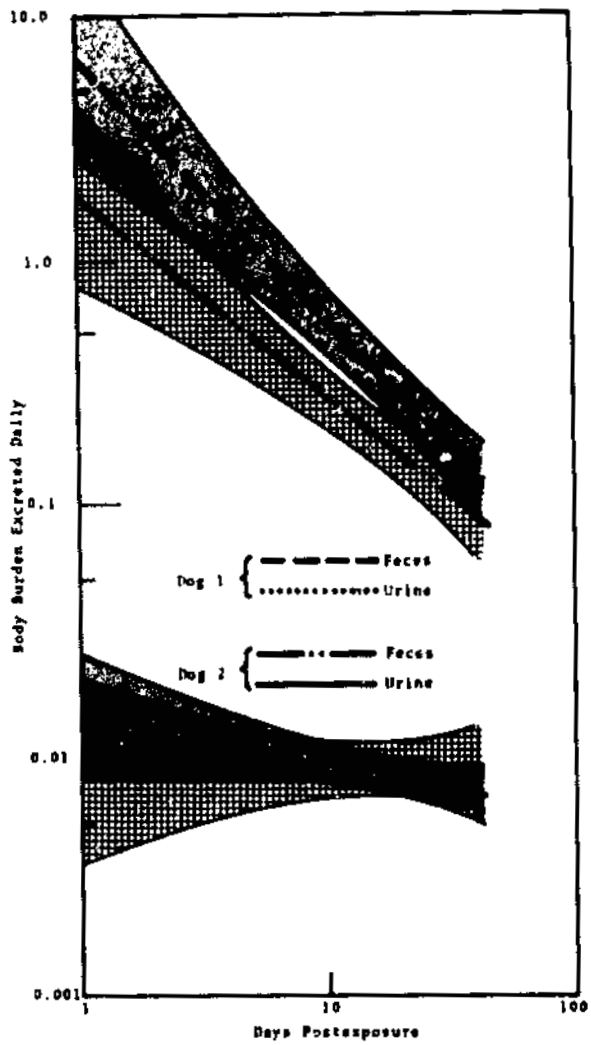

FIGURE 8. Excretion of ^{106}Ru-^{106}Rh by Beagle Dogs (95% confidence limits are shown)

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stationary urinary excretion rates at very low levels may be partly due to calculation of these data on the basis of the whole-body burden which decreased rapidly resulting from high fecal excretion rates.

Conclusions

The very high retention of ^{106}Ru-^{106}Rh in the lungs after inhalation of the calcined oxide is in marked contrast with earlier reports of inhaled ruthenium chloride. Dailey et al.[2] exposed rats to ruthenium tetraoxide vapor or aerosols of ruthenium chloride, obtaining lung burdens of only 10 and 7%, respectively, of the initial body burden 1 month after exposure. Hamilton[3] found 25% of the initial body burden in the lungs of rats 2 months after inhalation of ruthenium chloride. In comparison, more recent work in this laboratory showed 80 to 90% pulmonary retention in mice 5-1/2 months after exposure to aerosols of chemically prepared ^{106}Ru-$^{106}RhO_2$. The present findings also emphasize a very high level of pulmonary retention of this fission product in the form of the oxide, with very little translocation even to the tracheobronchial lymph nodes. This long-term pulmonary retention, together with an extremely small degree of in vivo solubility, as shown by very low radioactivity in other organs and minimal urinary excretions, support the concept of the lung as the critical organ following inhalation of ^{106}Ru-$^{106}RuO_2$. This uncommon immobility of an energetic gamma- and beta-emitting fission product in the lungs affords comparison with alpha-emitting heavy metal oxides such as $^{239}PuO_2$, which also has shown high pulmonary retention.

References

1. *Willard, D. H., L. A. Temple, and W. J. Bair. Turnover and Tissue Distribution of Radioruthenium Oxide in the Lungs of Mice, HW-52286. 1957.*

2. *Dailey, N., I. Wender, and R. Abrams. Tracer Studies with Inhaled 1.0 Year Ruthenium, MDDC-420 . 1945.*

3. *Hamilton, J. G.. Radiology, vol. 49, p. 325. 1947.*

CLEARANCE OF INHALED FERRIC OXIDE IN IRRADIATED RATS

Exposure of rats to 300 R whole-body X-irradiation had no effect on the clearance of inhaled $^{59}Fe_2O_3$ particles up to 25 days after the inhalation exposure.

Investigator:
J. V. Dilley
Technical Assistance:
D. L. Catt[*]
L. G. Michels[**]
M. D. Snyder

It has been reported[1] that 300 R whole-body X-irradiation just prior to inhalation of SiO_2 particles inhibits the clearance of these particles from the lungs of rats for periods of up to 25 days post inhalation. To determine the applicability of this observation to materials other than SiO_2, experiments were initiated involving the inhalation of a biologically inert material, calcined $^{59}Fe_2O_3$ particles.

[*] *On educational leave of absence.*

[**] *Summer employee.*

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Observations

Adult, female Sprague-Dawley rats were exposed to 300 R of 200 KVP whole-body X ray with physical characteristics closely approximating those employed by Ferin, et al. Both irradiated and control groups were then exposed to the $^{59}Fe_2O_3$ aerosol for 30 min.

In the first exposure half of the animals were sacrificed immediately following inhalation exposure. The remaining animals were sacrificed at 18 hr postexposure. In a second exposure half of the animals were sacrificed at 18 hr and the other half at 25 days postexposure. The results of this experiment are shown in Table I. The increased deposition noted in the second exposure was due to a higher aerosol concentration. There was no indication of a consistent difference between irradiated and control animals.

To confirm these findings a similar experiment was performed in which all animals were exposed simultaneously to the aerosol. The results of this experiment are shown in Table II. Again there was essentially no difference between irradiated and unirradiated groups in either initial deposition or retention of the particles.

Conclusions

In these experiments exposure to 300 R of whole-body X ray had no effect on the deposition or clearance of inhaled $^{59}Fe_2O_3$ particles. The apparent disagreement between these results and

TABLE I. Experiment 1 - Deposition and Retention of Inhaled $^{59}Fe_2O_3$ in Rats Following X-irradiation

Time after exposure	Lung burden ± standard error, μCi	Percent remaining in lung
Exposure no. 1		
0 hr controls	0.036 ± 0.002	100
18 hr controls	0.036 ± 0.004	100
0 hr irradiated	0.038 ± 0.005	100
18 hr irradiated	0.032 ± 0.003	85
Exposure no. 2		
18 hr controls	0.050 ± 0.004	100
25 days controls	0.034 ± 0.006	67
18 hr irradiated	0.064 ± 0.006	100
25 days irradiated	0.034 ± 0.008	53

TABLE II. Experiment 2 - Deposition and Retention of Inhaled $^{59}Fe_2O_3$ in Rats Following X-irradiation

Time after exposure	Lung burden ± standard error, μCi	Percent remaining in lung
0 hr controls	0.011 ± 0.004	100
18 hr controls	0.010 ± 0.004	94
25 days controls	0.005 ± 0.002	47
0 hr irradiated	0.011 ± 0.003	100
18 hr irradiated	0.010 ± 0.003	91
25 days irradiated	0.006 ± 0.003	55

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those reported by Ferin remains to be explained. The silica employed by Ferin was quite possibly metabolically active, while the ferric oxide employed in our experiments was most probably inert. Estimation of silica retention was based on chemical analysis while retention of ferric oxide in our experiments was based on more accurate and sensitive radiochemical assay procedures. Any conclusion as to the more general applicability of either of these conflicting observations must clearly await the accumulation of further data on other types of inhaled particles.

References

1. *Ferin, J., G. Urbankova, and A. Vlckova. Arch. Env. Health, vol. 10, pp. 790-795. 1965.*

EFFECT OF PHARMACOLOGICAL AGENTS ON INHALED PARTICLES

Treatment of rats with atropine sulfate prior to inhalation of $^{59}Fe_2O_3$ particles slightly decreased the quantity of material deposited in the lung. Treatment with 2,4-dinitrophenol had little, if any, effect on deposition of the inhaled particles.

Investigator:
J. V. Dilley
Technical Assistance:
D. L. Catt[a]
M. D. Snyder

Two physiological processes which may influence the deposition and retention of inhaled particles are the activity of the cilia lining the tracheobronchial tree and the character of the mucous blanket covering these cilia. These two functions can be separately modified by pharmacological agents to study the influence that each may have on the deposition and retention of particles.

Observations

The specific effect of atropine sulfate is to reduce the water content of the mucous lining of the tracheobronchial tree, markedly increasing the viscosity of this mucous blanket. Adult, female rats were intraperitoneally injected with this drug and, together with suitable controls, were exposed to an aerosol of $^{59}Fe_2O_3$ for 15 min. The animals were sacrificed at various time intervals following the inhalation exposure and the lungs and trachea removed and counted for radioactivity. The results of these studies are shown in Table I. In all but one instance the treated group had a lower lung burden than the control animals. However, the differences were small, even with massive doses of atropine, suggesting that the viscosity of the mucous blanket exerted only a small effect on particle deposition and retention.

A second experiment employed 2,4-dinitrophenol as a stimulant to the basic metabolic rate. This agent causes the uncoupling of oxidative phosphorylation; it should first increase the activity of the cilia in the respiratory tract but deplete energy stores for continued ciliary activity. Rats were injected intraperitoneally with 12.5 mg of 2,4-dinitrophenol per kilogram of body weight just prior to aerosol exposure. At intervals during

[a] *On education leave of absence.*

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TABLE I. Effect of Atropine Sulfate on Deposition and Retention of Inhaled Particles

Time postexposure	Dose of atropine, mg/kg of body weight	^{59}Fe in lungs, μCi[a] Treated	Controls
2.5 min	10	0.0058 ± 0.0002	0.012 ± 0.
2.5 min	10	0.010 ± 0.001	0.008 ± 0.
20 hr	10	0.009 ± 0.002	0.015 ± 0.
3 min	100	0.011 ± 0.003	0.014 ± 0.
3 hr	100	0.010 ± 0.0006	0.012 ± 0.
22 hr	100	0.012 ± 0.003	0.015 ± 0.

[a] *Average of five animals ± standard error.*

the exposure four treated and four control animals were removed from the exposure chamber, sacrificed, and lungs and trachea removed and assayed for radioactivity. The results of these studies are shown in Table II. The dinitrophenol-treated group showed, perhaps, a tendency toward increased deposition as the length of exposure increased, but differences were clearly not significant.

Conclusions

By employing large doses of potent pharmacological agents, it is possible to alter normal physiological functions concerned with the deposition and retention of inhaled particles. The results of the studies reported here indicated that increasing the v of the mucous blanket in the tory tree or the uncoupling tive phosphorylation had lit on the deposition of particl two functions may be conside important to the disposition particles than previously th

TABLE II. Effect of 2,4-din on the Deposition of Inhaled

Length of exposure, min	^{59}Fe in lungs, Treated	
30	0.012 ± 0.003	0.
45	0.027 ± 0.008	0.
60	0.038 ± 0.008	0.
90	0.112 ± 0.016	0.

[a] *Average of four animals ± standard er*

REMOVAL OF INHALED RADIOACTIVE PARTICLES

Of several agents administered as aerosols none were effective in removing inhaled $^{106}RuO_2$ from the lungs of rats.

Inv
D.

This is part of a continuing search for an agent useful in hastening the removal of inhaled radioactive particles, especially plutonium. In the present studies the beta-gamma emitter, ^{106}Ru-Rh, was used instead of ^{239} tate radiochemical analysis. tonium dioxide, ruthenium d relatively insoluble in the has a long biological half-

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Observations

Following exposure of rats to $^{106}RuO_2$ aerosols, the therapeutic agent was given by aerosol continuously over an approximately 5 hr period. Treatment was resumed for approximately 8 hr on the following day. Aerosols were produced by jet or ultrasonic atomization. Animals were killed and lungs analyzed for ^{106}Ru-Rh immediately 1 or 7 days after RuO_2 exposure. In a second test series, animals were killed immediately 3 or 14 days after exposure.

Data obtained at the longest time interval following exposure are summarized in Table 1. None of the agents tested caused a significant reduction in ^{106}Ru retention as compared with untreated controls. Several agents appeared to inhibit the natural clearance process. These included dimethylsulfoxide (DMSO), procaine, ethylenediaminetetraacetic acid (EDTA), thenoyltrifluoracetone (TTA), and diethylenetriaminepentaacetic acid (DTPA). In earlier tests DTPA had increased the rate of removal of inhaled $^{144}CeO_2$ from both rats and dogs, but was not effective in removing $^{239}PuO_2$.

Conclusions

These and earlier studies reported from this laboratory have not led to the identification of a significantly effective agent for reducing the lung burden of such insoluble compounds as RuO_2 or PuO_2. It would seem more profitable to seek a new approach, perhaps directed toward enhancing the role of the phagocyte in removing foreign particles from the lung.

TABLE I. ^{106}Ru Lung Burdens of Rats Treated with Test Aerosols (percent of initially deposited ^{106}Ru)

Test Aerosol	Treated	Controls
Series I[a]		
DMSO	81	70
DMSO before exposure	63	65
Silver nitrate	49	36
Adrenalin	54	53
Caffeine	68	56
Nicotine	76	77
Choline	60	54
Procaine	82	50
Series II[b]		
Carbopol	63	57
EDTA	83	67
Triton	63	58
TTA	75	57
DTPA	81	68
Oxime	65	84
Terpin ... drate	78	74

(a) Lung burdens 1 week after exposure.
(b) Lung burdens 2 weeks after exposure.

EFFECT OF CIGARETTE SMOKING ON FATE OF INHALED PARTICLES

The possible effects of chronic cigarette smoking on the clearance of particles from the lung were examined in beagle dogs smoking 20 cigarettes per day. Deposition and clearance of inhaled insoluble particles were tested periodically by exposing the dogs to aerosols of $^{59}Fe_2O_3$ or $^{51}Cr_2O_3$. No effects were seen in dogs after smoking cigarettes 5 days/week for over a year.

Investigators:
W. J. Bair
J. V. Dilley
Technical Assistance:
W. Skinner
M. D. Snyder

It is believed that the mucus blanket propelled by the ciliated cells of the respiratory tract epithelium plays an important role in the removal of inhaled particles from the lung. Several authors have reported that cigarette smoke inhibits mucus production, the beat of cilia, or the rate of mucus and particle transport on isolated segments of ciliated tracheal epithelium. These results of essentially in vitro studies have been interpreted to indicate that

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cigarette smoking inhibits the clearance of inhaled particles from the lung. The implications of such a conclusion can be far reaching. It could lead to the establishment for smokers of more conservative permissible limits for airborne contaminants. Therefore, it seemed necessary to determine directly whether cigarette smoking impairs the clearance of inhaled particles from the lung. The results of these preliminary experiments show no consistent gross effects of chronic cigarette smoking on either deposition or clearance of inhaled particles from the lung.

Observations

For this study a method was developed to simulate human smoking in dogs. The dogs were caused to inhale smoke directly from a cigarette through the mouth into the trachea. Blood pressure and heart rate changes, increased levels of thiocyanate in the urine, and the appearance of smoke in exhaled air

TABLE I. Protocol for Dog Experiments

Time, days after first ^{59}Fe test (a)	Event
0	First ^{59}Fe test
45	Introduction to smoking (1 cigarette/day)
75	Begin 2 cigarettes/day
85	Begin 6 cigarettes/day
95	Begin 12 cigarettes/day
105	Begin 18 to 20 cigarettes/day
310	^{51}Cr test
420	Second ^{59}Fe test

(a) *Dogs were 18 months old.*

$^{51}Cr_2O_3$ aerosols for deposition and clearance studies. The dogs were tested again 320 days after beginning to smoke 18 to 20 cigarettes per day, this time with $^{59}Fe_2O_3$. In these experiments lung clearance of ^{51}Cr and ^{59}Fe was measured by whole-body counting and also by longitudinal scanning of the dogs. Excreta and blood samples were also counted for ^{51}Cr and ^{59}Fe.

Figure 1 shows the results of the

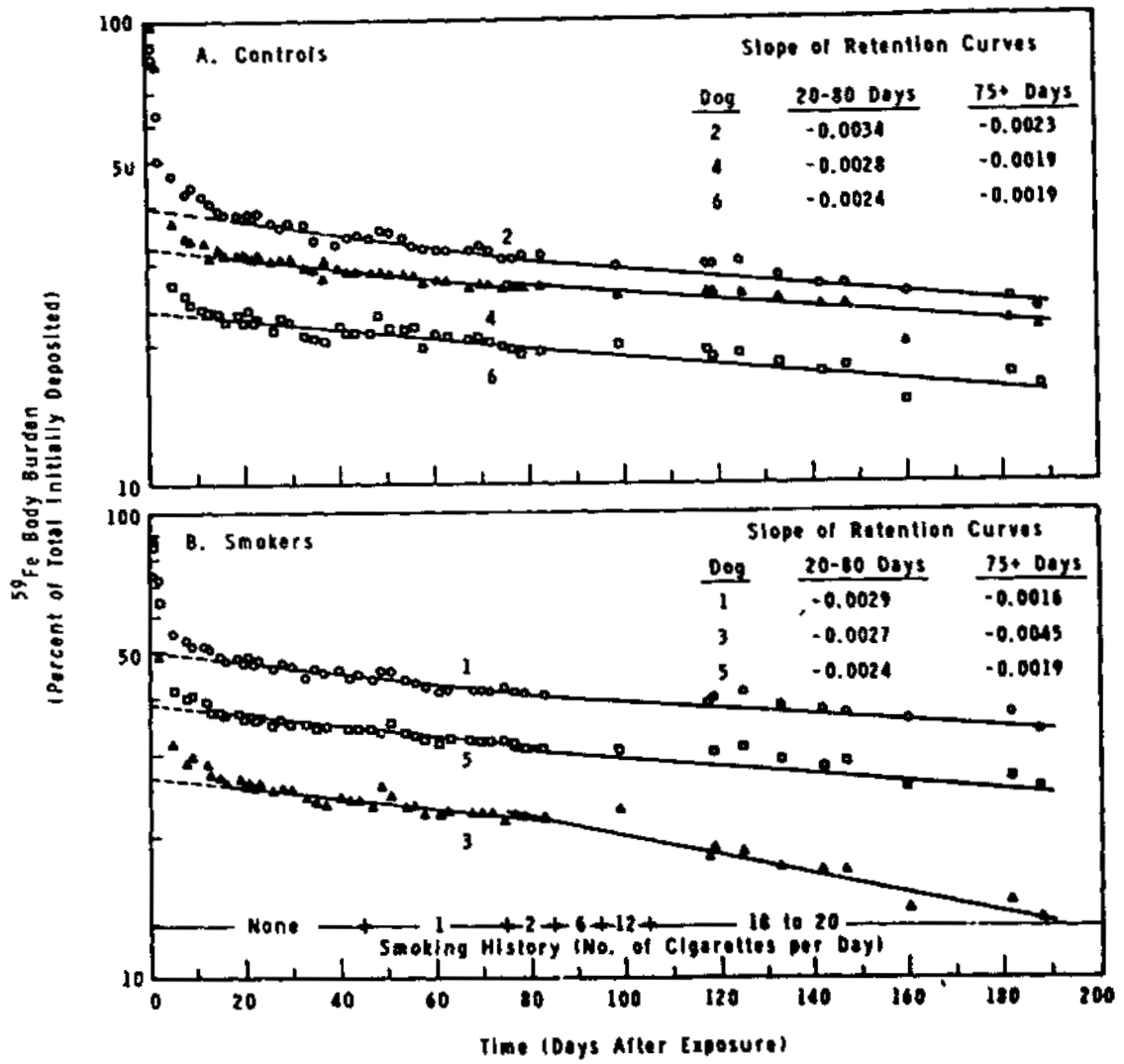

FIGURE 1. Whole-Body Retention of Inhaled ^{59}Fe in Dogs (Initial $^{59}Fe_2O_3$ Test)

In the postsmoking tests with ^{51}Cr and ^{59}Fe, the total amount inhaled was determined from whole-body counts and analyses of exhaled air. Percentage deposition was calculated for the total deposited and for that deposited in the lower respiratory tract or alveoli. Total deposition ranged between 55 and 85% in all dogs for both ^{51}Cr and ^{59}Fe with one exception: a deposition of 31% of inhaled ^{51}Cr in a smoking dog. There were no consistent gross differences in deposition between control and smoking dogs.

The particle size of the inhaled and exhaled aerosols were also compared in these experiments to determine whether smoking altered this aspect of deposition. The count median diameters (CMD) of the exhaled aerosols tended to be somewhat larger for the smoking dogs than for the controls. External scans showed considerable initial variation in deposition between head and lungs,

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but no clear distinction between smoking and nonsmoking dogs.

Long-term clearance following the second ^{59}Fe exposure, as obtained from whole-body counts, is shown in Figure 2. Since scans showed the body burden of ^{59}Fe to be concentrated in the lung region, these curves can be assumed to reflect lower respiratory tract clearance. The retention half-times ranged from 300 to 600 days for the period of 20 to 100 days after exposure. There were no significant differences between controls and smokers. Dog 3 in this test did not show the accelerated rate seen in the first ^{59}Fe test.

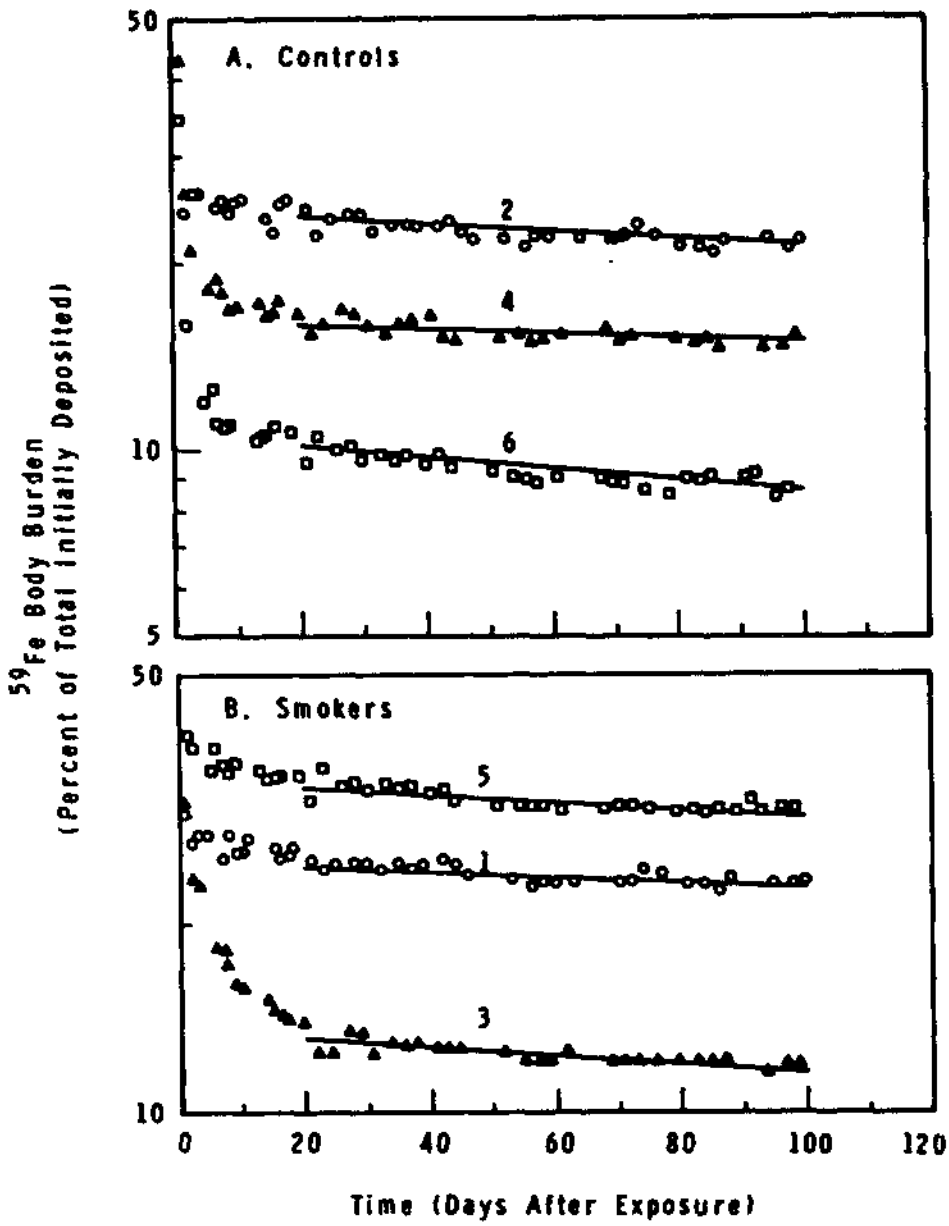

FIGURE 2. Whole-Body Retention of Inhaled ^{59}Fe in Dogs (Second $^{59}Fe_2O_3$ Test)

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The low rates of clearance of ^{59}Fe from the lower respiratory tract observed in these studies were similar to those found for ^{239}Pu. Such low rates were unexpected since other authors have published retention half times for iron oxide of about 60 days.[1] A more rapid clearance would have provided a better test for any inhibitory effects caused by smoking. This slow clearance may be due to specific chemical and physical properties of the $^{59}Fe_2O_3$ employed. To insure insolubility, it was calcined at 800 °C. The particles were irregular in shape. The iron oxides used by other authors were generally not calcined and were less irregular in shape. These differences in properties may account for the differences in retention.

Conclusions

These preliminary studies have demonstrated no gross effect of cigarette smoke on deposition and pulmonary clearance of inhaled particles in beagle dogs. These results are in contrast to a recent report from Czechoslovakia in which a single exposure of rats to cigarette smoke decreased the clearance of SiO_2 from the lung.[2] On the other hand, our results are in substantial agreement with data obtained in studies with rabbits at the Jefferson Medical College.[3] Our dogs have been subjected to further clearance tests at 480 days after start of heavy smoking and, although results are not complete, no effect of smoking seems apparent.

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EFFECT OF CYANIDE ON CLEARANCE OF INHALED PARTICLES

Cyanide, a potent ciliostatic agent and a principal component of the gaseous phase of cigarette smoke, had little effect on the early clearance of inhaled $^{59}Fe_2O_3$ particles from rat lungs. It significantly inhibited clearance over a 25-day period.

Investigator:
J. V. Dilley
Technical Assistance:
D. L. Catt[*]
L. G. Nichols[**]

The quantity of cyanide in 100 g of American cigarette tobacco has been estimated to be about 80 mg. A smoker who consumes 1-1/2 to 2 packs of cigarettes per day will have a daily intake of 12 to 16 mg of cyanide. This has been confirmed by increased excretion of thiocyanate in urine and increased levels of thiazolidine in saliva. Since cyanide is known to inhibit ciliary activity in the respiratory tract, it would seem likely that it might significantly alter the clearance of particles from the tracheobronchial tree.

Observations

Adult, female Sprague-Dawley rats were given potassium cyanide in their drinking water daily at levels of

[*] *On educational leave of absence.*
[**] *Summer employee.*

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100 mg per 100 ml of water. Sodium cyclamate was added to improve palatability. Control animals received only sodium cyclamate in their drinking water. Since cyanide is rapidly absorbed through pulmonary tissue, it seemed justifiable to administer it via drinking water, thus simplifying the administration and the regulation of daily intake.

After about 5 months on a cyanide regimen, treated and control animals were given a 15 min exposure to $^{59}Fe_2O_3$ aerosols and sacrificed in groups of four at time intervals extending to 2 hr postexposure. The lungs and trachea were counted separately from the skinned carcass for ^{59}Fe content. Results of these experiments are shown in Table I. There was little difference in rates of removal of the insoluble particles from the lungs of the experimental and control groups.

A second experiment was performed to study the slow component of pulmonary clearance. Control and cyanide-treated animals were exposed to aerosols of $^{59}Fe_2O_3$, simultaneously, for a period of 30 min. Equal groups of the control and the cyanide-treated animals were sacrificed at 0 hr, 18 hr, and 25 days postexposure. The mean ^{59}Fe lung burden for each group is shown in Table II.

TABLE I. Retention of Inhaled $^{59}Fe_2O_3$ Particles in Cyanide-Treated and Control Animals After a 15 min Aerosol Exposure

Time	Controls		Cyanide-treated	
	μCi in lung	Percent[a]	μCi in lung	Percent[a]
3 min	0.019 ± 0.005	31	0.019 ± 0.004	25
4 min	0.020 ± 0.004	22	0.020 ± 0.005	22
5 min	0.052 ± 0.009	14	0.037 ± 0.002	17
15 min	0.022 ± 0.004	24	0.024 ± 0.004	18
30 min	0.018 ± 0.002	25	0.022 ± 0.005	16
1 hr	0.041 ± 0.008	10	0.039 ± 0.005	11
2 hr	0.044 ± 0.009	10	0.052 ± 0.011	8

[a] *Expressed as percent of the total lung and skinned carcass burden that remained in the lung at the time of sacrifice. Each value represents the mean and standard error of five animals.*

TABLE II. Retention of Inhaled $^{59}Fe_2O_3$ Particles in Cyanide-Treated and Control Animals After a 30 min Aerosol Exposure

Time	Controls		Cyanide-treated	
	μCi in lung	Percent[a]	μCi in lung	Percent[a]
0 hr	0.0103 ± 0.004	100	0.0074 ± 0.001	100
18 hr	0.0098 ± 0.004	95	0.0068 ± 0.001	92
25 days	0.0045 ± 0.001	43	0.0054 ± 0.002	73

[a] *Expressed as percent of the lung deposition in animals sacrificed at 0 hr. Each value represents the mean and standard error of seven animals.*

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The lungs of the cyanide-drinking animals retained a larger percentage of the deposited ^{59}Fe, at 25 days, than did those of controls.

Conclusions

Emphasis has been placed on the importance of ciliary activity in removing inhaled particles from the respiratory system. It has been suggested that the metaplasia of the trachea seen in heavy cigarette smokers necessarily leads to an increase in the deposition and a decrease in the clearance of inhaled particles, although this has not been clearly demonstrated in human experience or in experimental animals. The results of these experiments seem to indicate that the cilia are of minor importance in removing deposited material from the lung. Considerable ^{59}Fe could have been removed by ciliary activity during the exposure period and gone unobserved under our experimental conditions; however, this seems unlikely in view of earlier experiments conducted in this laboratory in which the ciliated epithelium was damaged with aerosols of silver nitrate prior to exposures to particles of $^{51}CrO_2$. Partial denuding of the ciliated epithelium was confirmed histologically, but there was little difference in clearance of inhaled particles between treated and control animals.

The rats in this experiment had ingested cyanide in concentrations equivalent to that of a human being smoking cigarettes for about 10 years. The results suggest that a heavy smoker should have a reduced capability to clear inhaled particles from his lungs. However, tests in this laboratory with dogs trained to smoke cigarettes have failed to confirm this supposition.

Several questions concerning the mechanism of cyanide inhibition of particle clearance from the lung remain to be answered. These include the possibility of an alteration of lung structure at the cellular and subcellular levels. The detoxification of cyanide requires a readily available source of divalent sulfur and/or cysteine. Does this continual demand by this detoxifying mechanism bring about a depletion of the body sulfur reserves?

EFFECT OF CYANIDE ON LIVER ENZYME ACTIVITY

The daily ingestion of cyanide by mice for a period of 4 months depressed liver catalase activity by 35 to 40%. The major cyanide detoxifying enzyme, transsulfurase, was only slightly inhibited but was apparently changed in its tertiary structure as indicated by its increased lability to heat and 4M urea solutions.

Investigator:
J. V. Dilley
Technical Assistance:
D. L. Catt[*]
R. J. Franke
L. G. Michels[**]

Cigarette smoke has been implicated as a cause of lung cancer and other pulmonary disorders in man, but little is known of the possible causative components in tobacco smoke and their mechanisms of action. A possible working hypothesis is that a pharmacologically active component of cigarette smoke may alter normal biological function in

[*]*On educational leave of absence.*
[**]*Summer employee.*

such a manner that a second substance may be able to induce the observed biological effect. The purpose of this study was to determine whether cyanide could be such a pharmacologically active component of tobacco smoke.

Observations

One group of adult female CF_1 mice received 0.5 mg/ml of KCN and 0.125 grains/100 ml of saccharin in drinking water for 4 months. A second group received only saccharin and served as controls. The animals were sacrificed by decapitation and the livers immediately removed and weighed. Liver catalase activity was measured by the method of Fujimoto.[1] This method measures the quantity of H_2O_2 destroyed by the enzyme in an incubation mixture. The results of these studies are shown in Table I.

It is apparent that the cyanide-treated animals had a lower level of catalase activity than the controls. Recovery occurred but was not complete when cyanide exposure was terminated 24 hr prior to enzyme assay. The effect was even more apparent when total liver weights were compared. Liver weights in the cyanide-treated group were less than in the controls. Consequently the inhibition of total enzyme activity was greater than that indicated on a concentration basis.

TABLE I. Effect of Cyanide Ingestion on Liver Catalase Activity[a]

Temperature, °C	Cyanide-treated	Controls	Percent Inhibition
25	6.8 ± 0.2	10.8 ± 0.2	37
25[b]	25 ± 0.1	26.4 ± 0.2	5
Liver weight, g	1.45 ± 0.01	1.56 ± 0.14	

[a] Expressed as umoles of H_2O_2 destroyed/mg of liver tissue/10 min incubation at specified temperature. Each number is an average of 10 animals ± standard.

[b] No cyanide given for 24 hr prior to sacrifice.

Liver transsulfurase activity was measured according to the method of Saunders and Himwich,[2] except that a whole liver homogenate was used instead of a partially purified enzyme preparation. This enzyme is responsible for the detoxification of cyanide and the assay method measures the conversion of cyanide to thiocyanate. The assay was conducted at varying temperature and pH to determine the effect of these variables. Results are shown in Table II. Preparations from the cyanide-treated animals showed a smaller effect of temperature, suggesting an alteration in the enzyme resulting in a higher activation energy.

The transsulfurase activity was investigated for stability at higher temperatures by incubation at 45 °C for various periods of time prior to enzyme assay. Results of a typical experiment are shown in Table III. Preparations from the cyanide-treated animals were more sensitive to higher temperatures than were those from control animals.

These results suggested a change in the tertiary structure of the transsulfurase enzyme of the cyanide-treated mice which should involve changes in hydrogen bonding. To investigate this

TABLE II. Effect of Assay Incubation Temperature and pH on Transsulfurase Activity[a]

Temperature, °C	pH	Cyanide-treated	Control
1	7.4	4.2 ± 0.6	4.4 ± 0.27
10	7.4	5.7 ± 0.5	5.4 ± 1.03
18	7.4	7.7 ± 1.2	9.5 ± 2.42
25	7.4	14.1 ± 1.2	16.5 ± 1.10
1	8.4	4.3 ± 0.6	5.2 ± 0.5
10	8.4	6.8 ± 1.5	6.0 ± 1.7
18	8.4	7.2 ± 1.2	9.8 ± 1.6
25	8.4	15.6 ± 1.5	16.3 ± 1.0

[a] Expressed as umole of SCN produced/mg of liver tissue/per 5 min incubation. Each number is an average from five animals ± standard error.

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TABLE III. Effect of Incubation at 45 °C on Transsulfurase Activity[a]

Incubation time, min	Cyanide-treated	Control
15	1.1	1.1
30	1.1	1.2
45	0.9	1.3
60	0.9	1.4

[a] *Expressed as ug SCN formed/ml of incubation mixture/2 min. Each number is an average of five animals.*

TABLE IV. Effect of 4M Urea on Liver Transsulfurase Activity[a]

Preincubation	Cyanide-treated	Control
4M urea	0.49 ± 0.021	0.85 ± 0.18
No urea	1.05 ± 0.051	1.32 ± 0.06
Inhibition	54%	36%

[a] *Expressed as umoles SCN formed/mg liver tissue/2 min incubation. Each number is an average from eight animals ± standard error*

possibility, enzyme activity was assayed after 15 min incubation of the homogenate in 4M urea. Results, shown in Table IV, indicate that preparations from the cyanide-treated animals were more sensitive to urea incubation than were those from control animals. This supports the suggestion that there is a different quantity of hydrogen bonding in the tertiary structure of the enzyme protein.

Conclusions

Chronically ingested cyanide was shown to have an effect on at least two liver enzyme systems. The effect on catalase activity is especially interesting in view of a recent suggestion that it may be implicated in the onset of some carcinomas.[3] This effect of cyanide may create a biological environment which is more favorable to the influence of a carcinogenic agent.

Many toxic substances have the ability to induce the enzyme systems responsible for their detoxification. Cyanide was found to have the opposite effect on its major detoxifying pathway. Our studies suggest that this effect may be due to an alteration of the tertiary structure of the enzyme protein.

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EFFECT OF RADIATION ON LUNG TISSUE

In thoracic X-irradiated rats the lung collagen content increased between the second and fourteenth day postexposure, lung elastin was elevated between the tenth and twenty-fourth day, and total lung lipids were elevated between the eighth and eighteenth day.

Investigator:
E. G. Tombropoulos
Technical Assistance:
K. E. MacDonald

Numerous reports describe the lethality, histopathology, and clinical effects in animals exposed to intensive radiation of the thorax. However, the possibility of chemical changes in the lung tissue preceding the histopathologic changes has not been examined. This is a report of preliminary studies of the short-term effects of acute X-irradiation on lung tissue biochemisty.

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Observations

A group of fifty, 40-day-old Sprague-Dawley, female rats was exposed to 800 R of X-irradiation to the thoracic region. A similar unexposed group was kept as a control. The animals were killed by decapitation at intervals of 1, 2, 4, 8, 10, 14, 18, 24, 31, 38, 45, or 52 days postexposure. Lungs were removed, weighed, and homogenized, and the homogenates were extracted with chloroform and methanol (2:1). The defatted residue was analyzed for elastin and collagen. The collagen, elastin, and lipid values, expressed as percent of the corresponding values for control animals, are shown in Figure 1.

The lung collagen content of irradiated animals increased 100% by the second day postexposure and remained high for 2 weeks. The lung elastin content began to increase by the eighth day, attaining a maximum on the tenth day postexposure (100% above the controls). Although the total lung lipids increased in the irradiated animals, the ratio of phosphorus to total lipids remained essentially unchanged, suggesting that the gross composition of the lipids was unaltered.

Conclusions

Although the differences reported are statistically significant, more experiments are clearly needed before final conclusions can be drawn. If the observed transient elevation of collagen, elastin, and total lipids in the lungs of thoracic X-irradiated

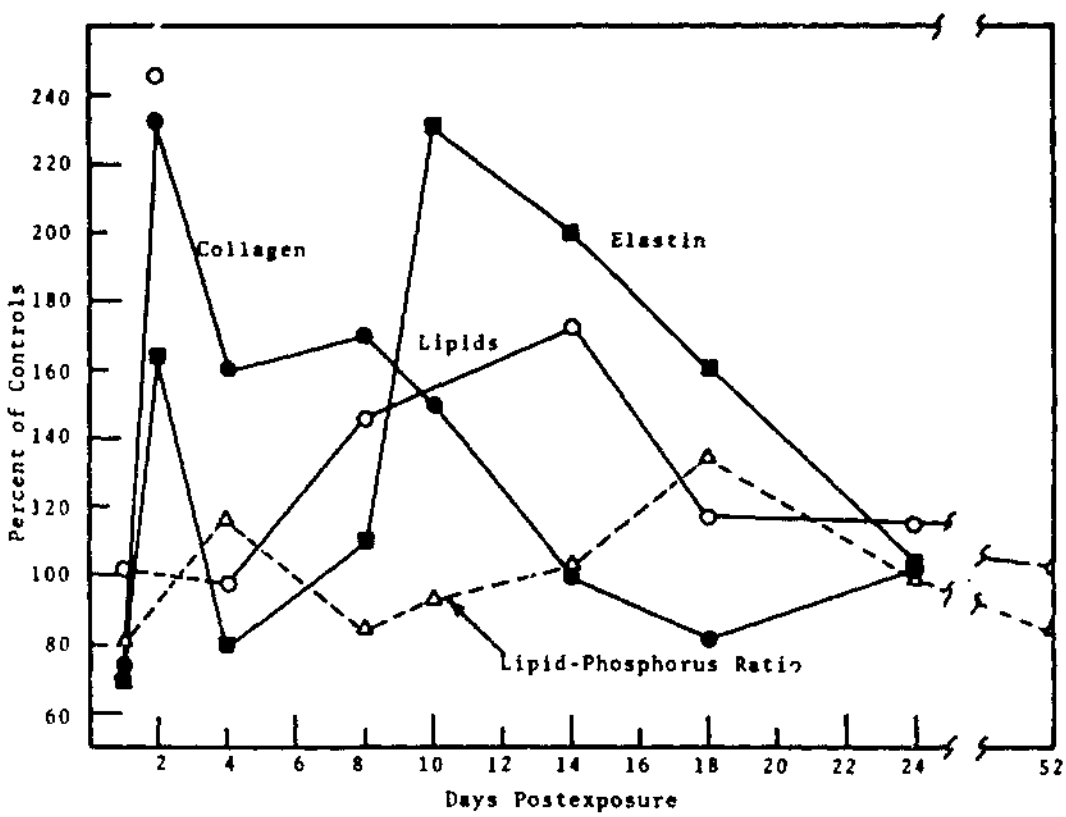

FIGURE 1. Lung Collagen, Elastin, and Lipid Content of Rats Following 800 R Thoracic X-irradiation

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animals is confirmed in subsequent experiments, several questions must be answered regarding scarring reactions or other repair processes. Does 800 R thoracic radiation produce permanent damage in the lung or does it produce only temporary changes by mobilizing collagen, elastin, and lipids or by increasing the synthesis of these compounds?

LUNG MITOCHONDRIA SYSTEM FOR FATTY ACID SYNTHESIS

The mitochondrial fraction from dog lung was shown to be the most active subcellular fraction for fatty acid synthesis. The reduction of nucleotides could be a rate-limiting step in this synthesis. Cofactor requirements, stimulators, and the distribution of ^{14}C within the product fatty acid indicate that fatty acid synthesis in rat lung mitochondria proceeds by elongation of preexisting shorter chain fatty acids.

Investigator:
E. C. Tombropoulos
Technical Assistance:
K. E. McDonald

We have reported that in rat and rabbit lung tissue the most important subcellular fraction for fatty acid synthesis is the mitochondrial fraction. The rat lung mitochondrial fraction requires ATP, CoA, NADH, and NADPH for optimal incorporation of acetate into long chain fatty acids. We have also shown that rat lung mitochondria synthesize fatty acids by elongation of preexisting shorter chain fatty acids (Annual Report, 1964). This work has been extended to the study of dog lung tissue, and further information obtained on the requirements for, and mechanisms involved in, fatty acid synthesis by rat lung mitochondria. Methods employed were similar to those previously described (Annual Report, 1964).

Observations

Lung tissue was obtained from a 4 year-old male beagle dog. Table I shows that the most active subcellular fraction for the incorporation of acetate into long-chain fatty acids was the mitochondrial fraction. These results are similar to those reported for rat and rabbit lung tissues.

To determine whether the reduction of nucleotides is a limiting step in fatty acid synthesis and whether reduced nucleotides can enter the mitochondria of our preparations, reduced and oxidized forms of nucleotides were compared as to their ability to stimulate fatty acid synthesis. The criterion for comparison was the amount of acetate incorporated into long-chain fatty acids by rat lung mitochondria (Table II).

Acetate incorporation was increased when the reduced rather than the oxidized nucleotides were added to the incubation medium. It would appear

TABLE I. Acetate-1-^{14}C Incorporation into Long-Chain Fatty Acids by Subcellular Fractions of Dog Lung Tissue

Fraction tested	Acetate incorporation after 40 min incubation, nanomoles/mg protein
Mitochondria-rich	1.10
Supernatant	0.015
Microsomal	0.11

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TABLE II. Requirement of Reduced Pyridine Nucleotides for Incorporation of Acetate-1-^{14}C into Long-Chain Fatty Acids by Rat Lung Mitochondria-Rich Fraction

Nucleotides in the incubation mixture	Acetate incorporation after 40 min incubation, nanomoles/mg protein
NADH (2 mmole) NAD (2 mmole)	
NADPH (2 mmole) NADP (2 mmole)	
NADH (2 mmole) + NADPH (0.5 mmole)	..31
NAD (2 mmole) + NADP (0.5 mmole)	0.40

TABLE III. Stimulation of Incorporation into Long-Cha Acids by Citrate, Isocitrat Glucose-6-Phosphate

Additions to the preparation	Acetate i after 40 nanomoles
None	1.
Citrate (5.0 mmole)	2.
Isocitrate (5.0 mmole)	2.
Glucose-6-phosphate (5.0 mmole)	0.
Citrate (5.0 mmole) + Glucose-6-phosphate (5.0 mmole)	1.
Isocitrate (5.0 mmole) + Glucose-6-phosphate (5.0 mmole)	1.

that under these experimental conditions, the reduction of nucleotides is a rate-limiting step. Furthermore, NADH is more important for the synthesis of long-chain fatty acids by our mitochondrial preparation than NADPH.

It has been reported that isocitrate and citrate stimulate the incorporation of acetate into long-chain fatty acids. Data are presented in Table III showing the effect of citrate, isocitrate, and glucose-6-phosphate in our system. Citrate and isocitrate stimulated the incorporation of acetate into long-chain fatty acids, but the effect was not comparable to that reported for particle-free preparations, where up to 60-fold stimulation, has been observed. This is not surprising since citrate in the particle-free preparations acts mainly by activating or stabilizing acetyl-CoA carboxylase. The fatty acid synthesis in lung mitochondria, as we will show, does not proceed through the carboxylation of acetyl-CoA. With our mitochondria-rich fraction, citrate and isocitrate apparently act as hydrogen donors. In other experiments we found that succinate, but not α-keto-glutarate, similarly stimul incorporation into long-cha The inability of glucose-6-p to stimulate fatty acid syn could be explained by the al of the appropriate enzyme in mitochondria.

Evidence has been provide on absence of HCO^-_3 requirem insensitivity to avidin, the lung mitochondrial fraction preexisting shorter-chain fa (Annual Report, 1964). To f examine this mechanism, the bution of ^{14}C activity withi product long-chain fatty aci examined. Theoretically the activity within the product acid derived from acetate-1- should be equally distribute the odd carbons if the malon pathway predominates, but wi confined to the -COOH end of acid chain if synthesis occu elongation. Degradation of acids produced by incubation rat lung mitochondria-rich f with acetate-1-^{14}C showed 60 total ^{14}C activity was in the boxyl group, and only 7.5% in methyl terminal acetate group

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Conclusions

The cofactor requirements of fatty acid synthesis, the magnitude of citrate stimulation, and the results from degradation studies all point to elongation of preexisting shorter-chain fatty acids as the main mechanism for fatty acid synthesis in lung mitochondria. These results are similar to those reported for liver tissue mitochondria where there is further evidence that this type of synthesis depends on the presence of endogenous fatty acids. Preliminary experiments in our laboratory indicate that the lung mitochondria-rich fraction is capable of esterifying fatty acids. It is therefore tempting to speculate that shorter-chain fatty acids carried to the lung by the circulation are modified by mitochondria, such as those of epithelial cells, to serve as a component of lung phospholipids. Lung mitochondria may incorporate these lipids into their structure before releasing them for other biological functions. Similar functions have been attributed to particulate fractions from other tissue, e.g., liver.

CONTROL OF DENTAL CALCULUS IN EXPERIMENTAL BEAGLES

Dental calculus was reduced in dogs when their normal soft diet was supplemented with one-half an oxtail weekly. Approximately 85% of the accumulated dental calculus was removed from the surface of the teeth after four oxtail feedings. Approximately 35% of the individual teeth of the dogs showed calculus deposits after the test period compared to 85% before oxtails were fed.

Investigators:
M. G. Brown
J. P. Park
Technical Assistance:
P. E. Bergam
J. C. Gaven
T. C. Kinnas

Experimental beagle dogs housed in runs and cages and fed soft food diets accumulate heavy deposits of dental calculus (tartar). Dental calculus may cause gingivitis, gingival lypoplasia, loose teeth, and loss of teeth in dogs. Manual removal of calculus is a laborious process requiring anesthesia, which is a risk to the dogs.

At the University of California at Davis, regular feeding of oxtails to dogs decreased calculus accumulation. Adoption of this in our colony 6 years ago confirmed its feasibility. However, it was necessary to determine the amount of oxtails and frequency of feeding required to obtain optimum effectiveness.

Observations

Four groups of six to eight dogs were removed from the regular oxtail diet for 5 weeks prior to the test period. Group I was fed one-half oxtail and Group III, one oxtail, at 7-day intervals. Group II was fed one-half oxtail and Group IV, one oxtail, at 14-day intervals. All groups were examined for calculus deposits before and after each oxtail feeding. Groups I and III were examined 7 days after the final oxtail feedings and Groups II and IV were examined 14

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days after the final oxtail feedings. The regular soft diet was not fed on days the oxtails were given to insure that the dogs chewed upon the oxtails.

Approximately 45 to 55% of all the dogs' teeth surfaces were covered with dental calculus at the beginning of the test period, before oxtails were fed. Approximately two-thirds of the accumulated calculus was removed 24 hr after the first oxtail feeding. In Groups I and III calculus accumulation dropped to approximately 5% of the total teeth surfaces after the second oxtail feeding and remained about the same throughout the test period. In Groups II and IV calculus accumulation dropped to about 15% after the second feeding and increased slightly at the end of the test period.

Approximately 75 to 90% of the individual teeth in each dog in all groups had calculus deposits at the beginning of the test period. Groups I and III dogs had about 30% less teeth with calculus deposits after the first oxtail feeding, and approximately 50% after the second and subsequent feedings. Groups II and IV dogs had about 20% less teeth with calculus deposits after the first oxtail feeding, and about 30% after the second feeding. However, in these dogs 70% of their teeth had accumulated calculus deposits 14 days after the final feeding. The canine and the first and second premolar teeth had 90% of the calculus deposits remaining at the end of the test period.

Only one dog, in Group IV, refused to chew oxtails initially, but was soon encouraged to do so. Fights occurred between some dogs housed together when oxtails were fed, but could be minimized if the dogs were separated the first 2 hr after given oxtails. Ten dogs used in the test above are still in our colony. They have received one-half oxtail every 7 days for the last 3 years. Calculus accumulation is approximately the same as was observed in Group I dogs in this test after oxtails were fed. No harmful effects of feeding oxtails have been observed in a colony of 200 dogs after more than 4 years.

Conclusions

This test confirmed the feasibility of preventing the accumulation of dental calculus in experimental dogs by regular feeding of oxtails. Manual removal of calculus was not required when dogs were fed one-half or one whole oxtail per week. Biweekly feeding of one-half to one oxtail was 20 to 30% less effective. No harmful effects of feeding oxtails have been observed.

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COMBATING DETRIMENTAL EFFECTS OF RADIATION

The three reports which follow do not represent our total effort in this category. Several reports concerned with the removal of inhaled radionuclides and with basic studies of pulmonary physiology are grouped in the previous section on inhalation studies.

The past year has been one of transition in this area, with a lessening of emphasis on the screening of new chelating agents for their possible effectiveness in removal of internally deposited radionuclides, and with increasing attention to the application of proven agents to practical problem situations. Due to personnel changes, our efforts of the past several years in the area of radiation protection by hematopoietic system transplants were terminated.

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REMOVAL OF INTERNALLY DEPOSITED RADIONUCLIDES

Deferoxamine (DFA) was not beneficial, by itself, or in combination with diethylenetriaminepentaacetic acid (DTPA), in preventing radioactive cerium deposition. Nor were vancomycin, cephalothin, or Pluronics F-68 of any benefit by themselves, or as an adjunct to DTPA treatment, in preventing plutonium deposition.

Investigator:
V. B. Smith
Technical Assistance:
B. P. Neal

The success of DFA in reducing the deposition of plutonium in bone and its additive effect with DTPA (Annual Report, 1964) prompted a test of the effectiveness of this treatment procedure in reducing the deposition of ^{144}Ce, which is physiologically similar to plutonium. Two chelating antibiotics were tested for possible effect on plutonium deposition.

Observations

Female, Sprague-Dawley rats, ten per treatment group, were injected intravenously with 4.9 or 1.1 μCi of ^{144}Ce-$^{144}PrCl_3$ at pH 3 or with ^{239}Pu as the pH 5.0 citrate complex. Two treatment regimens were followed; either one treatment given 1 hr after the radionuclide or three treatments at 1, 5, and 24 hr. For better counting statistics the larger ^{144}Ce dose was used in the multiple treatment experiment. At the end of the fifth day, the animals were killed by exsanguination under ether anesthesia, and tissues taken for radioanalyses.

Results of the ^{144}Ce studies are shown in Table I. Three treatments appear superior to a single treatment; however, this conclusion is not strictly valid because different isotope and total treatment doses were used. The DFA had a slight effect in the spleen and femur, but clearly was of no practical utility either alone or in combination with DTPA. Pluronics F-68, a biologically compatible, nonionic, surfactant and surface tension depressant, was ineffective though it had been hoped that it might make the cerium more accessible to the DTPA.

Cephalothin and vancomycin are two chelating antibiotics used to combat bone infections. As can be seen from Table II, they were of no value in preventing the deposition of plutonium, by themselves or in conjunction with DTPA.

In multiple treatment situations, Catsch[1] recommends use of the zinc salt of DTPA because it is less toxic,

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TABLE I. Effects of Treatment on ^{144}Ce-^{144}Pr Decorporation

Single treatment	Fraction of control concentrations (Average values from ten rats) Liver	Kidneys	Spleen	Femur	Excreta
DFA[a]	--[b]	--	0.58	--	--
DTPA[c]	0.36	0.41	0.58	0.50	1.5
DFA • DTPA	0.44	0.47	0.52	0.46	1.4
Pluronics F-68[d]	--	--	--	--	--
Pluronics F-68[d] • DTPA	0.31	0.48	0.56	0.49	1.6
Saline controls[e]	(33)	(1.3)	(0.48)	(0.76)	(16)
Three treatments					
DFA	--	--	0.65	0.79	--
DFA • DTPA	0.44	0.47	0.70	0.35	4
DTPA	0.45	0.51	0.70	0.40	4
Saline controls[e]	(22)	(0.91)	(0.58)	(0.72)	(15)

[a] *Desferal (CIBA, Ltd.) given im at 0.1 mmole per treatment; pH = 7.3.*

[b] *--Indicates not significantly different from the control values at the 95% confidence interval.*

[c] *DTPA given ip at 0.1 mmole per treatment with 0.09 mmole Ca (as Ca gluconate-Ca glunoheptonate, Abbot); pH = 7.2.*

[d] *Pluronics F-68 (Wyandotte Chemicals Corp.) given ip 600 mg in 3.6 cc H_2O per treatment; pH = 7.2.*

[e] *Three cc of physiological saline given ip per treatment. Values given are percent of administered dose.*

and almost as effective as the calcium salt. Our results (Table II) do not invalidate Catsch's recommendation since the number of treatments were too few and the duration of the treatment schedule too short, but the greater efficacy of the calcium salt for preventing plutonium deposition is aptly demonstrated.

Histologic examination of tissues from rats used in previously reported plutonium removal experiments (Annual Report, 1964) show greater damage resulting from combined DFA-DTPA treatment than when either agent was used alone. This does not contraindicate use of the combination for preventing deposition of plutonium, but caution is indicated if there is suspicion of kidney or liver disease, and the combination should be avoided if treatment is to extend over several months.

(The cephalothin and vancomycin were supplied through the courtesy of Dr. R. A. Griffith, Lilly Laboratory for Clinical Research, the deferoxamine through the courtesy of Dr. H. Bickel, CIBA, Ltd., and the Zn DTPA through the courtesy of Dr. M. W. Weiner, Geigy Research Laboratories.)

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TABLE II. Effect of Treatment of ^{239}Pu Deoorporation

Percent of Administered ^{239}Pu in Tissues and Excreta
(Average values from ten rats)

Treatment	Liver	Kidneys	Spleen	Femur	Cumulated excreta
Vancomycin[a]	12	1.1	0.34	3.3	7.2
Vancomycin + DTPA[b]	0.41	0.52	0.033	0.30	73
Cephalothin[c]	13	1.1	0.27	3.3	6.4
Cephalothin + DTPA	0.46	0.22	0.53	0.39	83
Zn DTPA[d]	2.7	0.43	0.21	1.4	53
DTPA[b]	0.39	0.22	0.038	0.35	90
Physiological saline	14	1.1	0.24	3.2	8.7

[a] *160 mg/kg of vancomycin (Lilly), pH 3.6, given ip at 1, 5, and 24 hr after the plutonium.*

[b] *0.1 mmole of DTPA with 0.9 mmole Ca, pH 7.0, given ip on above schedule.*

[c] *240 mg/kg of Keflin (Lilly), pH 6.6, given im on above schedule.*

[d] *0.1 mmole Na_3Zn DTPA: 4 1/2 H_2O (Geigy), pH 7.1, given ip on above schedule.*

TRANSLOCATION OF SUBCUTANEOUSLY DEPOSITED PLUTONIUM

Following subcutaneous injection of $^{239}PuO_2$ in miniature swine, soft tissue concentrations of ^{239}Pu showed a continuing increase over a 1 year period. Treatments designed to alter translocation of ^{239}Pu(IV)nitrate in rats were generally of little effect.

Investigators:
Beatrice J. MaClanahan
H. A. Ragan

Technical Assistance:
J. L. Beamer
P. L. Sheldon

In continuation of earlier work (Annual Report, 1964) miniature swine were injected subcutaneously on each foreleg with $^{239}PuO_2$ in 0.1% polypropyleneglycol ethyleneoxide polymer to obtain information on plutonium translocation that would be useful in evaluating cases of accidental exposure. Preliminary results were also obtained on the effects of diethylenetriaminepentaacetic acid (DTPA) and dimethylsulfoxide (DMSO) on translocation following subcutaneous injections of ^{239}Pu(IV) nitrate in rats.

Observations

Table I contains the summary of the data available on the ^{239}Pu content of swine tissues at different times after the subcutaneous injection of 3.8 to 7 μCi $^{239}PuO_2$. The rate of translocation was much lower when the plutonium was administered as the oxide rather than as the nitrate (Annual Report, 1963). However, the regional lymph nodes accumulated significant quantities of plutonium, suggesting that experiments should be undertaken to establish the effects of long-term alpha irradiation

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TABLE I. *^{239}Pu Concentrations in the Soft Tissue of Swine Following Subcutaneous Injection of $^{239}PuO_2$ (Percent of Injected Dose)*

Days after injection	No. of animals	Liver	Kidney	Spleen	Regional lymph nodes	Injection site
1	3	0.0094	0.00058	0.00029	1.1	40
7	2	0.0075	0.00034	0.00035	4.6	54
30	2	0.044	0.00053	0.00069	3.5	27
60	1	0.050	0.0021	0.00044	3.2	36
90	1	0.28	0.0019	0.023	0.75	34
372	1	0.48	0.0051	0.012	11	32

on these tissues. Additional data should be obtained to ascertain the turnover of particulate plutonium of very low solubility.

Figure 1 describes the influence of DMSO and DTPA on the migration of 3.7 μCi of subcutaneously deposited ^{239}Pu-(IV) nitrate in rats. The treatments were initiated 4 days after plutonium administration and were continued until the animals were killed after five daily treatments. One milliliter of 5% DMSO, administered subcutaneously at the injection site daily, was without effect on plutonium metabolism. When 0.25 ml DTPA, dissolved in 80% DMSO at a concentration of 42 mg/ml, was rubbed on the rat's skin at the site of injection four times a day, there was a significant reduction in the liver deposition. Since there were no animals that received DTPA without DMSO, it cannot be stated unequivocally that DMSO is necessary for percutaneous penetration of DTPA. The ^{239}Pu content of liver was reduced to the same extent when 14 mg/kg DTPA was injected subcutaneously once a day either at or away from the site of plutonium injection, or when it was administered intravenously. None of the treatments significantly affected the amount of the ^{239}Pu remaining at the site of injection. DTPA achieved an increased urinary excretion regardless of its mode of introduction.

Conclusions

Additional data should be accumulated on the metabolism of subcutaneously deposited $^{239}PuO_2$ to aid in more accurately evaluating its potential hazard. DMSO appeared to have no influence on plutonium metabolism under the conditions employed in this study. DTPA was equally effective in reducing liver and

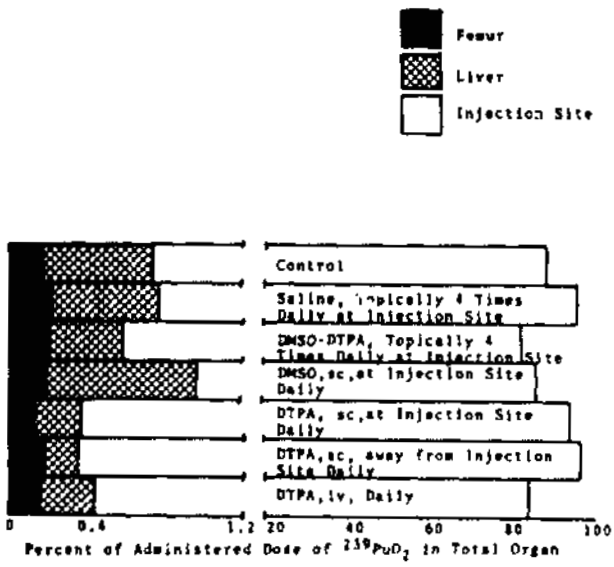

FIGURE 1. Effect of Dimethylsulfoxide (DMSO) and Diethylenetriaminepentaacetic Acid (DTPA) on Retention of ^{239}Pu in Rats

0012407

femur concentrations whether it was injected subcutaneously or intravenously, but it had no detectable influence on the amount of plutonium remaining at the site.

REMOVAL OF THYROID-BOUND IODINE

Thiazolidine-4-carboxylic acid was effective in removing thyroid-bound ^{131}I in rats. The most effective dose is well below the acute LD_{50} giving it an excellent chemotherapeutic index of over five.

Investigator:
J. V. Dilley
Technical Assistance:
*D. L. Catt**

An exposure to ^{131}I by any route will result in rapid uptake by, and irradiation of, the thyroid gland. Opportunities for therapeutic treatment involve blocking the thyroid iodine-trapping mechanism, hastening the turnover of the organic iodine, or increasing the rate of clearance of iodine from the thyroid. The effectiveness of these procedures would be enhanced if the excretion rate from the body was also elevated.

Observations

All experiments were performed on groups of ten female Sprague-Dawley rats weighing between 250 and 300 g. The ^{131}I and the drugs were administered by intraperitoneal injection. Animals were killed and thyroids counted 2 to 4 hr following the last treatment. Results of all experiments are shown in Table I.

The first, single dose experiment compared thiazolidine-4-carboxylic acid, a diuretic; ammonium chloride; and a pyretic, 2,4-dinitrophenol; a control group was treated with normal saline. The efficacy of thiazolidine was confirmed in a second experiment in which animals were treated at 1 and 20 hr after ^{131}I injection. Combined treatment with thiazolidine and ammonium chloride was even more effective.

The dose level of thiazolidine in the first two experiments approached a toxic level for a single injection. For this reason and because a fractional dose regimen is usually favored for drug administration, a third experiment was conducted to determine an optimal multiple dose regimen. The lowest level of thiazolidine (25 mg/kg) was the most effective; the highest dose, although effective

TABLE I. Effect of Various Pharmacological Agents on the Level of ^{131}I in the Thyroid of Rats

	mg/kg	^{131}I in thyroid % of control
Single Treatment 1 hr after ^{131}I injection		
Thiazolidine	100	85
Ammonium chloride	100	95
2,4-dinitrophenol	4	128
Treated at 1 and 20 hr after ^{131}I injection		
Thiazolidine	100	86
Thiazolidine + ammonium chloride	100	80
Treated at 3,24,48, and 68 hr after ^{131}I injection		
Thiazolidine	25	76
"	50	86
"	75	97
"	100	127
Treated at 1 and 20 hr after ^{131}I injection		
Thiazolidine	100	97
2,4-dinitrophenol	4	142

*On educational leave of absence.

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when given as a single injection, increased thyroid ^{131}I when given repeatedly. This latter observation is often seen when therapeutic agents are given at near toxic levels.

A fourth experiment was conducted to compare 2,4-dinitrophenol administered 1 and 20 hr after ^{131}I injection. The 2,4-dinitrophenol causes a profound increase in the basal metabolic rate of rats, resulting in a thyroid stimulation similar to TSH. Since 2,4-dinitrophenol consistently elicited an increase in the thyroid ^{131}I, the mechanism of action of the thiazolidine must not be one of increasing thyroid turnover of organic iodine.

Conclusions

These experiments demonstrate the effectiveness of a new type of chemical agent in removing ^{131}I from rat thyroid tissue. The effect obtained is comparable in magnitude to that obtained by others using a "shotgun therapy" with combinations of $KClO_4$, TSH, stable iodine, and a potent diuretic, hydrochlorthiazide.[1] Thiazolidine, alone or in combination with ammonium chloride, offers advantages of simplicity of administration and avoids the hazardous side effects of TSH, $KClO_4$, and Lugol's solution. Thiazolidines are known to have some radioprotective activity when given prior to X-irradiation and may thus afford an additional benefit unconnected with the effect on ^{131}I clearance rate.

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0012409

MOLECULAR AND CELLULAR LEVEL STUDIES

Although molecular and cellular level studies do not constitute a major feature of our program, it is our conviction that no modern biological laboratory can properly function without competence in this area. The two reports included in this section by no means reflect the total extent of our molecular and cellular effort. Additional studies are involved in support of other investigations, e.g., ultrastructural studies in connection with the investigation of the intestinal radiation syndrome, and in connection with studies of temperature acclimation in fish; microbiological studies related to the diseases of Columbia River fish; and basic biochemical studies of lung physiology in support of our inhalation program.

The two reports which follow deal with continuing studies in microbiological systems which are not only of fundamental biochemical interest, but which also promise to provide interesting model systems for the study of basic radiation effects.

0012410

THE EFFECT OF METHIONINE ON SYNTHESIS OF RIBONUCLEIC ACID IN ESCHERICHIA COLI

The relative rate of RNA synthesis by Escherichia coli is increased upon addition of methionine to a minimal medium. The effect is not confined to a single class of RNA. The relative rate of methylation of RNA is high in cells recovering from chloramphenicol poisoning, intermediate in cells grown in minimal medium, and low in cells grown in medium containing methionine. Methionine enhances the rate of conversion of chloramphenicol ribonucleoprotein particles to 70 S ribosomes. From these findings, we infer that methionine increases the relative rate of RNA synthesis by increasing the rate of conversion of ribonucleoprotein particles to ribosomes and thereby decreasing their availability for regulating RNA synthesis by feedback inhibition.

Investigator:
W. H. Matchett
Technical Assistance:
Laura S. Winn

We have previously reported an increase in the relative rate of RNA synthesis in E. coli when the single amino acid methionine was added to a minimal medium (Annual Report, 1964). The rate of growth of the cells was not increased significantly by this addition. These observations led us to postulate a peculiar role for methionine in the regulation of RNA synthesis. The experiments described here were carried out in an attempt to identify the role of methionine in the regulation of RNA synthesis.

Observations

It was of interest, first, to examine the RNA synthesized by cells in the presence of methionine and to compare this RNA with that synthesized by cells in minimal medium. For this purpose cells were grown for many generations in a nonradioactive medium, either minimal or methionine-rich. Cells were then transferred quickly to fresh media containing 2-^{14}C-uracil. The transfers were made from minimal media to minimal media, from minimal to methionine-rich, and from methionine-rich to minimal. Cultures were sampled at intervals, whole cells counted to assess the relative rate of RNA synthesis for each culture, and crude extracts of the cells examined by the method of zone centrifugation through sucrose density gradients. In all cases, there was a close correspondence between the optical density profile and the radioactivity profile,

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indicating that neither the shifts between media nor the presence of methionine caused the synthesis of an extraordinary species of RNA. The data on relative rates of synthesis of RNA in these cultures were consistent with previously reported results. Cells shifted from minimal to methionine medium showed the highest relative rate; cells shifted from minimal to fresh minimal medium showed an intermediate rate; and cells shifted from methionine to minimal medium showed the lowest relative rate of synthesis of RNA. These results showed that methionine increases the relative rate of synthesis of all classes of RNA.

The role of methionine as a donor of methyl groups is, of course, well known. It was, therefore, desirable to test the idea that methionine was enhancing the rate of methylation of RNA, or perhaps altering the amount of methylated RNA in the cells, and that this in turn resulted in an increased rate of synthesis of RNA. This question was approached by examining the relative rate of methylation of RNA in cells which had been grown either in minimal medium or in methionine-containing medium. It was assumed that cells with methyl-deficient RNA would show a higher initial relative rate of methylation when shifted to a medium containing ^{14}C-methyl-methionine. Conversely, it was assumed that cells with methyl-saturated RNA would show a lower initial relative rate of methylation. That this is indeed the case is shown by the data summarized in Figure 1. Cells poisoned with chloramphenicol (CAP) are known to synthesize RNA which is deficient in methyl groups; further, they are known to exhibit a higher than normal relative rate of methylation of RNA during recovery from CAP poisoning.(1) The initial relative rate of methylation of such cells, as shown in Figure 1, was much higher than that observed for cells grown in the presence of a large excess of methionine. Cells grown in a minimal medium showed a relative rate of methylation of RNA intermediate between these extremes. The inference drawn from these results is that cells grown in excess methionine have a relatively highly methylated RNA. The question now posed by these findings concerned the role of methylation of RNA in the overall regulation of the rate of synthesis of RNA.

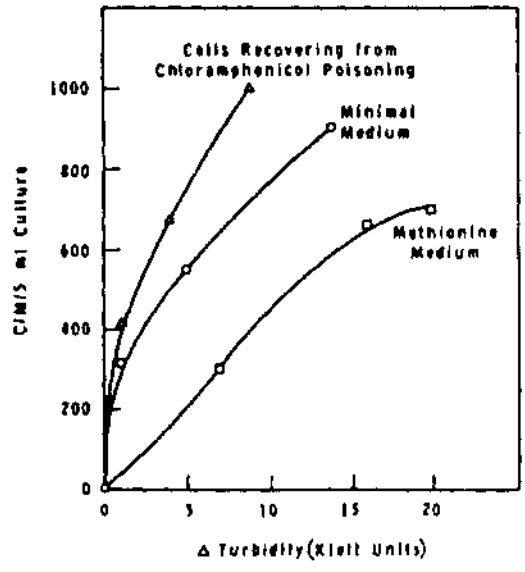


FIGURE 1. Relative Rate of Methylation of RNA in Cells as Indicated by ^{14}C Incorporation from ^{14}C-Methyl-Methionine

0012413

From the experiments of Borek,[2] it is known that methylation of RNA occurs at a step subsequent to polymerization of nucleotides. Gordon, Boman and Isaksson[1] showed that CAP particles [i.e., ribonucleoprotein (RNP) particles formed in the presence of CAP] were an excellent substrate for incorporation of methyl groups and that mature ribosomes were essentially devoid of any acceptor activity in an in vitro methylating system. Several workers have shown recently that CAP particles behave kinetically, as if they were the precursors of mature 70 S ribosomes. In view of these findings, it seemed desirable to test the effect of methionine on the rate of conversion of CAP particles to mature 70 S ribosomes. The results of such an experiment are presented in Figure 2.

For this experiment, cells grown in minimal medium were challenged with CAP (50 µg/ml). After 30 min, 2-^{14}C-uracil was added to the culture and the incubation was continued for 30 min. Under these conditions, about 60% of the total radioactive uracil incorporated by the cells is present in the form of CAP particles. The cells were then removed from the radioactive medium and washed with cold distilled water and cold fresh medium containing ^{12}C-uracil. These washes removed all traces of CAP and 2-^{14}C-uracil. The cells were then suspended in fresh minimal medium containing ^{12}C-uracil (10 µg/ml) and recovery from CAP poisoning was allowed to proceed. One sample of cells was quickly chilled, washed with buffer, sonically disrupted, and extracted with tris buffer containing 10^{-2}M Mg^{++}. The remaining cells were divided into three equal

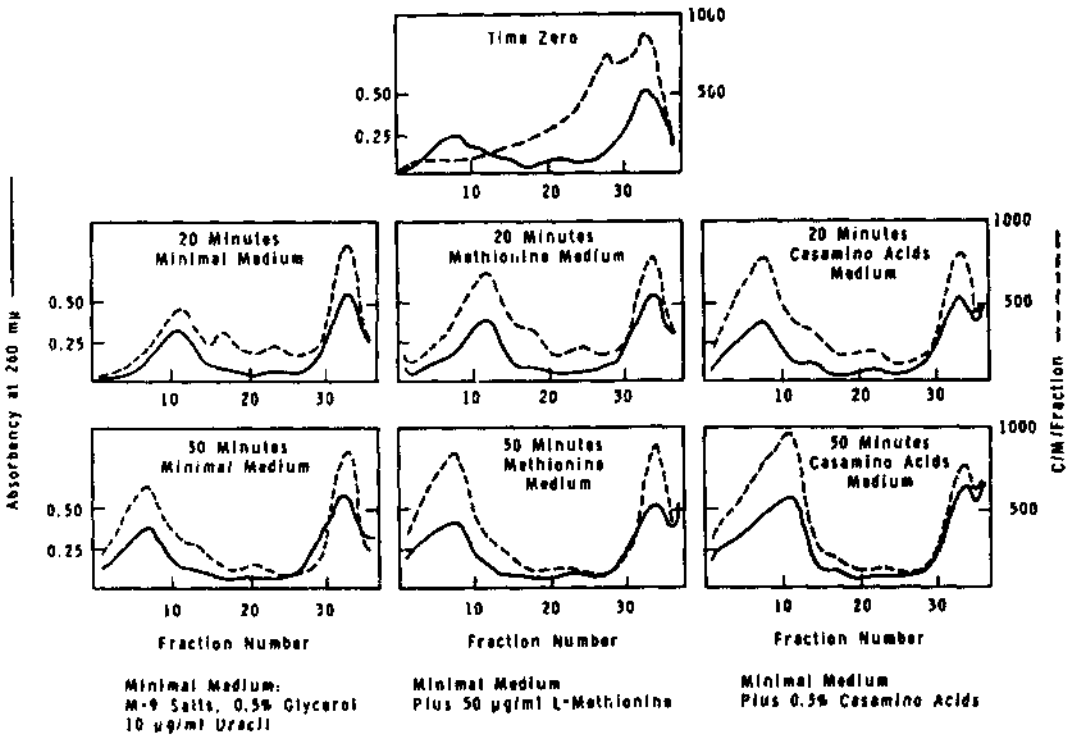


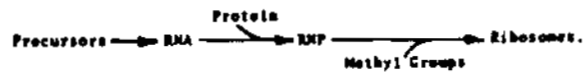
FIGURE 2. Conversion of Chloramphenicol Ribonucleoprotein Particles to Ribosomes in Various Recovery Media

0012414

portions. One of these received casamino acids (0.5%), another received methionine (50 µg/ml), and the third received no supplement. The incubation was continued and samples of each culture were removed after 20 and 50 min. Extracts were prepared in the manner described for the zero time sample. All extracts were examined by the method of zone centrifugation through density gradients of 5 to 20% sucrose. From the density gradient profiles shown in Figure 2, the CAP-RNP material present at time zero undergoes a gradual shift in sedimentation velocity (and therefore size) with time in all three cultures studied. This shift in sedimentation velocity has been interpreted elsewhere as the conversion of CAP-RNP particles to mature 70 S ribosomes.[3] The presence of casamino acids in the recovery medium increases the rate of this conversion. Similarly, addition of the single amino acid, methionine increases the rate of this conversion.

Conclusions

The clear implication of the experiments summarized in Figures 1 and 2 is that methionine enhances the rate of formation of 70 S ribosomes from CAP-RNP particles by stimulating the rate of methylation of these particles, or in other words, by relieving that part of the rate limitation imposed by the requirement of methylation of these particles. The increase in the overall rate of RNA synthesis observed in the presence of methionine and the results reported here suggest a model for regulation of RNA synthesis. The model can be visualized as follows:



It is necessary to assume that under certain conditions the rate of methylation limits the rate of conversion of RNP particles to ribosomes and that the accumulation of RNP particles limits the rate of synthesis of RNA from precursors. This model is attractive in that it permits interpretation of the inordinately long lags observed before resumption of normal relative rates of synthesis of RNA in cells recovering from CAP poisoning and in relaxed mutants recovering from amino acid starvation (such mutants accumulate large quantities of RNP particles under conditions of amino acid deprivation). According to the model these long lags would result from the repressive effects of high concentrations of RNP particles on RNA synthesis.

The model predicts that RNP particles should inhibit the transcription of RNA from DNA and therefore that they should inhibit the DNA-dependent RNA synthesis of a subcellular system. This prediction will be tested in future work.

References

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2. *Borek, E., Cold Spring Harbor Symposium Quantitative Biology, vol. 28, pp. 139-148. 1963.*

3. *Nomura, M. and K. Hosokawa. J. Mol. Biol., vol. 12, pp. 242-265. 1965.*

0012415

AMINO ACID TRANSPORT IN NEUROSPORA CRASSA

Tryptophan transport in N. crassa is mediated by a specific transport system. The process is stereospecific, shows the typical substrate saturation kinetics for enzymic reactions and a sharp decrease in the rate of tryptophan uptake at low temperatures. The structural requirements apparently necessary for reaction with the "binding site" are (1) an α-amino group next to a carboxyl, and (2) a free $-CH_2$ group in the β position.

Investigator:
W. R. Wiley
Technical Assistance:
Laura S. Winn

The mechanism of amino acid transport in fungi is unknown. The present investigations were undertaken to examine the biochemical properties of the trypotphan transport system in the bread mold, Neurospora crassa. We were especially concerned with obtaining evidence for a "binding site" which combines with tryptophan and controls its entry and with defining the structural and stereochemical specificity of this binding site.

Observations

Time Course of Tryptophan Uptake

As shown in Figure 1, tryptophan uptake begins immediately following the addition of tryptophan to the medium. Transport continues at a constant rate until the supply in the medium approaches depletion. Soluble "pool" tryptophan rises rapidly at first, remains constant for a period and finally decreases as the pool tryptophan is converted into protein. Incorporation of labeled tryptophan into protein or acid precipitable material is preceded by a 90 sec lag. These results demonstrate that tryptophan in the amino acid pool is available for protein synthesis. Further, the data show that passage of tryptophan into the pools is a necessary step in protein synthesis.

Dependence of the Rate of Uptake on Substrate Concentration

A criterion for establishing the existence of a specific transport system in living cells is the demonstration that the initial velocity of uptake does not increase indefinitely with increasing substrate concentration, but tends to become saturated. Figure 2 shows a double reciprocal plot of substrate concentration versus the initial velocity of tryptophan uptake by N. crassa. As shown, there is a

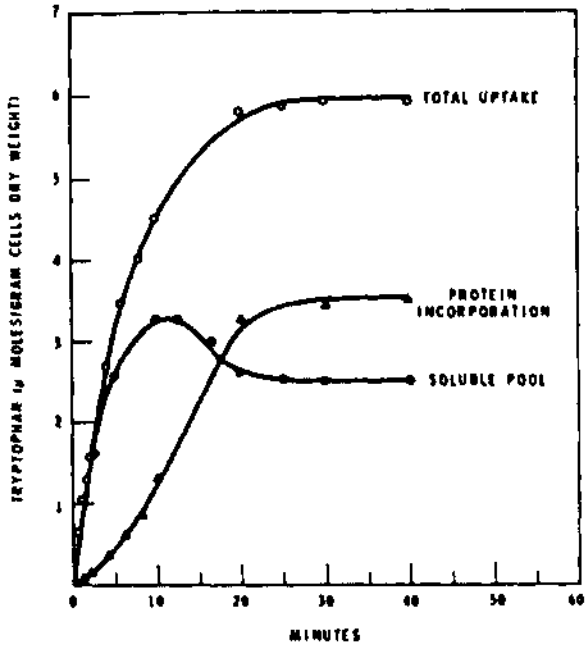

FIGURE 1. Time Course of Tryptophan Uptake and Incorporation into Protein

0012416

proportionality between the initial rate of uptake and the extracellular tryptophan concentration. The apparent Km is 5.0×10^{-5} M. These data provide strong support for the contention that tryptophan transport in N. crassa is mediated by a reactive site of limited availability.

Additional support for a binding site for transport is its stereospecificity, evidenced by the fact that D-tryptophan does not affect L-^{14}C tryptophan transport.

The possibility that we were measuring an intracellular binding site, independent of the transport site, was eliminated by demonstrating that the rate of tryptophan uptake is maximal at an external concentration, which is approximately 100-fold lower than the concentration at which the intracellular pool saturates.

The Effect of Temperature and pH on Transport

The effect of temperature on the rate of tryptophan uptake was investigated with results as shown in Figure 3. The Q_{10} between 20 and 40 °C is 2.01. The sharp decrease in the rate of transport at low temperatures is much greater than would be expected for thermal diffusion. This fact provides further support for the enzymic nature of the uptake process.

Although the rate of tryptophan uptake at 0 °C is practically nil, intracellular pools formed at 30 °C are completely stable. This is shown in Figure 4. The decrease in pool tryptophan at 30 °C is not a result of leakage from the pool but is probably a result of incorporation of tryptophan into protein and conversion to other intermediates of the tryptophan cycle.

Tryptophan transport is also pH dependent. The optimal range for transport is 5.8 to 6.0. The rate of uptake decreases sharply at pH values below 5.0 and above 7.5.

Effect of other Amino Acids on Tryptophan Transport

The effect of a number of other amino acids on tryptophan transport

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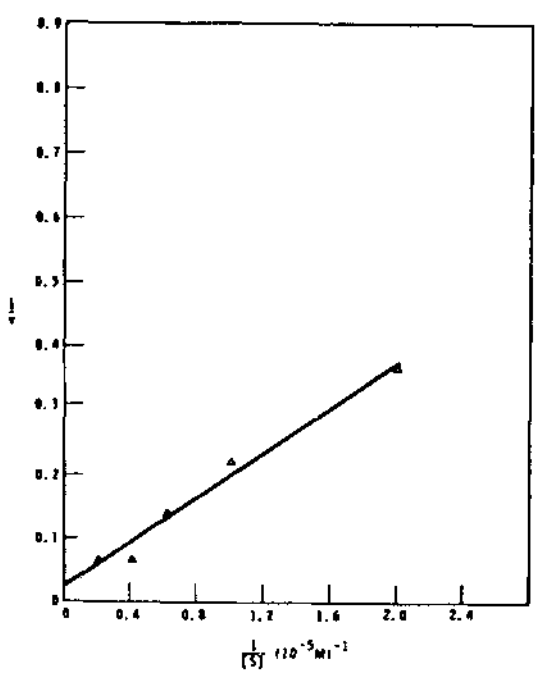

FIGURE 2. Effect of Tryptophan Concentration on Rate of Uptake

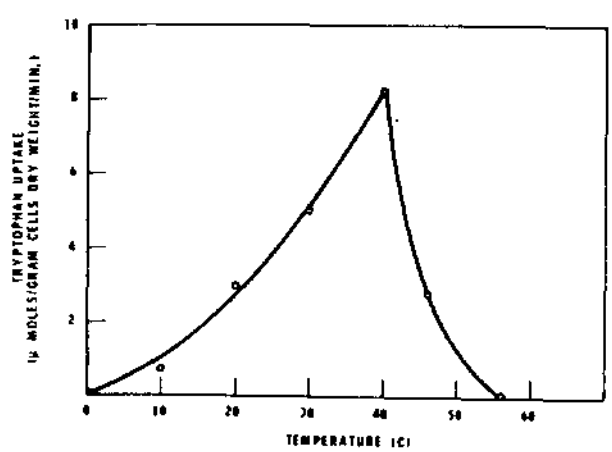

FIGURE 3. Effect of Temperature on Uptake

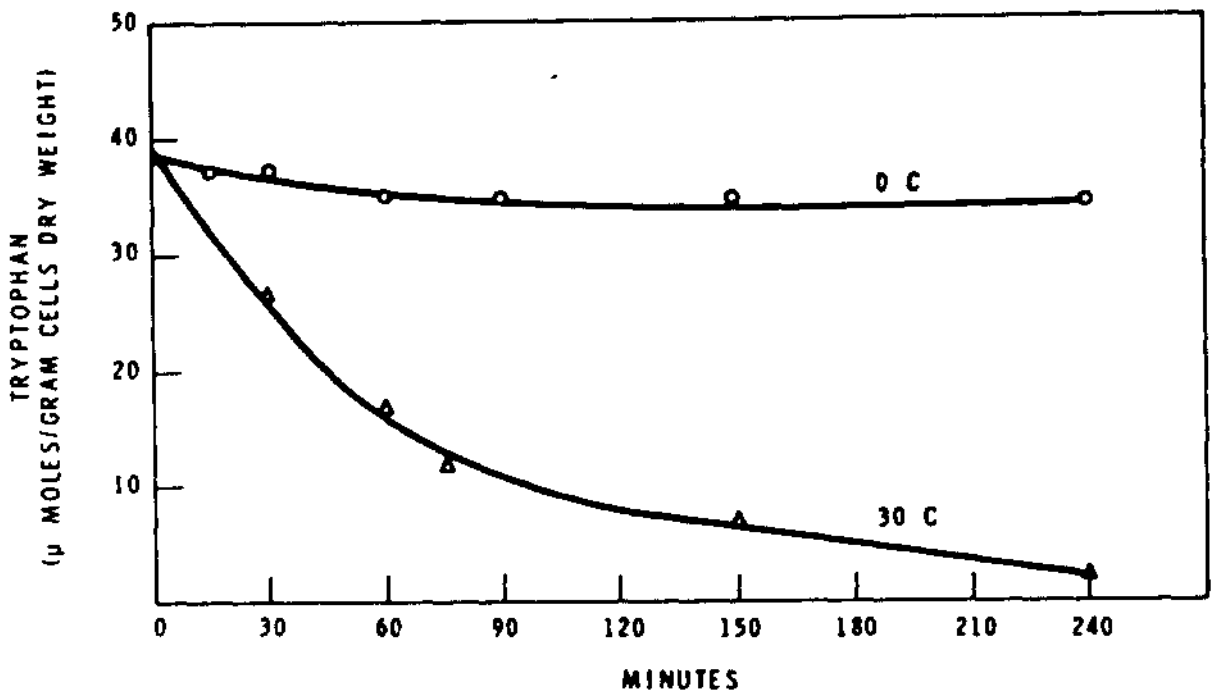

FIGURE 6. Retention of Preformed Tryptophan Pools at 0 °C

was investigated. As shown in Table I, leucine, methionine, phenylalanine, ethionine, serine, tyrosine, cysteine, and histidine showed a 50% or greater inhibition of tryptophan transport. In contrast, alanine, isoleucine, valine, and glycine affected transport only slightly, if at all.

The nature of leucine inhibition of tryptophan uptake was further investigated. As indicated by the Lineweaver-Burk double reciprocal plot shown in Figure 5, leucine competitively inhibits tryptophan transport. The apparent Ki for the process is 1.1 x 10^{-4}M. Leucine affects tryptophan transport, and not the subsequent metabolism of tryptophan, as evidenced by the fact that the utilization of preformed tryptophan pools is unaffected by extracellular leucine present at concentrations which provide a 90% inhibition in the rate of tryptophan uptake. All attempts to demonstrate a leakage of tryptophan from the pool, in the presence or absence of tryptophan, were uniformally negative.

The results suggest quite convincingly that leucine exerts its inhibitory effect on the entry process and not on the utilization or retention of tryptophan. It thus appears that tryptophan and leucine bind a common reactive site for transport.

Structural Requirements for the Inhibition of Tryptophan Uptake

By modifications in the molecular structures to both leucine and tryptophan, it was possible to show that an α-amino group next to a carboxyl and a $-CH_2$ group in the β position are necessary requirements for combining with the tryptophan transport site. It should be emphasized that indole is

0012418

TABLE I. Effect of Other Amino Acids on Tryptophan Uptake (External tryptophan concentration 1 x 10^{-5} M)

Inhibitor	Concentration	% Inhibition of the rate of uptake
L-leucine	5 x 10^{-4}	90
L-methionine	4 x 10^{-4}	82
L-ethionine	4 x 10^{-4}	82
L-phenylalanine	4 x 10^{-4}	95
L-tyrosine	4 x 10^{-4}	72
L-serine	4 x 10^{-4}	53
L-histidine	5 x 10^{-4}	47
L-cysteine	4 x 10^{-4}	77
L-isoleucine	4 x 10^{-4}	11
L-alanine	5 x 10^{-4}	20
L-valine	5 x 10^{-4}	9
Glycine	5 x 10^{-4}	0

not an inhibitor of tryptophan uptake. As shown in Table I, alanine is also not an inhibitor of tryptophan uptake, which demonstrates the extreme specificity of the binding site.

Conclusions

The evidence presented demonstrates quite conclusively that tryptophan transport in Neurospora crassa is mediated by a specific transport system, enzymic in nature, and stereospecific. The competitive inhibition of tryptophan transport by leucine and other amino acids with similar structural

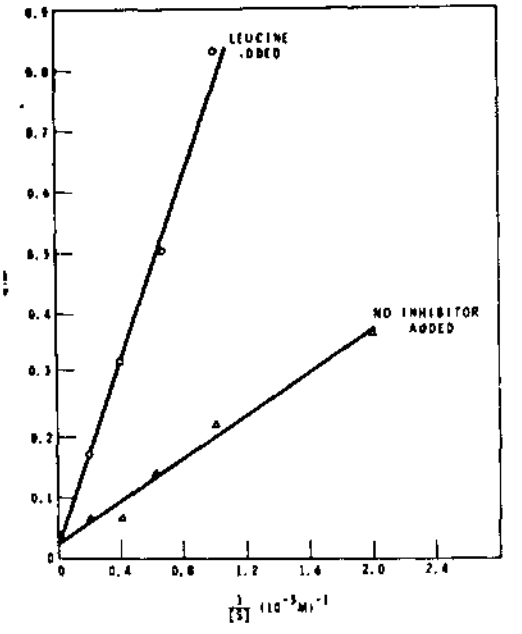

FIGURE 5. Competitive Inhibition by Leucine of Tryptophan Transport

relationships in the vicinity of the α-amino carboxyl suggests that "families" of amino acids are transported by the same reactive site. Further studies presently underway are expected to define more concisely the mechanism of amino acid transport in N. crassa.

0012419

ENVIRONMENTAL RADIATION STUDIES - TERRESTRIAL

Our environmental studies programs, both terrestrial and aquatic, were severely handicapped as a result of the destruction of their principal laboratory facilities, by fire, in November, 1964. By mid-1965, however, temporary facilities were in place and most programs were again in full swing.

The fourth in our series of annual Hanford Symposia was held May 3-5, 1965, on the subject, "Radiation and Terrestrial Ecosystems." Forty-three papers presented at this symposium were published as a special December, 1965, issue of Health Physics.

The first two reports in this section reflect our continuing concern with the unusual arctic ecosystem which results in relatively high deposition of fallout ^{137}Cs in some of the natives of far-northern lands. These studies, while expensive and somewhat disruptive of our more normal program of desert ecology studies, are, we feel, of vital concern and must be continued.

Our plant physiology program is poorly represented in this report due to the absence on short-term leave of the principal investigator primarily concerned with work in this area.

0012420

CESIUM-137 IN THE FOOD CHAIN OF ESKIMOS

Cesium-137 body burdens of Anaktuvuk Pass, Alaska residents during the summer of 1965 were about 30% less than during 1964. Lower amounts of ^{137}Cs in the people reflected a similar decrease of ^{137}Cs concentrations in caribou flesh, which serves as the natives' food base.

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

Cesium-137 body burdens in Eskimo residents at Anaktuvuk Pass, Alaska were measured during the summer of 1965, continuing a study of radionuclides in Alaskan arctic ecosystems begun during 1962.

Observations

Measurements were made with a 53 x 76 mm NaI(Tl) crystal and photomultiplier tube connected to a compact single-channel gamma analyzer-scaler. Two consecutive 1-min counts were made of each individual; variation between these was consistently less than 5%.

Whole-body counts of all available village residents were made on July 23-24 and August 24. Cesium-137 body burdens for three age categories are presented in Table I. There was essentially no difference between July and August values for the adult and minor categories; however, the children's ^{137}Cs body burdens decreased during the elapsed month, probably because several subjects participated in the U.S. Government's Project Head Start, which included a school lunch program featuring processed food containing lower ^{137}Cs concentrations than their usual diet.

Cesium-137 body burdens of the Anaktuvuk Pass residents were essentially constant during July and August at a level about 30% less than during the same period of 1964. This reflects a lower ^{137}Cs concentration in caribou flesh, which forms the food base of these people. Samples of caribou flesh obtained from representative family food caches contained 30% less ^{137}Cs than similar samples measured in 1964, although ^{137}Cs concentrations in lichens at Anaktuvuk Pass increased from about 24 pCi/g standard dry weight during 1964 to about 32 pCi/g in 1965. The maximum ^{137}Cs body burden was found in the same man who has usually had the highest burden. He contained 1710 nCi ^{137}Cs on July 24, 1740 nCi on August 24 and 1740 on September 1. These values are 30% less than his body burden during the summer of 1964.

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TABLE I. Cesium-137 Body Burdens in Various Age Categories of Anaktuvuk Pass, Alaska, Residents During July and August, 1966

Age category	Cesium-137, nCi					
	July			August		
	Persons	Body burden	Per kg body wt	Persons	Body burden	Per kg body wt
Adults (>21 years)	38	920 ± 49[a]	15.1 ± 0.8	23	920 ± 58	15.7 ± 1
Minors (15-20 years)	8	560 ± 58	9.7 ± 0.9	5	490 ± 42	9.5 ± 1
Children (3-14 years)	36	200 ± 18	7.8 ± 0.4	22	170 ± 17	6.2 ± 0.3

[a] *Mean ± one standard error.*

Conclusions

The decrease of ^{137}Cs body burdens in Anaktuvuk Pass Eskimos and of ^{137}Cs concentrations in caribou flesh serving as the food base for these people is inconsistent with the trend of ^{137}Cs concentrations in lichens, which serve as the major winter food of caribou. A similar inconsistency was noted during the summer of 1964, when ^{137}Cs concentrations in caribou flesh and in people increased to twice the 1963 summer values at a time when concentrations in lichens increased only slightly. This emphasizes the need for information about caribou migration patterns through the Anaktuvuk Pass region from winter ranges possessing various ^{137}Cs levels in lichens.

BIOLOGICAL HALF-TIME OF CESIUM-137 IN ESKIMOS

The biological half-time of ^{137}Cs in Alaskan Eskimos was investigated by substituting domestic meat for caribou and sheep in their normal diet for 4 weeks. Whole-body counts and excreta measurements led to average half-time estimates of 40 days for children, 52 days for minors, and 64 days for adults.

Investigators:
W. C. Hanson
L. L. Eberhardt
Technical Assistance:
Dorothy D. Wade

During August, 1965, the biological half-time of ^{137}Cs retention in Anaktuvuk Pass Eskimos was measured in an effort to evaluate the effect of age and weight in persons who had naturally accumulated significant amounts of the radionuclide. Four families cooperated in the study by agreeing to substitute domestic meat for caribou and Dall sheep flesh. In addition, their needs for flour, sugar, cooking oils, canned milk, butter, rice, noodles, and macaroni were supplied.

Observations

Total 24-hr urine samples were collected from each individual on 2 days of each week; a single feces sample was obtained from most participants at

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the beginning of the experiment to compare urinary and fecal ^{137}Cs excretion. Whole-body counts, obtained at weekly intervals, consisted of two consecutive 1-min counts made with a 53 x 76 mm NaI(Tl) crystal connected to a compact single-channel analyzer-scaler.

A more intensive study was made on two 20-year-old males, one on a normal Eskimo diet containing 5 to 6 kg of caribou meat per week and the other on the domestic meat diet. Measurements of ^{137}Cs excretion via urine and feces and whole-body counts were made daily for a 12 day period.

In a few instances, there is a suspicion that caribou was eaten surreptitiously, and additional study of excretion data is underway. Apparently acceptable results were obtained on ^{137}Cs half-time in 17 of the 28 persons studied. Values derived from whole-body counts obtained before and after the dietary change showed a consistent ranking with age and weight as indicated in Figures 1 and 2. Mean values were 40 days for children (age 4 to 12 years), 52 days for minors (15 to 20 years) and 64 days for adults (>21 years). Further treatment of all the data will refine these values.

A biological half-life of 41 to 52 days was observed for the ^{137}Cs retention of the 20-year-old male on the special diet from whom daily excretion and whole-body ^{137}Cs measurements were obtained. His excretion data are compared in Table I with those from another male of the same age on the normal diet of caribou meat.

Conclusions

Although the results await refinement, they are in accord with several

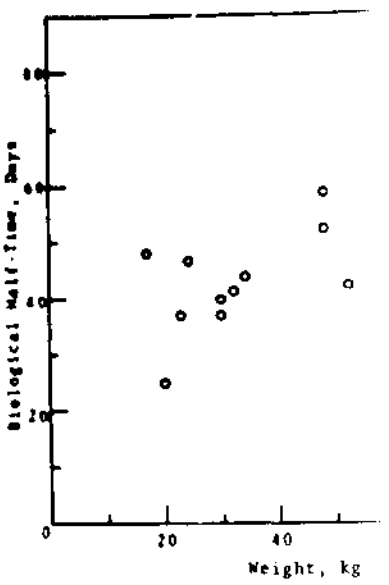

FIGURE 1. Cesium-137 Re
Times as a Function of B

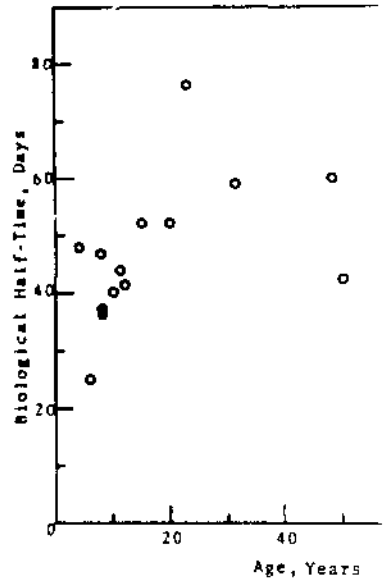

FIGURE 2. Cesium-137 Re
Times as a Function of Ag

other investigations whic
shorter half-lives for ^{13}
to northern peoples as co
temperate zone inhabitant
children as compared with
very limited data from t
suggest that about one-fo

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excretion occurs via the feces, and that this fraction is not greatly influenced by the current level of ^{137}Cs intake.

TABLE I. Cesium-137 Excretion Data Obtained from Daily Measurements of Two Male Subjects

Subject	Observation period	Average percent of body burdens excreted/day		Fraction of total excretion in feces
		Urine	Feces	
DH Special diet	13 days	0.55	0.23	0.29
MM Caribou diet	13 days	0.69	0.21	0.23

COMPONENTS OF VARIABILITY IN RADIONUCLIDE ABUNDANCE IN NATURAL ENVIRONMENTS

Variability due to analytical methods, differences between individual animals, and environmental factors is considered. Preliminary findings suggest a major importance of the environmental aspects.

Investigator:
L. L. Eberhardt

Studies of radionuclide abundance in natural environments necessarily depend on sampling methods for basic data. The purposes of such studies may differ greatly, including research on mineral cycling, monitoring of effluents or fallout, construction of mathemathical or computer models, prospects for "scaling up" or projecting present knowledge to future hazards; but all such concerns must deal with variability as encountered in the field. The present report deals with preliminary investigations as to the components or sources of variability in radionuclide concentrations in samples from natural environments.

Observations

The work reported here is based on both field studies (Annual Report, 1964) conducted directly for the purposes discussed here and on review of other work in the Biology Department and in the literature. It is sufficiently difficult and expensive to obtain satisfactory samples so that dictates of good sampling practice are rarely met. An attempt has been made to restrict the data considered here to time periods short enough to avoid seasonal cycles and to areas on the order of a few square miles. Sample materials used cover a wide range, including both plant and animal tissues, and such diverse forms as fish, pine needles, and fecal pellets. Principal radionuclides considered have been ^{137}Cs, ^{90}Sr, ^{32}P, and ^{65}Zn. Results have been expressed thus far in terms of the sample coefficient of variation (standard deviation divided by the mean).

Sample substances, radionuclides considered, analytical methods, etc., obviously affect results, but a partial classification of components of

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variability is suggested as follows:

Source	Coefficient of variation
Replicate measurements	0.04
Between individual organisms	0.09
Environmental ("small-scale")	0.30 to 0.40

Replication of measurements (or "instrument error") may be greatly influenced by homogeneity of the material used--muscle tissue of large animals (elk, caribou) provides perhaps the best source by way of homogeneity. Components due to differences between individual animals (essentially physiological differences) may vary as the history of the animal, and two wild animals rarely have the same temporal record. Hence useful data on this source have been considered to be those on the variability of radionuclides measured in fecal pellets from the same "band" of elk, as this reflects relatively short-term feeding and uptake differences. Landscape effects depend on a wide range of factors, few of which are well defined. In considering fallout data, the most evident point is, of course, precipitational differences, so that size of area sampled at some point blends into what might be called "geographic" differences.

Conclusions

As yet, too little is known about the variability in distribution of radionuclides to support any firm conclusions. It is clear that grouping of sample data frequency classes yields skewed distributions, and there is an interesting prospect that the coefficient of variation may be relatively constant for a wide range of materials. It appears that "instrument error" (measurement errors) may be a satisfactorily small fraction of the total variability (as it should be), and it seems most likely that a major fraction may be assigned to what has here been called "landscape" variability--not due to differences between individual animals or to measurement, nor to large-scale influences (e.g., precipitation regimes), but to a combination of intermediate factors. A much better understanding of the mechanisms is basic to any kind of study on a natural system, and thus extends well beyond radionuclide investigations, including, for example, such problems as those having to do with pesticides and pollution.

CESIUM-137 IN WINTER AND SUMMER ANNUALS OF A DESERT STEPPE ENVIRONMENT

Annual plants of arid and semi-arid regions can be used as biological indicators of yearly or subyearly deposition of ^{137}Cs.

Investigator
W. H. Rickard

Radioactive debris is deposited on the earth's vegetative cover from the atmosphere under dry conditions but more is brought down with precipitation. In the semi-arid environment of south-central Washington, precipitation is

largely confined to fall and winter months. Most of the plant species of this region grow during cool, moist winter and spring months and aestivate during the hot, dry summer. Only a few species of herbaceous plants are able to grow in summer by exploiting winter moisture stored in the soil profile below the reach of other plants. By harvesting winter and summer growing plants at their time of seed maturation, it is possible to compare these different kinds of plants as to their annual accumulation of radioactive fallout under different precipitation exposures.

Observations

The concentration of ^{137}Cs as determined by gamma spectroscopy in a winter annual grass, Bromus tectorum (cheatgrass), and a summer annual forb, Salsola kali (russian thistle), growing on the same quadrats of an abandoned field habitat on the Hanford Reservation, Benton County, Washington during the 1964-65 growing season is reported here (Table I). During a life cycle of approximately 6 months, B. tectorum averaged 15 times more ^{137}Cs per gram dry tissue than did S. kali during its 5 month life cycle. The precipitation exposure of B. tectorum totaled more than 4 in. as compared to less than 1 in. for S. kali (Table I). These data show that annual plants growing on the same habitat accumulate fallout in different amounts according to differences in ecological growth requirements.

Conclusions

Much of the information on fallout interception by plants of temperate latitudes has been concerned with vegetables or perennial pasture grasses. Little information is published concerning fallout concentrations by wild plants, especially annuals of arid and semi-arid regions. Radiological surveys using plants as bioindicators of the concentrations of radioactive matter in the environment often use aboveground parts of perennial plants for analyses. These parts often are of unknown age and therefore their aerial

TABLE I. Growing Season of Bromus tectorum and Salsola kali in Relation to Precipitation and ^{137}Cs Concentrations During 1964-65

	Precipitation, in.	
October, 1964	0.28	
November, 1964	0.94	Bromus tectorum growing season
December, 1964	2.34	Total precipitation = 4.34 in.
January, 1965	0.93	^{137}Cs pCi/g dry wt = 2.3 ± 0.80 (SE) n = 10
February, 1965	0.14	
March, 1965	0.03	
April, 1965	0.09	
May, 1965	0.15	
June, 1965	0.49	Salsola kali growing season
July, 1965	0.11	Total precipitation = 0.87 in.
August, 1965	0.03	^{137}Cs pCi/g dry wt = 0.15 ± 0.12 (SE) n = 10
September, 1965	0.00	

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exposure time is also not known. Long-lived radionuclides like ^{137}Cs tend to accumulate on the more aged tissues thereby mixing several years of fall-out contributions. Annual plants, on the other hand, are of known age and these can be used as a bioindicator of the yearly or subyearly accumulations of ^{137}Cs.

POPULATION CHANGES IN DARKLING BEETLES

A 3 year decline in beetle population on the Hanford Reservation may be related to the concurrent decline in yield of herbaceous plants.

Investigator:
W. H. Rickard

One of the more obvious recurring biological events in the sagebrush-dominated vegetation of the Hanford Reservation is the autumn emergence of large populations of darkling beetles. These appear in September and remain active on the ground surface until early December when they are killed by low temperatures and snow. The life history of this kind of beetle has not been studied, but it is probable that as larvae they feed upon dead plant material. The beetles are probably eaten by other kinds of animals but this has not been observed. A purpose of these studies was to estimate numbers of beetles present in a representative sagebrush habitat during an emergence period and also to determine year-to-year changes in populations as related to environmental variables such as primary productivity.

Observations

Pitfall trapping of darkling beetles was conducted throughout the emergence period in the autumn of 1963, 1964, and 1965. Forty-nine traps arranged in a 7 by 7 pattern with 3 m spacing between traps comprised the trap grid. No other studies were conducted on the plot to minimize trampling disturbances, and the beetles were released after each trap period. Results are illustrated in Figure 1. The highest beetle catch was made in 1963 and followed two consecutive years of high grass yield. The lowest catch was made on the second of two consecutive years of low grass yield.

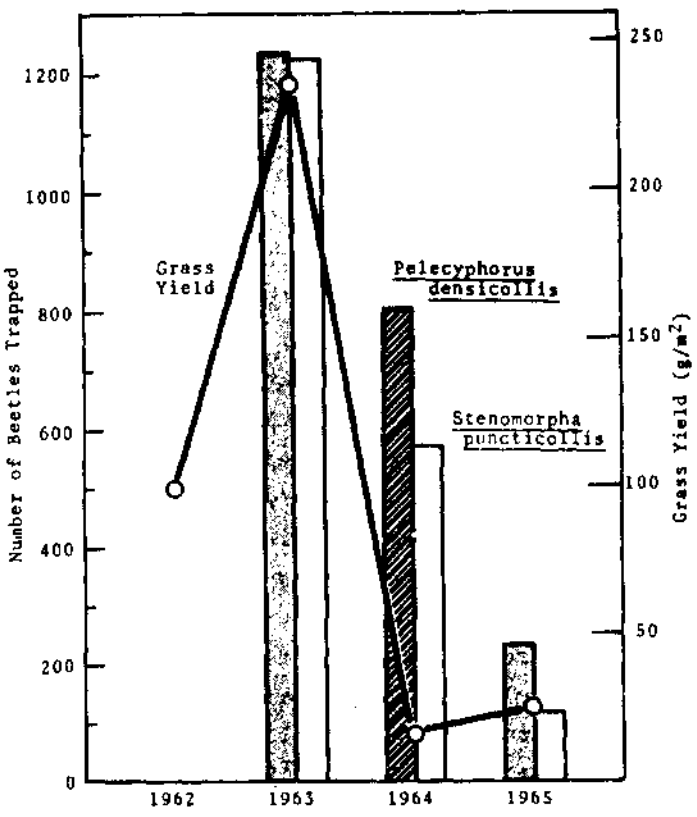

FIGURE 1. Darkling Beetle Populations and Grass Yields for 1962-1965

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Conclusions

Darkling beetles are a conspicuous element of desert steppe ecosystems that lend themselves to population studies by pitfall trapping. The present study suggests that there is a relationship between grass yields and beetle populations, but additional years of data will be required to establish the reliability of such observations. The greater reduction of beetles in the second year of reduced grass yield suggests a 2 year life cycle for the beetles with the first year being a primary feeding period and emergence occurring in the second year. Stenomorpha populations were more drastically reduced than were Pelecyphorus populations, which may indicate that Pelecyphorus is less adversely affected by low grass yield than is Stenomorpha.

RADIOIODINE ENVIRONMENTAL RELEASE STUDIES

The effective half-time of ^{131}I retention on pasture grass was about 8 days. Peak thyroid concentrations of ^{131}I in three grazing dairy cattle occurred on the fourth day.

Investigators:
C. R. Watson
J. F. Cline

Approximately 5 Ci of $^{131}I_2$ vapor were released at the base of the meteorology tower in a multi-disciplinary study of ^{131}I distribution and retention. This is a report of retention on pasture grass and uptake and retention in thyroids of three cows, pastured 1600 m downwind. Other aspects of ^{131}I distribution, including milk data, require further analysis.

Observations

Pasture grass samples were obtained at intervals following ^{131}I release within a 10 acre pasture on the 1600 m arc of the meteorology grid. One end of the pasture was initially contaminated with as much as 5 nCi/m^2; little ^{131}I was detected at the other end. Figure 1 shows the retention of ^{131}I activity on one area of the pasture. The effective half-life was approximately 8 days, in contrast to a 5 day effective half-life observed in a similar study in 1963 (Annual Report, 1963). The ambient temperature was 10 to 15 °F lower during the present study than in 1963, and the humidity was higher the first few days following release.

Three Holstein cows in the final 4 months of pregnancy grazed on the pasture for several weeks prior to the release to minimize the effect of dietary iodine on ^{131}I uptake. Supplementary feed was limited to small quantities of hay and grain fed at milking. Noniodized salt was available ad lib. Daily sprinkler irrigation of the pasture ended the day before the release. The cows were removed just prior to the ^{131}I release and returned to the pasture 4 hr after release to minimize inhalation of the radionuclide. The scintillation probe and the calibration technique for in vivo ^{131}I monitoring of cow thyroids was described in the 1962 Annual Report.

This experiment may be regarded as a practical extension of the 1962

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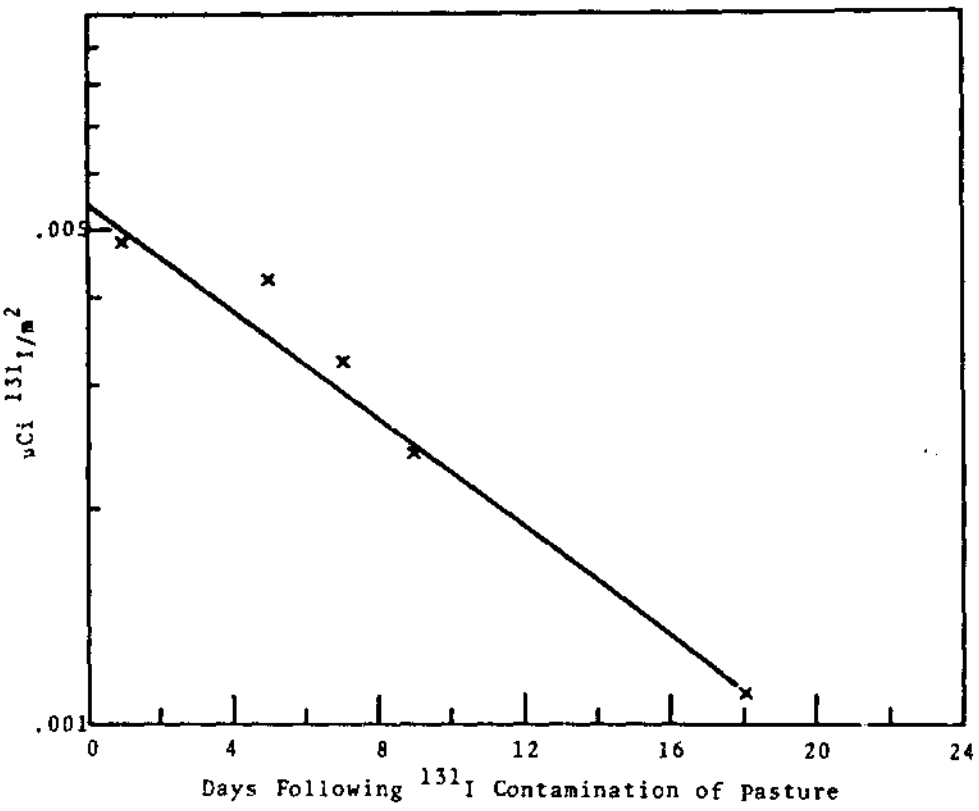

FIGURE 1. Retention of ^{131}I on a Plot of the 1600 Meter Arc Pasture after Airborne Deposition

single contamination simulation in which a 30 days' supply of cattle feed samples was spiked on Day 1 with 10 uCi of ^{131}I per sample (Annual Report, 1962). The patterns of ^{131}I activity in the thyroids, shown in Figure 2, were different in the pasture and laboratory contamination cases. The laboratory cows' thyroids had a peak ^{131}I concentration after the first week, remained constant the second week, followed by a gradual decrease (effective half-life around 15 days). Thyroid values in the present study peaked earlier (Day 4) and decreased faster (effective half-life about 8 days).

Conclusions

The longer effective half-life of ^{131}I on pasture grass observed in this study is probably due to climatic differences. High temperature has been shown to enhance the removal of ^{131}I from foliar surfaces. The shorter retention time of ^{131}I in these cattle thyroids is probably due to nonuniform contamination of the pasture. The cows grazed in the contaminated end of the pasture at the start of the experiment, then shifted to the noncontaminated end. These studies emphasize the difficulty of correlating laboratory and field data, and raise the further question as to which m[illegible] more relevant in hazard analysis predictions.

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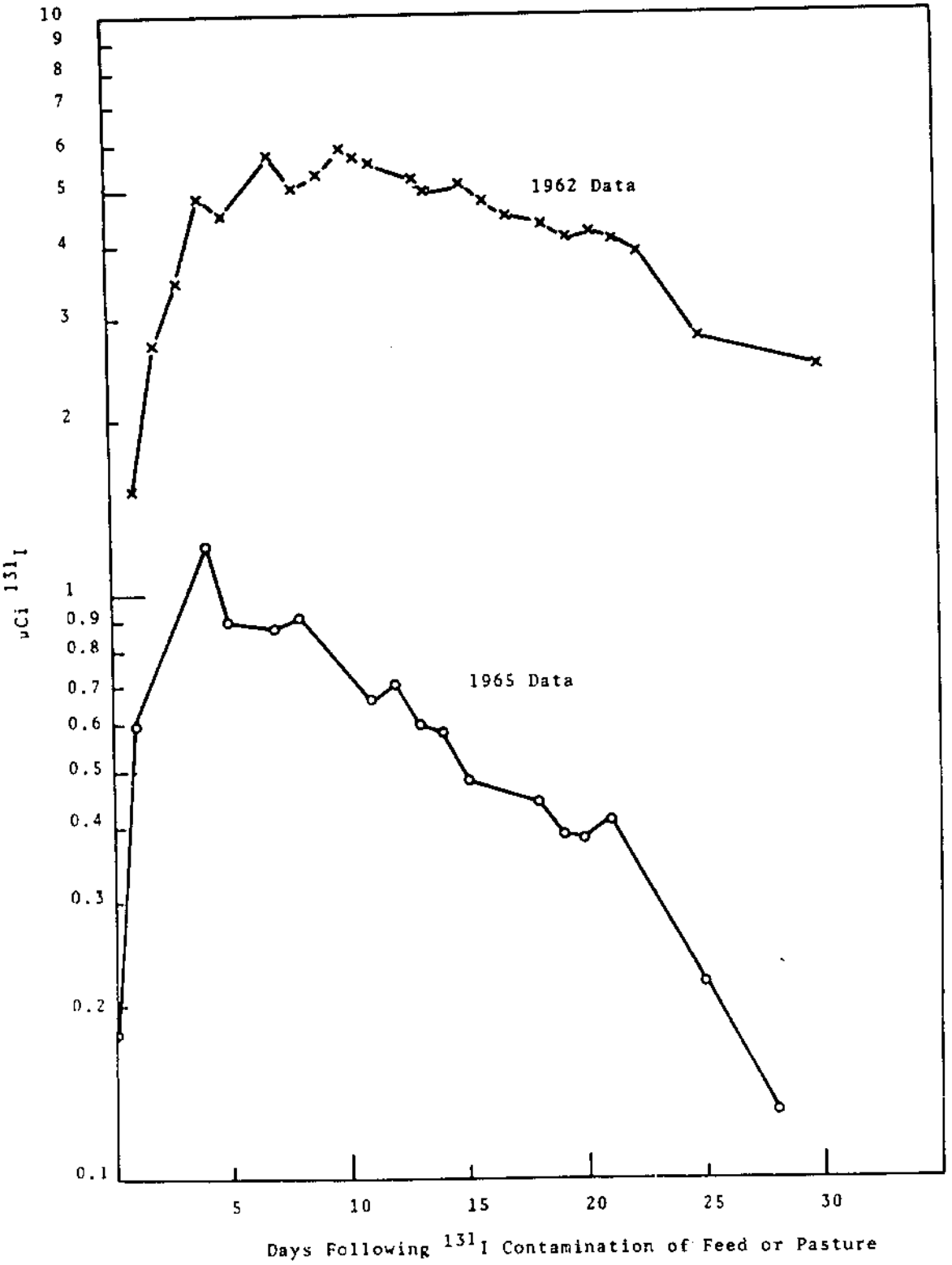

FIGURE 2. ^{131}I in Dairy Cattle Thyroid Following Contamination of Feed or Pasture (Upper curve—1962 Laboratory Experiment. Lower Curve—Present Field Study)

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EFFECT OF CLAY PARTICLES ON UPTAKE OF CESIUM BY PLANTS

Cesium-137 moves easily from bentonite clay particles to plant roots when a complete and agitated water bridge connects the particles to the root.

Investigator:
J. F. Cline
Technical Assistance:
Mary W. Magula

Cesium-137 is tightly sorbed to clay particles and only slightly available to plants growing in soil. Plants growing in soil flooded with water at time of and subsequent to contamination, however, take up relatively more ^{137}Cs. The question is whether the ^{137}Cs moves directly from a dissolved state to the plant surface or is the dissolved ^{137}Cs first adsorbed to soil particles and subsequently exchanged through the solution to the plant surface. An experiment was performed to determine whether ^{137}Cs is dissociable from the soil surface and under flooding can move by convection currents to the plant surface where uptake occurs.

Observations

Bentonite clay was contaminated with ^{137}Cs and then suspended in a nutrient solution in which bean plants were growing. The solution was aerated during the 15 days of exposure and the amount of clay was small enough so that relatively little sedimented to the bottom of the pan. Concentrations of ^{137}Cs in plant tissues harvested at the end of the test were no different from concentrations in control plants cultured in the same way but with the ^{137}Cs added directly to the nutrient solution and no clay present (Table I).

Although some of the clay did deposit on the root surfaces, a significant portion remained suspended in the nutrient solution and there was relatively little direct contact between clay particle and root. If the clay had retained the ^{137}Cs in a tightly bound form, there should have been a lower uptake in plants grown in solutions to which the ^{137}Cs was added already sorbed to clay.

It appeared possible that the ready availability of the ^{137}Cs from the clay to the plant was a consequence of the relatively large concentration of diverse ions present in the nutrient solution in which the clay was suspended. To check this, a similar test was made using plant roots washed and suspended in distilled water. In water, the uptake of the ^{137}Cs from the clay particles was greater than when nutrient solution was present (Table II.)

Conclusions

The data of Tables I and II extend previous observations in pointing out that under water-soaked conditions the ^{137}Cs is readily available to the plant

TABLE I. Effect of Suspended Clay Particles and Carrier Cesium on Uptake of ^{137}Cs by Bean Plants

Treatment			nCi ^{137}Cs/g dry tissue[a]		
	Specific activity				
g Clay/liter	uCi ^{137}Cs	mg Cs	Leaf	Stem	Root
0	1	0	7.3	5.6	7.9
0.17	1	0	6.5	4.6	10.7
0.17	1	1	7	5.2	8.2
0.17	1	20	6.7	4.4	6.6
1	1	80	6.5	4.6	8
1.34	1	80	6.1	4.4	7.9

[a] Average of duplicate experiments.

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TABLE II. Effect of Nutrient Ions on Plant Uptake of ^{137}Cs Adsorbed to Clay Particles suspended in a Nutrient Culture

^{137}Cs and clay treatment	nCi ^{137}Cs/g dry tissue[a]			
	Leaf + Stem		Root	
	24 hr	48 hr	24 hr	48 hr
In Hoagland's solution	27	38	82	263
In distilled water	94	122	72	216

[a] *Average of duplicate experiments.*

even if sorption occurs first on soil particles. It is no longer necessary to infer a direct initial sorption on the plant. Rapid transport from the soil particle to the plant occurs by mass flow through the standing water with normal convection currents acting to facilitate this transport.

ION MIGRATION IN HOMOGENEOUS SOIL

A general empirical equation is proposed to describe the ion migration front and the amount of ions carried by infiltrating water into soil.

Investigator:
J. J. C. Hsieh

A mathematical description of ion movement into and within soil is important to the prediction of the availability of a given radionuclide to plant roots. An attempt was made to find a simple empirical equation to describe the one-dimensional ion movement in transient.

A mathematical expression for ion movement in a homogeneous soil is proposed as

$$\bar{Y} = mt^n,$$

where $\bar{Y}$ represents either the quantity of ions transported by the infiltrating water or the distance of ion migration front from the source at time t; both m and n are parameters. The general equation for a straight line on log coordinate paper is of the form

$$\bar{X} = mZ^n,$$

$$\log \bar{X} = \log m + n \log Z.$$

The validity of the proposed ion flow equation was tested by plotting the log of the distance of ion migration, or the log of the quantity of ions infiltrated into the soil, against the log of the corresponding time.

Observations

Air-dried and sifted Cinebar soil (particle size <1 mm) was packed into a glass cylinder. The labeled ion solution in a calibrated burette was introduced at the top of the packed soil column with a constant hydrostatic head of 1.5 cm. The amount of ion solution remaining in the calibrated burette, the distance of water advance, and the time were recorded. At the termination of the experiment, the wet soil samples were removed from the distal end of the soil cylinder at centimeter increments and were counted for radioactivity.

Two iodine solutions were tested; one with carrier (250 μCi ^{131}I with 5 mg of NaI in 405 ml of water), another without carrier (250 μCi ^{131}I in 405 ml of water). The results are summarized in Figure 1. The criterion for the presence of iodine in a given soil sample was arbitrarily chosen as 1000 counts/min (~1 nCi of ^{131}I).

Two cobalt solutions were tested; one with chelating agent (40.9 μCi ^{60}Co in 150 ml of 1% disodium EDTA plus

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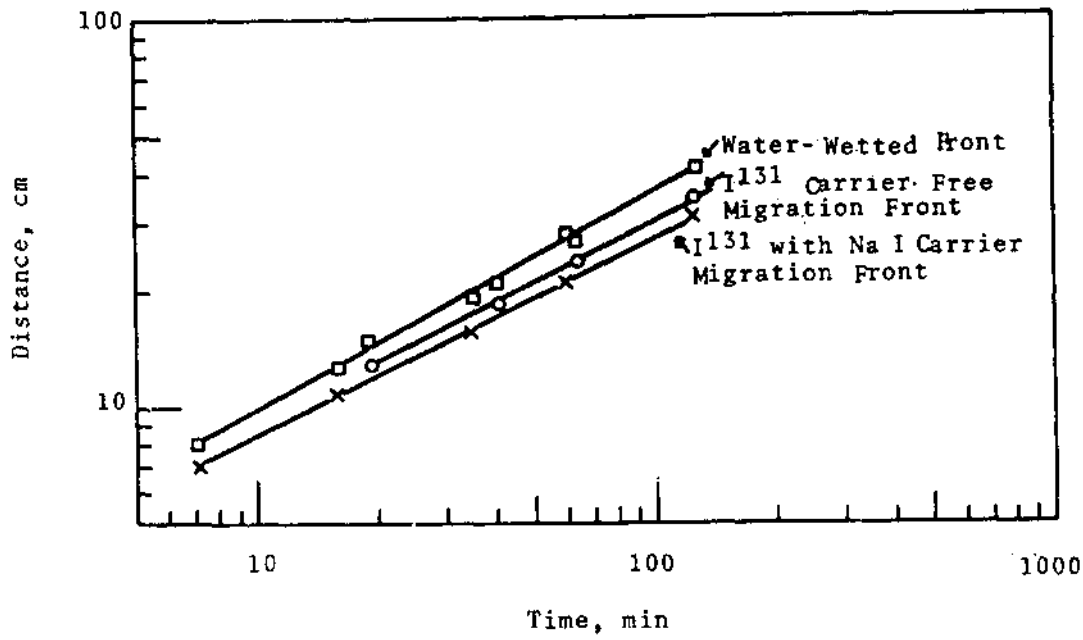

FIGURE 1. Rate of Movement of Water-Wetted Front and ^{131}I on Soil Column (Values for the water-wetted front are given for the tests with and without carrier.)

350 ml of 1N HCl), the second with 40.8 μCi ^{60}Co in 150 ml of water plus 350 ml of 1N HCl. The criterion for the presence of cobalt in a given soil sample was arbitrarily chosen as 1000 counts/min (~2 nCi of ^{60}Co). The results are summarized in Figure 2.

Conclusions

The experimental data seemingly fit the straight line drawn on log coordinate paper. Therefore, the empirical equation $\overline{Y} = mt^n$ may be used to describe the ion movement in soil under the limitations of the experimental condition tested. Also, the advancement of water-wetted front confirms the empirical equation of Bell and Cameron[1] for water movement $y^n = Kt$.

It is expected that the type of soil, size of aggregation, packing. etc., would affect the slope of the lines obtained; however, the form of the proposed equation may not be affected. Further studies will be required to evaluate this preliminary finding.

References

1. *Bell, J. M. and F. K. Cameron. J. Phys. Chem., vol. 10, pp. 658-674. 1906.*

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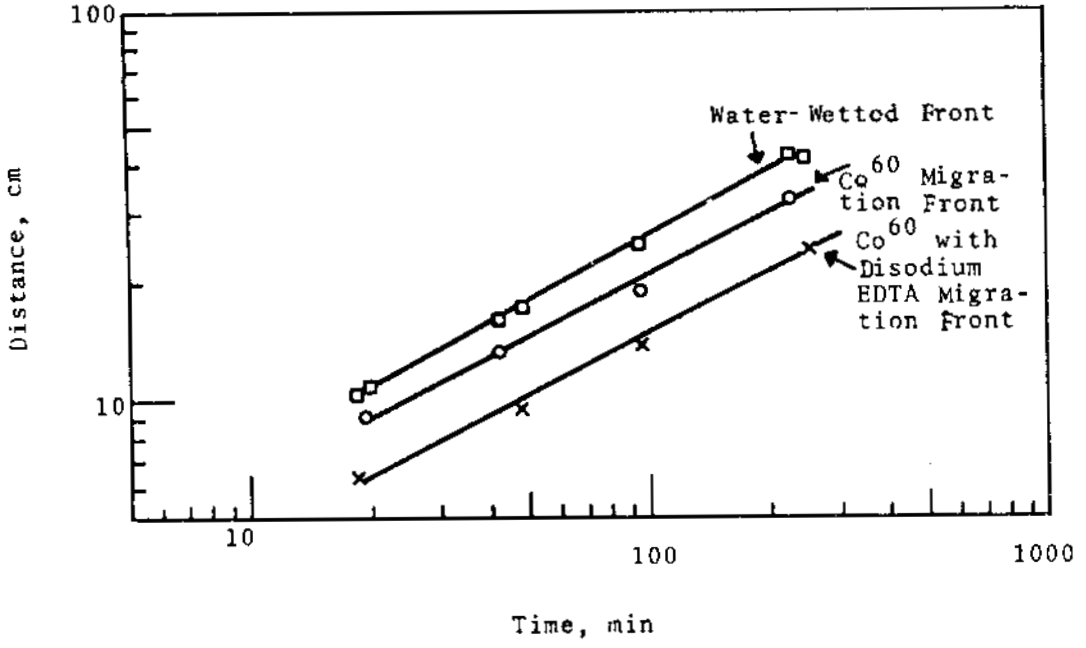

FIGURE 2. Rate of Movement of Water-Wetted Front and ^{60}Co on Soil Column (Water-wetted front points are for both tests with and without EDTA.)

ENVIRONMENTAL RADIATION
STUDIES - AQUATIC

All indoor fish and fish-holding facilities were destroyed in the fire of November 1964. By mid-1965, these were back in some semblance of operation and restocked from outdoor ponds and with generous assistance from the University of Washington.

The decision was reached in late 1965 to concentrate a greater proportion of our environmental studies effort on a comprehensive ecological survey of the Columbia River. This decision was prompted, in part, by the opportunity to study impending changes in this ecosystem which will result from curtailed reactor operations and from the impoundment of the river within the Hanford Reservation if a projected dam is constructed.

Of special interest among the reports in this section are the first three, which deal with metabolic effects of temperature acclimation in trout. Although quite preliminary, these reports describe three different approaches to this problem. The possibility of relating cellular ultrastructure to function in a system where temperature can be varied within wide, normal limits is a particularly exciting prospect.

TEMPERATURE EFFECTS ON CESIUM METABOLISM IN TROUT

Cesium-137, injected intravenously into trout acclimated to 5 °C, is retained with a half-life of about 20 days, a much longer half-life than previously measured in 18 °C acclimated fish.

Investigators:
J. M. Dean
R. F. Nakatani
Technical Assistance:
R. G. Genoway

Previous studies in this laboratory (Annual Report, 1964) showed that ^{137}Cs injected intravenously into trout was retained by most soft tissues with a half-life of about 6 days. A much longer half-life was exhibited by the ^{137}Cs in white muscle. These earlier studies employed fish acclimated to 18 °C. To determine the effect of environmental temperature, these studies were repeated employing fish acclimated to 5 °C.

Observations

Trout were acclimated to 5 °C for 4 weeks prior to injection with 10 μCi of ^{137}Cs. Four fish were sacrificed at each of several postinjection time periods and samples of the following tissues were taken: blood, gills, liver, heart, brain, kidney, gut, red muscle, and white muscle. These tissues and the remainder of the fish were digested in acid and assayed for ^{137}Cs content. Retention curves derived from these data are shown in Figure 1.

The results for the 5 °C fish show a retention half-life for ^{137}Cs of 20 days as compared with the value of about 10 days previously observed for

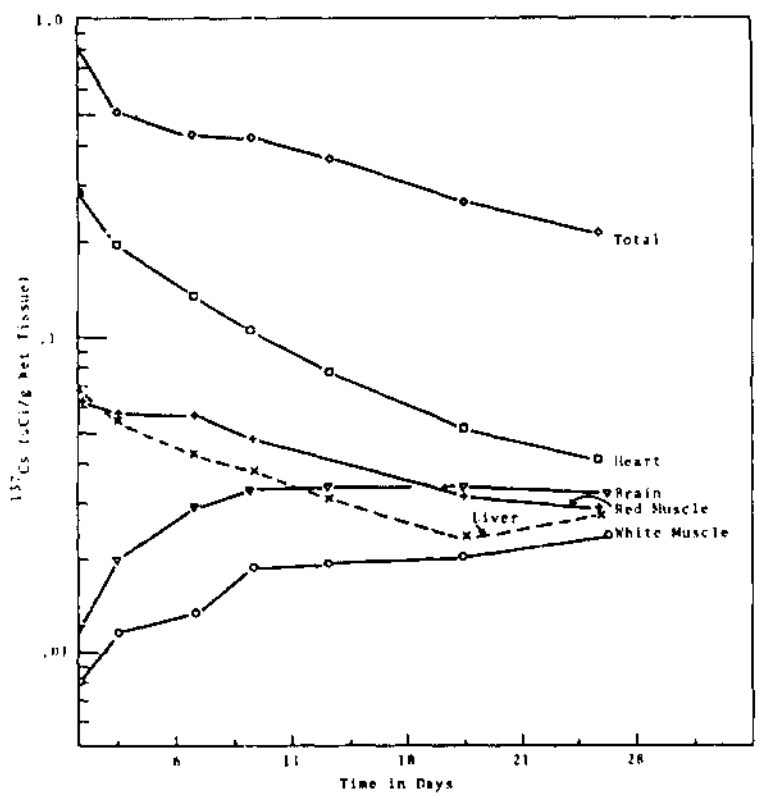

FIGURE 1. Retention of ^{137}Cs in Trout at 5 °C

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18 °C fish. All soft tissues, with the exception of nerve tissue, white and cardiac muscle, exhibit a half-life of 19 days. The measured half-lives in representative tissues from both 5 and 18 °C fish are shown in Table I. The half-life in white muscle was not determined as ^{137}Cs levels were still increasing when the experiment was terminated at 28 days postinjection. Because the white muscle may constitute 60 to 70% of the mass of the fish, it accounts for a substantial fraction of the total ^{137}Cs activity, even though it shows the lowest concentration on a μCi/g basis. There is a rapid uptake of ^{137}Cs by nerve tissue but little turnover after the plateau is established.

TABLE I. Effect of Temperature on Effective Half-life of ^{137}Cs in Trout

Tissue	Half-life, days	
	5 °C Fish	18 °C Fish
Whole animal	20	10
Heart	11	6
Liver	19	6
Kidney	19	6
Gill	19	6
Red muscle	19	6

Conclusions

It is well known that a relationship exists between environmental temperature and the metabolism of radionuclides. From the present study it is apparent that tissues such as cardiac muscle may have different metabolic characteristics at one temperature than at another. This then is a response to temperature at the tissue level. Knowledge of the turnover of radionuclides in white muscle is of special pragmatic interest because the white muscle of food fishes is an important dietary constituent of man. From these results it can be seen that fish concentrating ^{137}Cs in colder waters will tend to develop higher tissue concentrations and thus may play an enhanced role in the transfer of fallout radionuclides through the food chain.

TEMPERATURE EFFECTS ON ENERGY RESERVES IN TROUT

Higher levels of lipid reserves were observed in cardiac muscle of trout acclimated at 5 °C than at 18 °C, but red muscle, white muscle and liver showed no significant effect of temperature. The 5 °C fish had significantly higher glycogen levels in liver and cardiac muscle but showed no difference in red and white muscle.

Investigator:
J. M. Dean
Technical Assistance:
Diana G. Bauer
J. D. Mauleby

Studies of the utilization of energy reserves by poikilotherms at different temperatures yield information on changes in the metabolism of these animals as they respond to environmental shifts in temperature. The utilization of these reserves is especially important to survival during periods of stress such as reproduction and strenuous physical activity. Such stress can be compounded by the metabolic changes induced by temperature extremes.

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Observations

Rainbow trout were acclimated to 5 and 18 °C for 4 weeks in river water. Levels of total lipids and glycogen were determined in livers, cardiac, red muscle, and white muscle of the acclimated fish. Results are shown in Table I. It is generally assumed that with acclimation to low temperatures there is a quantitative increase in lipids, increase in chain length, and greater unsaturation. The results in Table I show, on the average, higher levels of total lipids in all tissues at low temperature, but from a statistical viewpoint only the lipids of cardiac muscle are significantly different. The glycogen is increased significantly in both liver and white muscle at low temperature, but not in other tissues.

To determine if the biochemical results were expressed morphologically, observations were made using histochemistry and electron microscopy. No difference in the fine structure between the red and white muscle tissues was observed, but ultrastructural differences were observed in the liver. These differences are discussed in the following report. Muscle tissue from exercised and unexercised fish acclimated to 5 and 18 °C showed no difference in subcellular structure.

Cellular localization of lipids, glycogen, and cytochrome oxidase was determined in the 5 and 18 °C fish by histochemical methods. The intracellular quantitative shifts can be correlated with those observed biochemically. Also, the distribution of the energy reserves substantiates the electron microscopic observations.

The biochemical, histochemical, and morphological characteristics of the tissues may be described as follows:

- Red muscle has a high lipid content, is rich in cytochrome oxidase, has an abundant supply of glycogen, numerous mitochondria, and short narrow fibers.
- White muscle has a low lipid level, little cytochrome oxidase, some glycogen, few mitochondria, and long broad fibers.
- The liver has a low lipid content, relatively few mitochondria, a low level of cytochrome oxidase, and is exceedingly rich in glycogen.

Since cytochrome oxidase is associated with the electron transport system and therefore with mitochondria, it is reasonable that tissues with few mitochondria have a low level of cytochrome oxidase.

Conclusions

These results suggest that red muscle would functionally be well suited for long-term, low-level, sustained activity, under which conditions oxygen could be easily supplied and an aerobic

TABLE I. Comparison of Total Lipids and Glycogen in Tissues of Fish Acclimated to 5 and 18 °C

Tissue	Sample Size	Glycogen, μg/mg N 5 °C	Glycogen, μg/mg N 18 °C
Red muscle	17	55	41
White muscle	18	24	9.2
Liver	18	300[a]	152[a]
Cardiac muscle	17	42[a]	27[a]
		Lipid, μg/mg N	
		5 °C	18 °C
Red muscle	20	417	292
White muscle	21	44	34
Liver	21	94	74
Cardiac muscle	23	182[b]	109[b]

[a] Difference significant at 0.05 level.
[b] Difference significant at 0.01 level.

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state maintained. Under such conditions lipids could provide a rich supply of energy. The structure of white muscle would, however, indicate that it is primarily dependent upon the aerobic glycolytic pathways for energy and as such would be best suited for activities requiring short bursts of energy. It has been observed that some species of fish at lower temperature are less subject to exhaustion. The increased energy reserves and higher cytochrome oxidase in tissues of fish acclimated to low temperatures may explain some of these results.

ULTRASTRUCTURAL CHANGES IN FISH LIVERS ASSOCIATED WITH TEMPERATURE ACCLIMATION

Morphological expression of proteinaceous materials, one of which may be plasma albumin, is increased in hepatocytes from cold acclimated fish livers as compared to similar cells from warm acclimated fish. This observation may have critical significance to an understanding of temperature acclimation in poikilotherms.

Investigators
J. D. Berlin
J. M. Dean
Technical Assistance:
R. R. Adee

In rat hepatocytes fibrillar and granular materials, thought to represent proteins, have been reported by Bruni and Porter[1] to be transported from the endoplasmic reticulum to the Golgi and, further, to microbodies and the space of Disse. According to these authors, the materials represent microbody enzymes and plasma albumin, respectively. The present investigation was initiated to determine if a similar morphological expression could be found in a poikilothermic liver, and, if so, to determine the effect of temperature acclimation on the process.

Observations

Salmo gairdneri (rainbow trout) were acclimated either to 18 or 5 °C. Livers were excised and prepared for electron microscopy by either glutaraldehyde-post-osmium or permanganate fixation and embedded in Epon.

Hepatocyte fine structure in warm acclimated fish is similar in many respects to that reported in rat liver cells. Materials in the endoplasmic reticulum cisternae and in the Golgi of a hepatocyte from a warm acclimated fish are shown in Figure 1. In a similar area from a hepatocyte of a cold acclimated fish, increased amounts of fibrillar and granular materials are associated with the Golgi (Figure 2). The concentrating activity of the Golgi is thought to be the cause of the Golgi-associated fibrillar material appearing as an amorphous electron dense substance.

The granular materials are clearly apparent after permanganate fixation (Figure 3). The appearance of these granules after $KMnO_4$ fixation suggests that they are proteineous; however, it is impossible to identify them as albumin by this procedure. They might, for example, be lipoproteins. The supposition that these granules are albumin (Bruni and Porter) is based on morphological observations and the fact that the liver is the sole site of albumin synthesis. Although the direction of movement of the various materials in hepatocytes is thought to be away from the endoplasmic reticulum,

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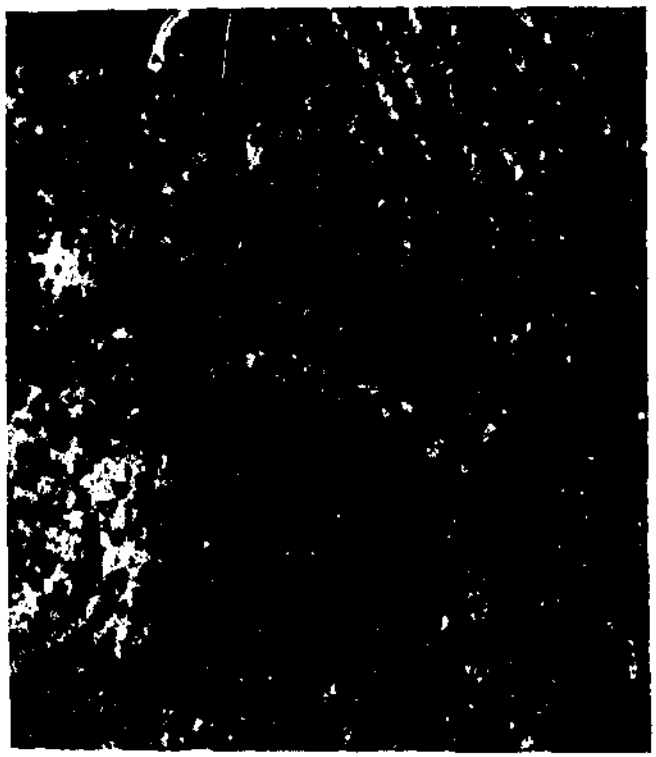

FIGURE 1. A Portion of a Hepatocyte from a Warm Acclimated Rainbow Trout [The Golgi (Go) consists of slightly dilated cisternae containing granular (g) and fibrillar (f) materials, the latter being condensed in the Golgi to an amorphous electron dense material. The Golgi associated granules, similar to granules (g) in the cisternal elements of the endoplasmic reticulum (ER), are thought to be synthesized in the endoplasmic reticulum and transported in vesicles (v) to the Golgi. The same interpretation applies to the fibrillar material; however, this material is not observed in the endoplasmic reticulum at this low magnification. The Golgi is believed to separate and concentrate these granular and fibrillar materials before they are further transported to the space of Disse and microbodies, respectively. Glutaraldehyde-osmium fixation.]

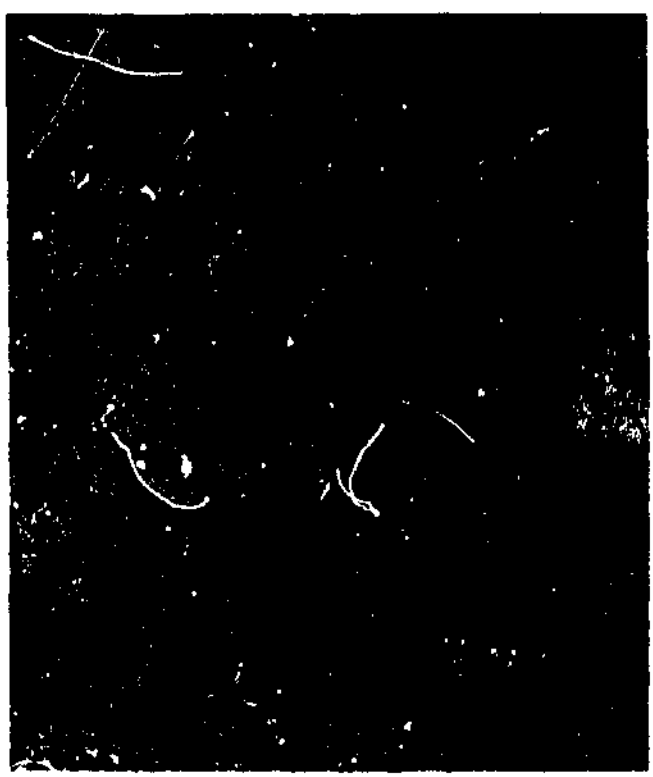

FIGURE 2. A Portion of a Hepatocyte from a Cold Acclimated Rainbow Trout [The Golgi (Go) cisternae are highly dilated and contain more granular (g) and fibrillar (f) materials than found in hepatocyte Golgi of warm acclimated fish. The difference in the amount of intracisternal Golgi materials in hepatocytes of warm and cold acclimated trout is thought to be the result of increased intracellular protein movement in hepatocytes of cold acclimated trout brought about by low temperature adaptation. Glutaraldehyde-osmium fixation.]

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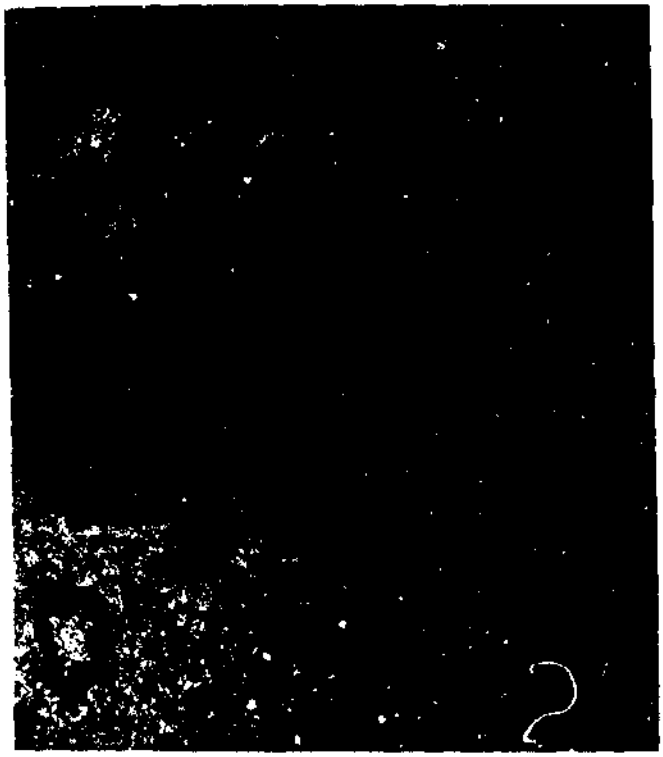

FIGURE 3. A Portion of a Hepatocyte Golgi from a Cold Acclimated Rainbow Trout [The granules (g) in the intracisternal Golgi spaces are apparent. These granules are thought to be albumin and their preservation after permanganate fixation suggests that they are proteinaceous. It is impossible to identify the granules specifically as albumin by this procedure and studies are presently underway to determine the identity of these granules.]

the reverse direction of movement is possible.

Conclusions

The temperature-induced hepatocyte changes observed in rainbow trout afford a unique model which will allow the investigation of certain aspects of hepatocyte structure and function. In future work, emphasis will be placed on the chemical characterization of the granular materials and upon determination of the direction of intracellular protein movement in these hepatocytes. On the basis of present results there is a difference in the amount of proteins found in the intracisternal Golgi spaces in hepatocytes of warm and cold acclimated rainbow trout, and it would appear that intracellular movement of proteins is increased in cold acclimated trout. This initial demonstration of morphological changes at the subcellular level may be an important step in understanding the mechanism of temperature acclimation in poikilotherms.

References

1. *Bruni, C. and K. R. Porter. Amer. J. Pathol. vol. 46, pp. 691-755. 1965.*

METABOLISM OF ZINC IN A MARINE BENTHIC AMPHIPOD

The metabolism of ^{65}Zn was examined in the marine benthic amphipod Anonyx sp. The uptake of ^{65}Zn from seawater was temperature dependent but did not vary significantly within the normal environmental temperature range. Anonyx sp. apparently cannot remove significant amounts of ^{65}Zn tightly bound to sediments.

Investigators:
*F. A. Cross**
J. M. Dean
R. E. Nakatani

Marine benthic amphipods, commonly called "sand fleas," are abundant on the continental shelf in boreal areas. These amphipods fill an important biological role in the ecology of the seas by serving as food for fishes as well as efficient scavengers of dead organisms. Radioanalysis of gammarid amphipods collected off the mouth of the Columbia River has identified ^{65}Zn as a dominant radionuclide, but little is known of the zinc metabolism of these organisms.

Observations

Test animals were collected by means of baited traps at a depth of 80 m off Depoe Bay on the Oregon coast. Temperature effects on ^{65}Zn uptake were studied by serial killing of amphipods acclimated to temperatures of 3, 7.5, and 12.5 °C and held in filtered seawater containing 25 μCi/liter of carrier-free ^{65}Zn. Figure 1 shows the uptake curves for each of the temperature treatment groups. Each point represents the average of six animals. The increase in uptake with higher temperature was expected. Differences between 12.5 and 7.5 °C groups are of special interest because these temperatures approximate the range of seasonal change in the normal marine environment. Longer experiments are clearly needed to determine maximal uptake levels. Concentration factors at the end of 96 hr were approximately 73, 55 and 12 for the 12.5, 7.5, and 3 °C groups, respectively.

To determine the effect of temperature on zinc retention, three groups of amphipods (12 animals/group) were placed in ^{65}Zn contaminated seawater at 3, 7.5, and 12.5 °C for a period of 8 to 10 days. Each amphipod was then weighed, counted for radioactivity and returned to individual containers of ^{65}Zn-free seawater of the same temperature as that to which they were initially exposed. The loss of ^{65}Zn was measured over a period of 29 days. Seawater was replaced on a weekly basis to prevent buildup of ^{65}Zn in the containers. Retention curves are shown in Figure 2. The 7.5 and 12.5 °C curves are essentially identical; the rate of loss at 3 °C is markedly less.

Because Anonyx sp. are benthic, feed on detritus, and burrow in the sediment, it might be assumed that they remove and incorporate ^{65}Zn complexed in the sediment. An experiment was performed to determine how the three major components of the sediment, i.e., bacteria, organic detritus and inorganic fraction, contribute to the labeling of the amphipods. Each of three groups of amphipods was exposed to one of three differently treated sediments for 7 days at 7.5 °C. The experimental sediments,

*Graduate student in the Department of Oceanography, Oregon State University, supported by the predoctoral AEC Richland Graduate Fellowship program.

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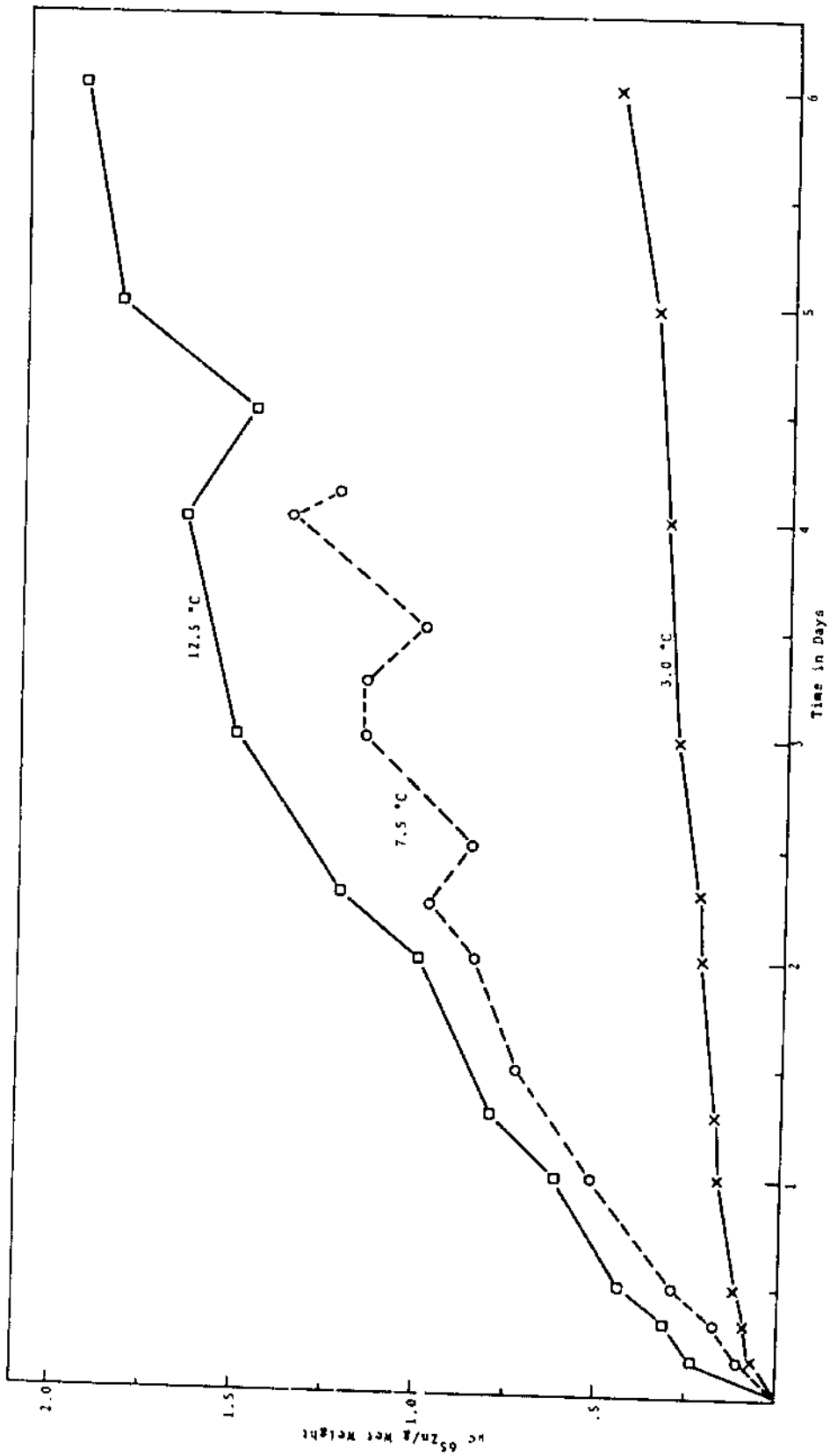

FIGURE 1. Uptake of ^{65}Zn by Anonyx sp. at Several Temperatures

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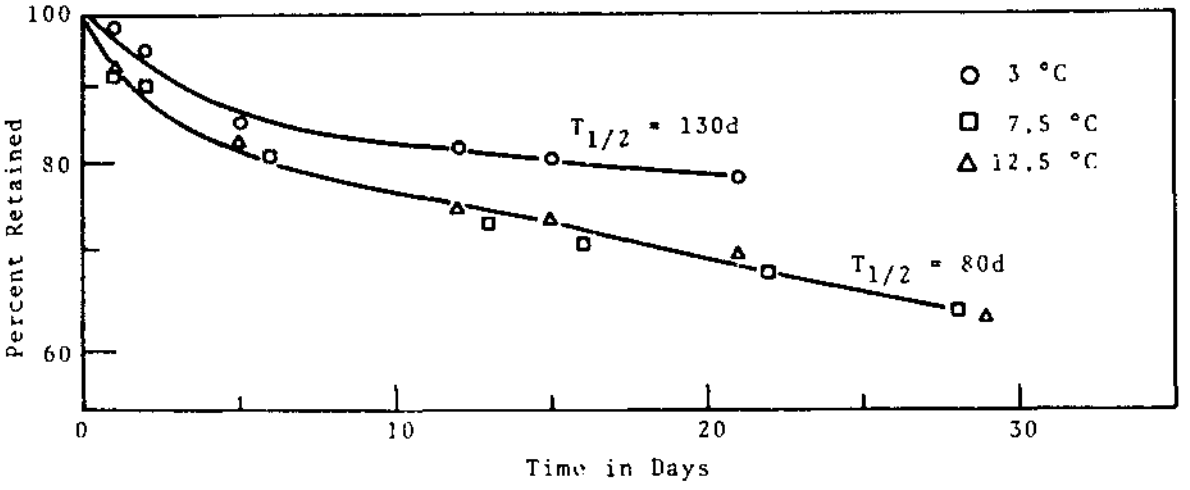

FIGURE 2. Retention of ^{65}Zn by Anonyx sp. at Several Temperatures

originally collected at 160 m, were untreated, sterilized by autoclaving, or oxidized with permanganate. The sediments were labeled by standing overnight in ^{65}Zn solution and were rinsed with seawater to remove the loosely-bound zinc before presentation to the animals. Radioanalysis showed only slight labeling of the serially killed animals and little difference among the groups. The ^{65}Zn incorporation into the amphipods could probably be attributed entirely to leaching of ^{65}Zn from the sediments.

Conclusions

Elevated temperatures increase the uptake of ^{65}Zn in marine amphipods, as expected for a poikilothermic organism. However, the usual temperature changes (7.5 to 12.5 °C) experienced off the Oregon coast should cause no sharp seasonal changes in the body burden of ^{65}Zn in these animals. Uptake of ^{65}Zn occurs primarily from the seawater rather than from the sediments. Because these amphipods are believed to be abundant and do not lose ^{65}Zn rapidly, they may form a significant reservoir of ^{65}Zn as food for fishes.

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A SYSTEM FOR MEASURING RADIONUCLIDE UPTAKE AND TURNOVER IN PERIPHYTON

An apparatus is described which will continuously measure uptake and cycling of radionuclides between periphyton and a controlled aqueous environment.

Investigators:
C. E. Cushing
N. S. Porter[*]

Previous investigations of radionuclide accumulation and productivity by periphyton in the Columbia River (Annual Report, 1964) were hampered by difficulties in control and measurement of the varying environmental conditions, especially those concerned with radionuclide concentration in the water. The uptake and cycling of radionuclides within aquatic communities could best be studied in a series of controlled, experimental channels. Lacking these, a laboratory approach to the problem was developed. The experimental system was designed to avoid the shortcomings inherent in typical aquaria experiments, e.g., lack of continuous flow of water over the algae, lack of ability to continuously measure radionuclide movement without destruction or sub-sampling of the community, and lack of flexible control of ambient isotope concentration and other environmental parameters.

Observations

The system as presently developed consists of two algal exposure chambers connected in series with the water supply and enclosed in an incubator. Each exposure chamber consists of a glass tube fitted over a waterproof radiation detector which is connected via a waterproof plug to a count-rate meter and decade scaler (Figure 1). The influent water supply is connected to a flow-rate meter so that the effects of varying velocities can be evaluated and also to aid in calculating isotope concentrations in the system. An automatic pipetting machine injects the radionuclide solution into the water line

[*]*Applied Physics and Electronics Department*

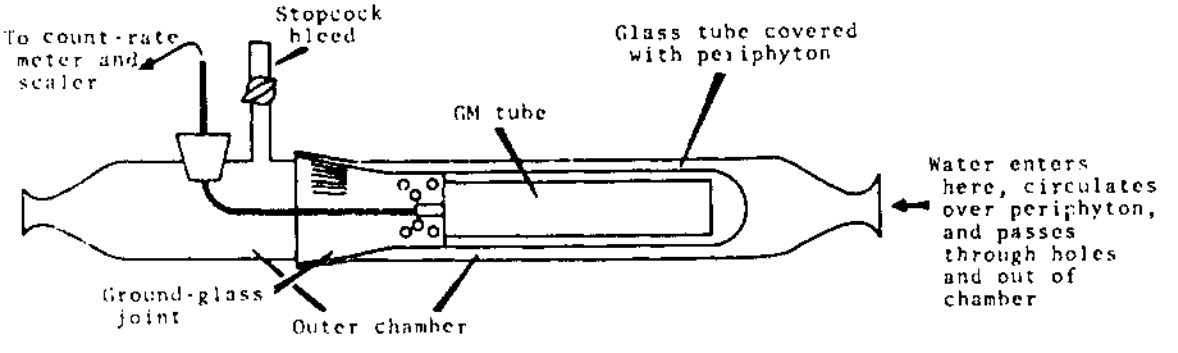

FIGURE 1. Apparatus for Measurement of Radionuclide Uptake and Turnover in Periphyton

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so that radionuclide concentration in the water can be controlled. Provisions are also included so that the system can be closed and the water recirculated by a vibrostaltic pump. Both chambers are enclosed in the incubator so that light and temperature effects can be evaluated.

In use, the glass tube in one chamber is covered with periphyton accumulated during previous exposure of the tube in the river. The second chamber contains a bare glass tube. The circuitry is such that the count-rate meter indicates the net count in the periphyton. The count rate from the blank tube, exposed to the same radionuclide concentration in the water, is automatically subtracted from the count rate from the algae-covered tube. Timed counts of either tube can be made at any time by switching to the decade scaler.

A single test has been conducted, designed primarily to check functioning of the electronics. A vibrostaltic pump recirculated 350 ml of filtered river water containing 1.03 μCi ^{65}Zn. Light and temperature were not controlled. One-minute counts were taken at 5-min intervals for 1 hr. In this cursory test, radionuclide content of the algae appeared to reach an equilibrium after about 30 min. At the end of the test, the algae were analyzed and found to contain 0.36 μCi ^{65}Zn or 1.1 μCi/g dry weight.

Conclusions

The perfection and application of this apparatus should permit the study of many variables influencing radionuclide uptake and retention by periphyton under conditions not subject to control in a river environment.

POSITIVE PHOTOTAXIS IN FIRST INSTAR CADDIS LARVAE

Positive phototaxis was observed in newly hatched Hydropsyche cockerelli larvae despite a recognized negative phototaxis in later larval instars.

Investigator:
C. C. Coutant

Larvae of the caddis fly Hydropsyche cockerelli Banks are some of the most abundant macroinvertebrate organisms in the Columbia River. The biology of this insect species is under study because its presence and behavior undoubtedly affect downstream transport of radionuclides. River caddis larvae are secretive organisms living between and under bottom stones. They are generally considered to be negatively phototaxic. Observations made during the summer of 1965 indicate positive phototaxis in newly hatched, first instar Hydropsyche larvae. This anomalous behavior may be of ecological significance to the species.

Observations

Egg masses presumed to be those of H. cockerelli (because of intense mating and ovipositing activity of this species in the area at the time) were collected from the Columbia River near Richland, Washington. The eggs had been deposited on asbestos sheets placed in flowing water at a depth of

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about 3 m. Hatching of the larvae occurred in unstirred tap water after about 48 hr at a temperature of 24 °C.

Positive phototaxis was first suspected during casual observation of the newly hatched larvae illuminated under a dissecting microscope. Curiosity prompted a series of experiments in which newly hatched larvae, in a beaker of water, were subjected to a directional light source which was moved at half-hour intervals. At the end of each interval the positions of swimming larvae with respect to the light were noted. The data conclusively demonstrated that larvae were attracted to the beam of light.

Conclusions

A swimming, positively phototaxic early larva of a predominantly benthic organism is strongly reminiscent of behavior found in many marine invertebrates. A probable advantage of this activity to marine sessile benthos is dispersal. A necessity for such dispersal can also be recognized for the Columbia River. Adult H. cockerelli emerge and carry out much of their oviposition during periods of high river flows in early summer. Most egg masses are laid on bottom stones in relatively shallow, near-shore water rather than in mid-channel. As water levels decline this near-shore area is exposed and the larvae must seek center channel or perish. A mechanism by which the hatchlings can enter river currents, such as swimming toward the lighted surface of the river, would serve to accomplish this end. Presumably a reversal of phototaxis occurs at a later instar.

Active dispersal of caddis larvae into river currents provides one additional mode of transport of radionuclides from the Hanford Reservation to downstream regions. The magnitude of transport by this means is, however, certainly quite small.

MIGRATION OF COLUMBIA RIVER FISH

The movement of resident Columbia River fish upstream from Hanford reactor outfalls or into a tributary stream was studied. The presence of ^{65}Zn, acquired by fish when downstream from reactor outfalls, served as a radioactive tag.

Investigator:
D. G. Watson
Technical Assistance:
G. W. Johnson
I. L. Newcomb
Dorothy D. Wade

The upstream movement of resident Columbia River fish has been known for some time through the enumeration of fish ascending river dams and through the detection of fish tagged with reactor effluent radionuclides upstream from the Hanford reactors. The present study sought to define the extent and time of resident fish movement upstream from the Hanford reactor effluent outfalls and into a tributary stream, the Yakima River, which joins the Columbia downstream from the reactor areas.

Observations

Fish were routinely collected with gill nets. Large samples of one species, the whitefish (Prosopium williamsoni), were obtained once yearly when the upstream dam fish ladders were drained for maintenance. The assumption that a fish had migrated from an area downstream from the Hanford reac-

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tors was based on detection of ^{65}Zn in the eyes and gastrointestinal tract. Radiozinc, one of several reactor effluent radionuclides that could have been employed, was selected as the indicator because of its ease of assay, its relatively long half-life (245 days), and its previously studied tendency to concentrate in the eyes and gastrointestinal tract of trout (Salmo gairdneri) (Annual Report, 1963). Although ^{65}Zn is present in worldwide fallout, fish collected from the Columbia over 160 km upstream from the Hanford facility had no detectable amounts of this isotope in their tissues.

The sampling was selective in that fish capture depended on the movement of fish through areas of the river where nets could be fished. The proportion of ^{65}Zn-tagged individuals among the three species most consistently collected are listed in Table I. The concentration of ^{65}Zn in the eyes of these species was consistently greater than that in the gastrointestinal tract. This is in contrast to carp (Cyprinus carpio) in which the gut always had more ^{65}Zn than eyes, and to whitefish where the eye and gastrointestinal tract contained nearly equal amounts of ^{65}Zn. No pronounced seasonal trends in the proportion of marked individuals is evident at Location 1, 17 km upstream from reactor outfalls. The sucker data, perhaps, indicate more upstream movement during late spring and winter. At Location 1, more than 50% of the fish usually showed evidence of prior exposure to reactor effluents; all of the chiselmouth taken at this site had measurable amounts of ^{65}Zn. Continuity of sampling at Location 2, in the stream tributary to the Columbia, was

TABLE I. Columbia River Fish Tagged with ^{65}Zn, 1965-66

	Sucker[a]				Squawfish[b]				Chiselmouth[c]			
	Location 1[d]		Location 2[e]		Location 1		Location 2		Location 1		Location 2	
Date	Total Number	% Tagged	Total Number	% Tagged	Total Number	% Tagged	Total Number	% Tagged	Total Number	% Tagged	Total Number	% Tagged
1965 May	8	88			3	33						
June	14	93			15	66			2	100	29	86
July	15	67	8	87	22	68	9	66	4	100	9	62
August	5	60	3	33	14	93	2	0	1	100	15	87
September	20	70	20	30	19	47	1	0	12	100	1	100
October	5	40	1	0	10	80	1	0	9	100		
November	11	66	1	0	11	73			8	100		
December	4	50			3	66	1	0	7	100		
1966 January	4	100										

[a] Largescale Sucker - Catostomus macrocheilus Girard
[b] Northern Squawfish - Ptychocheilus oregonensis (Richardson)
[c] Chiselmouth - Acrocheilus alutaceus Agassiz & Pickering
[d] 17 km upstream from reactor outfalls
[e] 28 km upstream, Yakima River

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not as good as at Location 1. There is some indication of an influx of ^{65}Zn-tagged suckers and squawfish in July.

In Figure 1, the seasonal variation in ^{65}Zn in two species of Columbia River fish collected upstream from the reactors is compared with that of fish collected downstream from effluent outfalls. There is a pronounced decrease in ^{65}Zn content during the summer. This is due to the greater dilution of reactor effluents by the increased river flow during the freshet period of early summer. Minimum levels of ^{65}Zn in chiselmouth and squawfish occur about a month later upstream from the reactors than below the reactors. The nearly equal quantities of ^{65}Zn in chiselmouth during the months of June, July, and August perhaps indicate a rapid upstream movement of this species during this period.

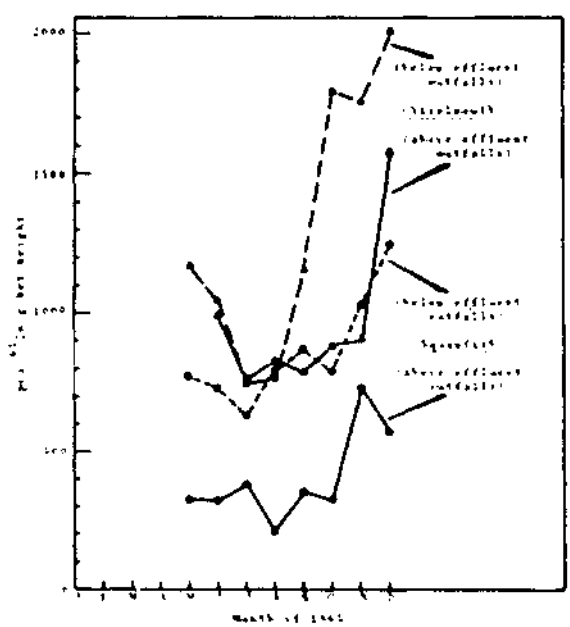

FIGURE 1. Zinc-65 in Eyes of Columbia River Fish

Samples of approximately 100 whitefish, a locally important sport and food fish, were collected 18 km upstream from the reactors in January and December of 1965. In January, 70%, and in December, 60%, contained measurable amounts of ^{65}Zn. The average concentration of ^{65}Zn in the eye and gastrointestinal tract of the tagged animals in January was 780 and 1100 pCi/g wet weight, respectively; and in December, 1060 and 1400 pCi/g wet weight, respectively. Upstream movement of this species starts in September in the Columbia River, and in July in the tributary. The distance of migration was not determined throughout the year. A single winter sampling from approximately 42 km upstream from the reactors showed only 8% tagged as opposed to 60% tagged at 18 km above the reactors.

Seasonal movement of whitefish from the Columbia to the Yakima River is very rapid. December Yakima River values for the eye and gastrointestinal tract were 1300 and 680 pCi ^{65}Zn/g wet weight, respectively, as compared to 540 and 1200 pCi ^{65}Zn/g at Hanford. At certain times of the year Yakima River fish may be as significant a source of ^{65}Zn and ^{32}P to local fishermen as those caught downstream from the reactors in the Columbia River.

Conclusions

The migration of resident fish appears to be a significant means of translocating reactor-produced radionuclides. Over 50% of the fish collected in the Priest Rapids area showed

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prior exposure to reactor effluents. The levels of ^{65}Zn in these tagged fish were usually greater than half, and often approached the levels measured in samples downstream from effluent outfalls. More extensive sampling, both in terms of sample size and collecting sites, along with controlled measurements of interspecies differences in zinc metabolism, will be required before seasonal migration patterns and quantity of radioelements translocated by migrating fish populations can be precisely determined.

RADIONUCLIDES IN COLUMBIA RIVER BIOTA

Measurements of the concentration of several reactor-produced radionuclides in Columbia River biota were made before and after closure of three Hanford reactors. A noticeable reduction of radionuclides in the biota was evident after reactor shutdown, but this may have been influenced by river temperature changes and channeling of effluent.

Investigators:
D. G. Watson
C. E. Cushing
R. W. Perkins[4]
Technical Assistance:
G. W. Johnson
I. L. Newcomb
Dorothy D. Wade

In October and November of 1964 and 1965, a variety of Columbia River organisms, collected from the same location downstream from the reactor effluent outfalls, was analyzed for 18 radionuclides. The purpose of this study was to evaluate the change in radionuclide burden in the biota after the closure of three production reactors immediately upstream from the sampling site, during the first half of 1965.

Observations

The concentrations of the six radionuclides that made up over 90% of the gamma emitters in representative organisms are shown in Table I. There was a general decrease in the concentration of radioisotopes from 1964 to 1965. Chromium-51, an element of little biological importance, decreased less than the other radioisotopes. In fact, there appeared to be an increase in concentration of this isotope in many organisms. Phosphorus-32, an isotope of major biological significance, was not measured in 1965 due to an analytical error, preventing measurement of change in concentration of this nuclide.

The average river temperatures during the fall were about 1 °C higher in 1965 than in 1964. This would tend to decrease the magnitude of change in isotope concentration, particularly in those forms with a temperature-dependent metabolic rate. The persistence of the closest upstream reactor effluent plume, carrying slightly warmer water past the sampling location in 1964, may have eliminated or reduced the effect of the temperature difference between the 2 years.

There was a marked decrease in concentration of several reactor radionuclides in the river between 1964 and 1965. No measurements were made of the radioactivity in the water at the sampling site; but at a point several kilo-

[4] *Environmental and Radiological Sciences Department*

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TABLE I. Concentration and Percent Change, pCi/g Wet Wt, F-1 Station, 1964-65

		Net Plankton	Filamentous algae[a]	Sponge[b]	Caddisfly larvae[c]	Chironomids	Limpets[d] soft parts	Limpets shell	Clam[e] soft parts	Clam shell	Crayfish[f]	Shiners[g]
^{95}Zr	1964	1,414	397	169	764	1,250	1,595	844	393	136	955	775
	1965	63	149	101	476	441	685	451	538	83	138	406
	% Change	-96	-63	-40	-58	-59	-57	-33	+16	-32	-84	-47
^{51}Cr	1964	57,500	43,400	10,200	5,590	1,390	1,940	1,080	620	181	534	733
	1965	28,600	33,900	10,900	4,890	2,660	2,260	1,350	599	184	473	N.D.
	% Change	-51	-24	+61	+36	+91	+16	+25	-3	+2	-12	---
^{95}Nb	1964	291,000	96,300	12,200	10,450	4,490	1,780	7,480	556	617	982	69
	1965	10,100	8,660	4,200	1,890	1,120	-1,590	669	+820	+366	77	21
	% Change	-96	-91	-65	-85	-81	---	-91	---	---	-92	-65
^{99}Ru	1964	101,000	36,000	47,400	8,970	5,560	1,500	2,230	3,150	585	748	N.D.
	1965	14,300	9,150	21,400	2,610	4,100	1,010	865	1,270	582	181	85
	% Change	-86	-75	-55	-71	-58	-65	-61	+65	+52	-76	---
^{95}La	1964	14,000	8,870	3,070	1,970	2,080	2,820	633	1,100	881	811	1,100
	1965	2,910	3,250	2,500	1,770	1,370	1,360	346	1,190	585	545	1,220
	% Change	-79	-63	-19	-10	-34	-52	-47	+8	-22	-33	+1
^{140}La	1964	5,300	1,610	950	223	-97	73	115	47	5	12	15
	1965	2,010	2,760	1,525	322	211	107	107	89	12	20	P.33
	% Change	-66	+9	+59	+44	---	+46	-7	+89	+139	+64	-99

[a] *Stigeoclonium* sp.
[b] *Spongilla* sp.
[c] *Hydropsyche cockerelli* Banks

servations suggested the possibility of using immune response to identify columnaris exposure in natural fish populations and led to the study reported here on the distribution of agglutinating titers and titer magnitude in blood sera of Columbia River fishes.

Observations

Fish were captured by gill nets every month for a year at four sites: Bonneville, McNary, Hanford, and Wenatchee. Seasonal variability in gill net catches complicates the study of titer distribution on an annual basis for some species, e.g., whitefish, Prosopium williamsoni, which were not caught consistently throughout the year. Other fish such as the coarse-scaled sucker, Catostomus macrocheilus, were gill netted in large numbers throughout the year at most of the sampling sites. Only the sucker data are presented in detail; the more limited data for most other species showed a similar pattern.

The results from over 900 suckers summarized in Figure 1 show the percentage of fish with titers, by season and by sampling site. The percentage of fish with agglutinating titers varied between 20 and 100% during the year. All sites showed a similar, annual cycle. No consistent relationship between the percentage of fish with titers and location of site is apparent. At no time did the percentage of fish showing a titer reach zero. Residual antibodies are carried by the suckers during the winter months when probability of exposure is slight.

Figure 2 illustrates the variation in titer magnitude for suckers by season and sampling site. The average agglutinating titer ranged from about 20-80 during most of the year, but rose sharply during the late fall months. Exceptionally high agglutinating values of 1:2560 were observed for some individual fish, but 1:640 was the more common high value observed for river fish. The sharp rise in titer values is most likely related to re-exposure and also coincides with the period when larger numbers of fish show a titer.

Blood sera sampled from several hundred whitefish showed no agglutinating titers. In the laboratory, however, whitefish challenged intraperitoneally with a vaccine (heat-killed columnaris) plus complete Freund's adjuvant developed antibodies within a month. The titers ranged from 1:10 to 1:160. When the vaccine was administered a second time, titers up to 1:1280 were produced within a week. The lack of titers in whitefish captured from the river is unexplained and requires further study.

Scrapings and smears from gills and lesions were cultured to determine both presence of organisms and gross infection of fish at each site. The results at Bonneville, Hanford, and Wenatchee were similar to those obtained in previous years' surveys; organisms were cultured from 6.2 to 9.2% of the fish sampled, and gross infection was evident in 1.9 to 2.2%. Much higher values were found for fish sampled at McNary, where organisms were cultured from 12.2%, and gross infection was evident in 5.7%.

Conclusions

Both the titer and the incidence of fish with titer against C. columnaris show an annual cycle, related,

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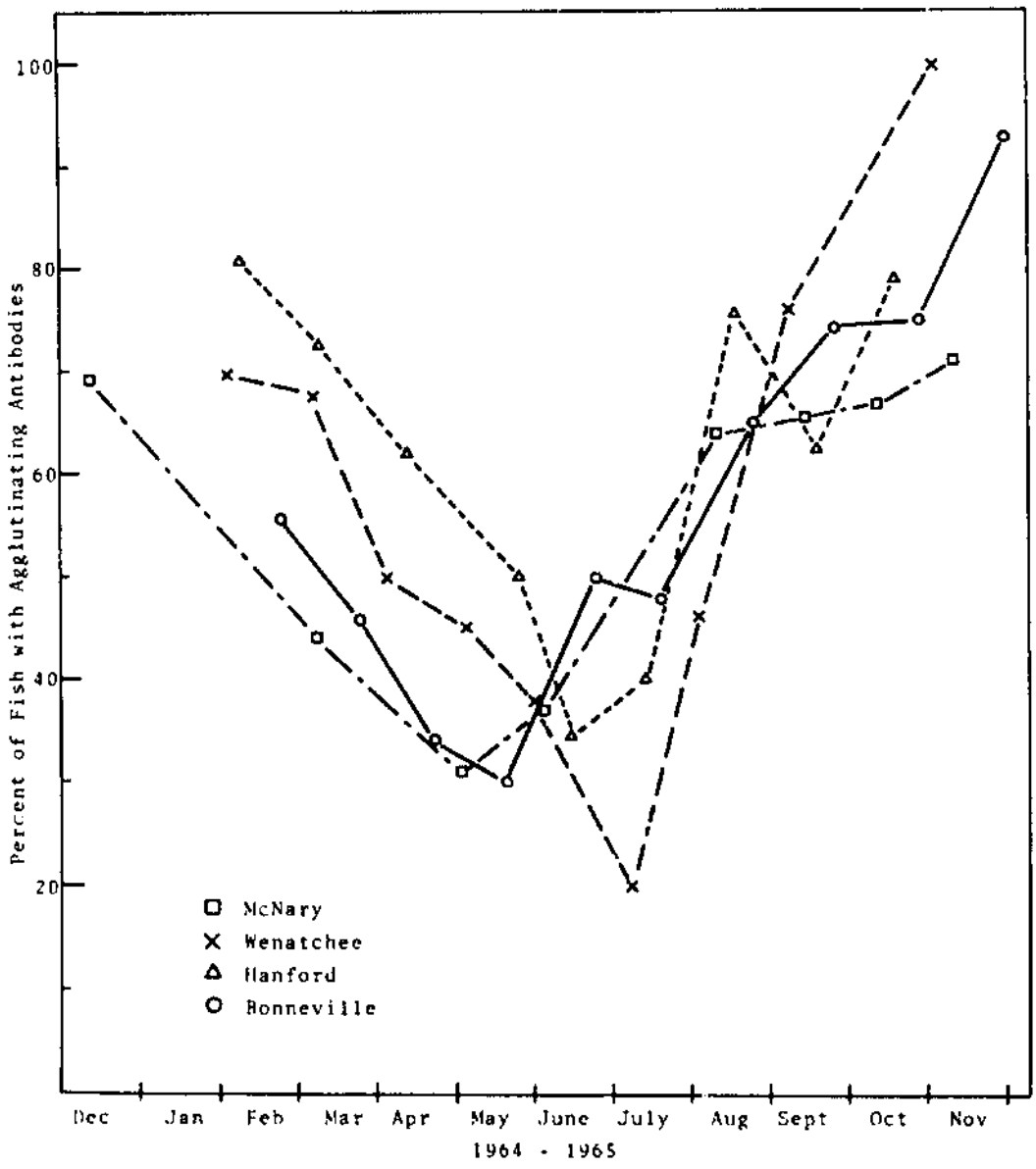

FIGURE 1. Number of Suckers (Catostomus macrocheilus) with Agglutinating Antibodies Against Columnaris

no doubt, to the seasonal changes in water temperatures and the re-exposure of fish to columnaris organisms. While the production of antibodies against columnaris has been demonstrated for most river fish, the degree of protection afforded by these antibodies is unknown. Studies are planned to investigate this question.

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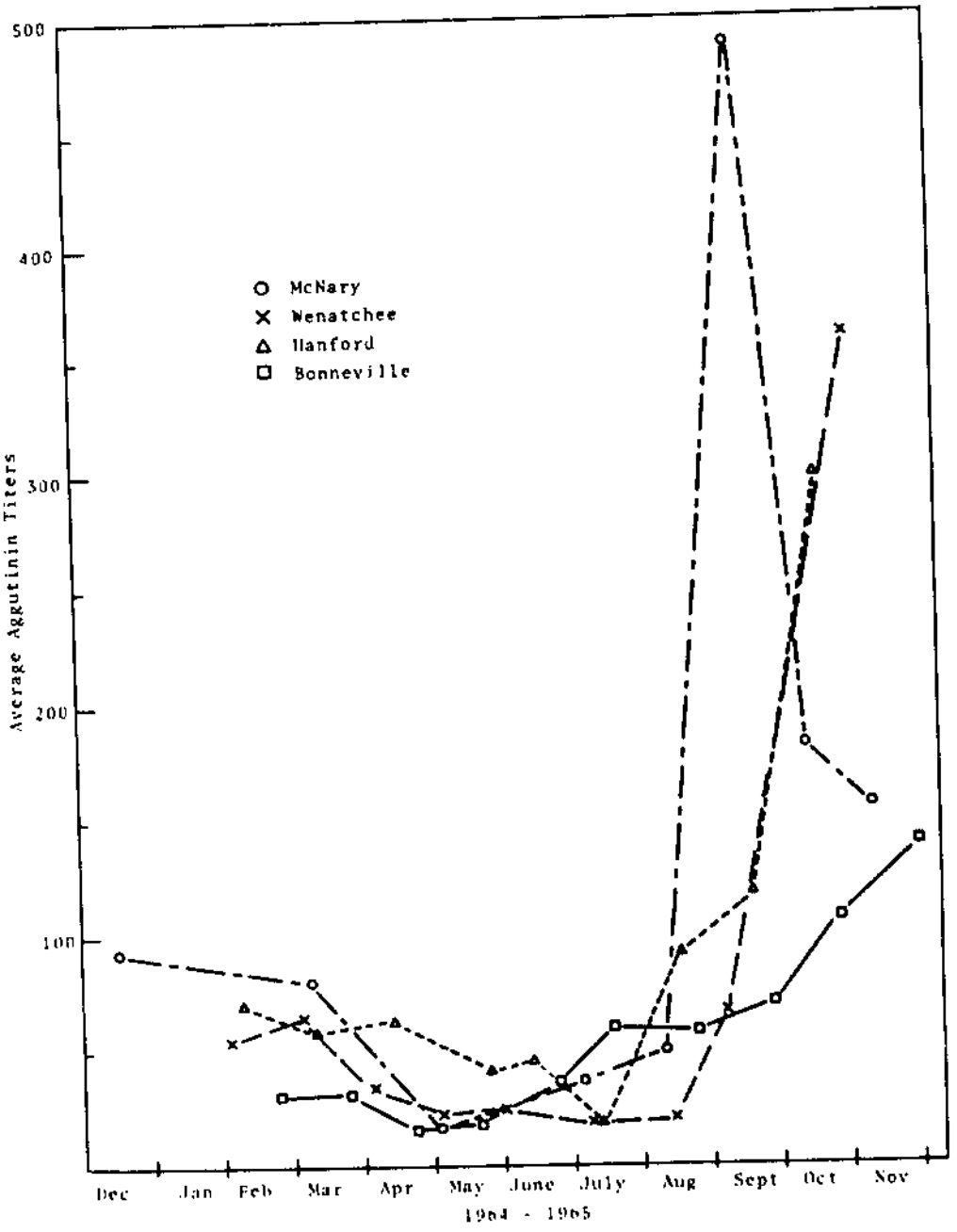

FIGURE 2. Seasonal Changes in Magnitude of Agglutinating Antibodies Against Columnaris in Suckers (Catostomus macrocheilus)

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COLUMBIA RIVER CHINOOK SALMON SPAWNING NEAR HANFORD

Investigator:
D. G. Watson

A record number of 1768 salmon nests were observed in the section of the Columbia River near the Hanford Project. This is approximately 300 more than the previous high of the past 19 years.

The section of the Columbia River within the Hanford Project is one of the few remaining parts of the mainstem river used for spawning by salmon. Aerial surveys of the locally spawning race of chinook salmon are made annually to evaluate possible effects of reactor effluents on the survival of these fish.

Observations

In 1965 four surveys were made of the Columbia River from Richland to Priest Rapids Dam. A total of 1768 nests were observed, a 19-year record. The previous high count of 1485 occurred in 1958; last year's count was 1443. The numbers of fall chinook ascending Bonneville Dam in 1958, 1964, and 1965 were 249,314; 172,463; and 157,839, respectively.

No salmon nests were observed out of water as they had been in the past several years. The surveys in 1965, however, were all made in the afternoon during periods of high river flow. Diurnal river level fluctuations due to operation of Priest Rapids Dam were in excess of 6 feet immediately downstream from the dam.

Conclusions

The long-term trends in numbers of salmon nests observed near Hanford are compared with the fall chinook run over Bonneville Dam in Figure 1. The numbers of local salmon have increased

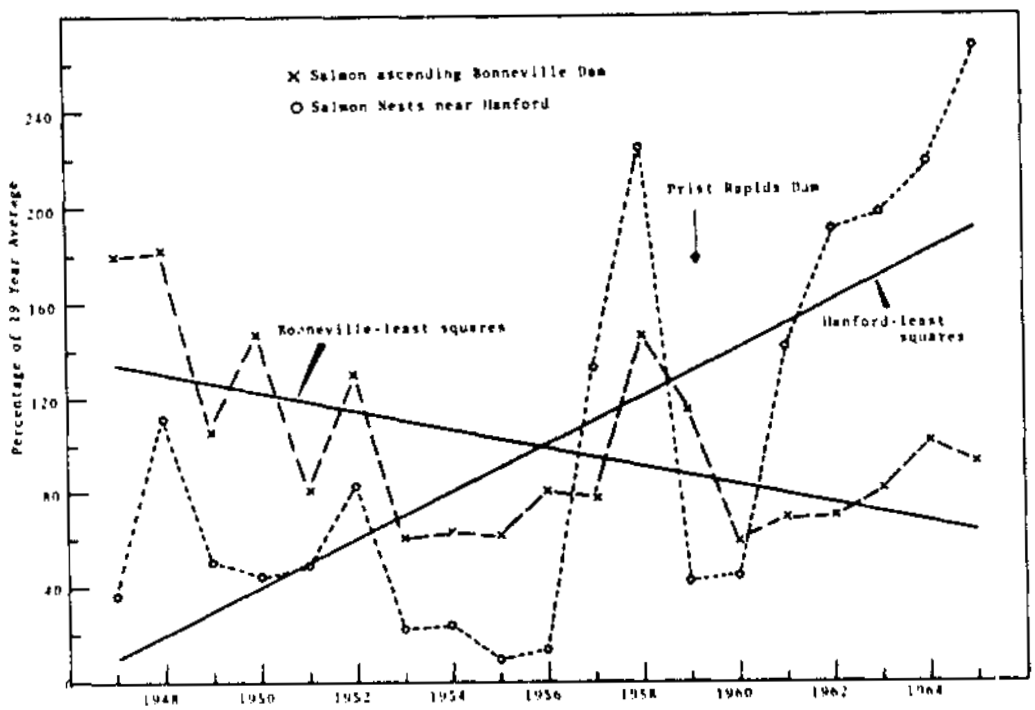

FIGURE 1. Trends of Fall Chinook Salmon

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over the past 19 years while the overall run in the river has declined. The increase in local spawning may be due to a partial barrier effect of Priest Rapids Dam. The spawning area immediately below the dam continues to be very heavily seeded, perhaps to the extent of overutilization. The major spawning areas near reactor outfalls had near record numbers of spawning fish in 1965, an indication that the success of the local population of salmon was better than that in the Columbia River generally.

REACTOR EFFLUENT MONITORING - 1965

Young chinook salmon were reared in 0, 2 and 4% reactor effluent at three different temperatures to determine effect on mortality, growth and uptake of radionuclides. There was no significant effect of temperature or effluent concentration on mortality, but warmer waters accelerated growth regardless of effluent concentration. The radionuclides detected in whole fish were related to effluent concentration, but not to temperature.

Investigator:
P. A. Olson
Technical Assistance:
R. G. Genoway
C. O'Malley

The decision made over 20 years ago to monitor Hanford reactor effluent with fish is especially significant today in view of national concern with water pollution control and abatement. A continuing surveillance is required because of the variation in upstream water usage and technical changes in reactor operations. The tests reported here were designed to evaluate the interaction effect of water temperature and effluent concentration on mortality, growth and uptake of radionuclides in young chinook salmon, Oncorhynchus tshawytscha.

Observations

The experimental array presented in Table I shows the three treatment levels for temperature and effluent concentration. Water temperatures were not constant but controlled to vary with the regular river temperature. River water served as the base temperature pattern and a small heat increment was added to this pattern for the other two temperature treatments as indicated in Table I.

Test subjects were obtained from local Columbia River adult chinook salmon captured at Priest Rapids Dam. About 1250 newly fertilized eggs were distributed into each of the eight treatment groups in December 1964. The test terminated 6 months later when the fish were downstream migrant-sized fingerlings.

TABLE I. Experimental Array and Average Mortality and Growth by Treatments

Temperature	Effluent concentration, % 0	2	4
River water	n = 1254 m = 22.4 w = 0.82	n = 1310 m = 19.8 w = 0.88	n = 1252 m = 21.6 w = 0.92
River water +ΔH	n = 1222 m = 20.9 w = 1.13	n = 1294 m = 23.0 w = 1.11	n = 1252 m = 15.4 w = 1.14
River water +2ΔH	No observations	n = 1226 m = 18.5 w = 1.34	n = 1299 m = 21.8 w = 1.35

n = observations per cell
m = mortality
w = average weight
ΔH = heat increment contributed by 2% effluent
2ΔH = heat increment contributed by 4% effluent

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The results on mortality and growth are summarized in Table I. The mortality data did not show any significant effect of temperature or effluent concentration. Analysis of variance of the growth data, however, showed that warmer waters accelerated growth regardless of effluent concentration. The interaction effect was not significant.

The length-weight relationship was analyzed by using the following model: $W = aL^b$, where W = weight; a = a constant; L = standard length; and b = an exponent, usually about 3 for salmonids. Analyses inferred no statistical difference between the treatment groups in length-weight relationships. The computed overall mean value of b was 3.3974.

The results of gamma scans of pooled samples from each treatment group are summarized in Table II. The concentrations of the three major gamma emitters, ^{24}Na, ^{51}Cr and ^{65}Zn, are directly related to effluent concentration but no temperature effect was observed. The presented values estimate the amount of radioisotope the young fish can accumulate directly from the water. The mode of entry is primarily through the gills since freshwater fish drink little or no water and the prepared diet contained no radioisotope. Because reactors are located upstream from the hatchery water intake, some radioactivity is observed in the zero percent effluent group.

Conclusions

Results of the 1965 tests agree in general with past years' studies although much of the past data was from work with a Puget Sound race of chinooks instead of with local stock. The effect of the relatively small heat increment associated with highly diluted reactor effluent in the Columbia River is difficult to assess even when simulated under controlled laboratory conditions. Much of the classical work in this field was done at widely separated and steady temperatures and is of very limited value in assessing field problems. More detailed work is needed to evaluate the biological significance of imposing a small heat increment to the varying seasonal temperature profile of a river system.

TABLE II. Concentration of Gamma Emitters in Chinook Fingerlings After 6 Months Exposure to 0, 2, and 4% Effluent at Different Temperatures

Effluent concentration, %	Water temperature	pCi/g wet weight ^{24}Na	^{51}Cr	^{65}Zn
0	River	12	11	3
	River +ΔH	11	14	4
2	River	854	64	16
	River +ΔH	945	77	18
	River +2(ΔH)	845	49	12
4	River	1669	94	26
	River +ΔH	1840	96	28
	River +2(ΔH)	1720	72	24

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