BNWL-1306
Part 1
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PACIFIC NORTHWEST LABORATORY ANNUAL REPORT FOR 1969 TO THE USAEC DIVISION OF BIOLOGY AND MEDICINE

VOLUME I: LIFE SCIENCES
PART 1. BIOLOGICAL SCIENCES

August 1970

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AEC RESEARCH & DEVELOPMENT REPORT

BNWL-1306
Part 1

BATTELLE NORTHWEST
BATTELLE MEMORIAL INSTITUTE
PACIFIC NORTHWEST LABORATORIES
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BNWL-1306
Part 1
UC-48, Biology and Medicine

PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1969
TO THE
USAEC DIVISION OF BIOLOGY AND MEDICINE

VOLUME I LIFE SCIENCES
PART 1 BIOLOGICAL SCIENCES

by

The Staff of the
Biology Department

Edited by
R. C. Thompson

August 1970

BATTELLE MEMORIAL INSTITUTE
PACIFIC NORTHWEST LABORATORIES
RICHLAND, WASHINGTON 99352

This is the 19th Annual Report of the Biology Department. Previous volumes are: HW-25021 (1951), HW-28636 (1952), HW-30437 (1953), HW-35917 (1954), HW-41500 (1955), HW-47500 (1956), HW-53500 (1957), HW-59500 (1958), HW-65500 (1959), HW-69500 (1960), HW-72500 (1961), HW-76000 (1962), HW-80500 (1963), BNWL-122 (1964), BNWL-280 (1965), BNWL-480 (1966), BNWL-714 (1967), and BNWL-1050 (1968)

Part 2 of this volume, BNWL-1306 Part 2, covers work in the ecological sciences. Volume II of this report covers work in the physical sciences and is issued in three parts: Atmospheric Sciences, BNWL-1307, Part 1; Radiological Sciences, BNWL-1307, Part 2; and Instrumentation, BNWL-1307, Part 3.

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FOREWORD

This is the nineteenth in a continuing series of Biology Department Annual Reports. This report departs substantially from the format of previous years. Reports are much shorter--to save your time in reading, to save our time in writing, and to facilitate earlier issuance of these reports. We continue to look upon publication in the open literature as our primary vehicle for dissemination of research results.

Reports, this year, are titled to correspond with AEC Budget Submission titles (189 forms). In many instances we have subdivided these titles to facilitate the organization of results.

During the past year the Environmental and Life Sciences Division acquired a new manager, Dr. Edward L. Alpen. The former manager, Mr. Herbert M. Parker, returned to his position as Consultant to the Director. The only major organization change within the Biology Department was the establishment of a new Cytology Section under the management of Dr. James C. Hampton. This is a small section, concerned primarily with electron microscopy studies. A complete listing of Biology Department personnel, by section, is included at the end of this report, together with lists of 1969 publications and presentations.

A highlight of the year was the Symposium on Radiation Biology of the Fetal and Juvenile Mammal, held in Richland, May 5-8, jointly sponsored by the AEC Division of Biology and Medicine and Battelle-Northwest. Scheduled for June 2-5, 1970, is the Tenth Annual Hanford Biology Symposium on Pollution and Lung Biochemistry.

Construction on the new Biology Laboratory continued throughout the year. Before the end of another year, we hope to be well-established in these new quarters.

W. J. Bair, Manager
Biology Department

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BNWL-1306
Part 1

GASTROINTESTINAL IRRADIATION STUDIES

This long-continuing project has included studies of morphologic damage, functional disturbances, permeability changes, and the influence of secretions on the irradiated alimentary tract. Our experimental animal has been principally the rat, but we have more recently been interested in species comparisons employing miniature swine, rabbits, mice, and guinea pigs. The importance of fluid and electrolyte loss in the survival of animals exposed to intestinal irradiation has been given further consideration in our recent studies.

In addition to the research described in the reports which follow, we have conducted studies for the National Aeronautics and Space Administration that are closely related to our AEC program. These studies indicate that irradiation of the gastrointestinal tract is largely responsible for the nausea and vomiting that occurs in all miniature swine shortly following 1000 rad whole-body gamma irradiation. Maximal response was noted after lower-body exposures of 1500 rad; there was a lesser response both above and below this dose. Substantial quantities of fluid and electrolytes are lost by this route. If these losses are critical to survival following intestinal irradiation, studies with swine may be more extrapolatable to the human than studies with rodents, which lack a vomiting center.

GASTROINTESTINAL RADIATION SYNDROME IN THE MINIATURE SWINE

Investigators:

B. W. Wachholz, T. D. Mahoney and M. F. Sullivan*

Technical Assistance:

D. L. Catt and S. A. Hughes, Jr.

Morphologic factors involved in the response of the intestine to irradiation have been shown to differ markedly in different species. The small intestine has usually been thought to be the critical segment in assessing the radiosensitivity of the gastrointestinal tract, but our studies have shown that this is true only for mucosal damage which usually results in an early death during the first week after exposure. In a large animal, such as the swine, which resembles man in body size and in many of the anatomical features of the gastrointestinal tract, delayed changes occur in the intestine which appear to be related to the injured vasculature in either the lower small intestine or in the large bowel, and these may cause death a few months after exposure. These observations suggest that the radiosensitivity of the vasculature contributes significantly to intestinal radiation injury and may be consequential in radiotherapy when loops of bowel are included in the exposure field.

To further our knowledge in this area we have accumulated further data on the acute and sub-acute effects in miniature swine exposed bilaterally to ^{60}Co gamma radiation. (Tables 1 and 2). Some were exposed to whole-body irradiation and others to lower-body irradiation only. The ratio of the median radiation dose causing acute death following lower-body exposure to that which caused death after whole-body exposure was about 8 to 1, in contrast to the ratio of 2 or 3 to 1 seen in small

* *Consultant Pathologist, Medical Arts Building, Richland, Washington*

PUBLICATIONS: GASTROINTESTINAL IRRADIATION STUDIES

McKENNEY, J.R. "Electrolyte Fluxes and Electrical Potentials in Isolated Rat Intestine," Skornya (ed.) Intestinal Absorption of Metal Ions, Pergamon Press, Oxford, pp. 81-100. 1969.

McKENNEY, J. R. and M. F. SULLIVAN. "Electrolyte Transport and Voltage Measurements of Rat Intestine in vitro," Am. J. Physiol., vol. 217, pp. 1728-1735. 1969.

WACHHOLZ, B. W., T. D. MAHONY, and M. F. SULLIVAN. "Intestinal Radiation Injury in Miniature Swine," Radiation Research, vol. 39, p. 526. (Abstract). 1969.

laboratory animals. This indicates a higher resistance of the swine gastrointestinal tract to acute radiation effects. Acute death usually did not occur in miniature swine until doses to the lower-body reached about 2500 rad. Mucosal injury reached its greatest severity at about 6 days postexposure which is a few days later than observed in the rat.

TABLE 1. Mortality Data on Whole-Body Irradiated Miniature Swine (^{60}Co, bilateral exposure)

Midline Dose, rad	Number Exposed	Postexposure Survival, days
205	1	survives at 600 days
245	1	18
285	1	survives at 600 days
326	3	16(2), 18
360	1	15
410	1	17
570	1	11
940	2	9, 10
1220	1	9
1550	1	7
1670	1	6
1800	1	5
2050	1	5
2850	1	6

TABLE 2. Mortality Data on Partial-Body Irradiated Miniature Swine (lower-body, bilateral, ^{60}Co)

Midline Dose, rad	Number Exposed	Fate
950	1	killed accidentally at 30 days
1250	2	survive at 600 days
1550	1	died at 85 days
1650	2	survive at 600 days
2000	33	2 died at 6 days 1 died at 15 days 1 died at 33 days 22 killed for histology at 2-10 days 7 killed for histology at 69-133 days
2250	1	died at 69 days
2450	5	died at 6, 7, 8, 10, and 11 days
2900	4	died at 7(2), 8, and 9 days

PROTEIN LOSS FROM THE IRRADIATED INTESTINE

Investigators:

M. F. Sullivan and B. W. Wachholz

Technical Assistance:

Alma L. Crosby and M. L. Smith

From the standpoint of both morphologic and histologic observations, vascular injury appeared to be more prominent in the irradiated intestine of swine than we have observed, or than has been usually reported, for rodents. In previous studies we attempted to quantitate these effects on the vasculature by measuring the permeability of the intestine to macromolecules moving from the blood to the lumen. Polyvinylpyrrolidone (PVP) labeled with ^{131}I was used as a "stand-in" for plasma protein. We have now extended these studies to quantitatively compare vascular injury in rats, rabbits, guinea pigs, mice and swine.

Plasma protein was labeled with ^{51}Cr injected 2 days following exposure of the lower body to various radiation doses known to cause severe intestinal radiation injury (Figure 1). Irradiated rats (1000 to 1500 R) lost about three times as much protein into the lumen of the intestine as did unirradiated controls in agreement with previous observations employing PVP. A similarly increased leakage was seen in the irradiated pig intestine, but the percentage of injected ^{51}Cr excreted by the unirradiated pig was only one-fifth that excreted by the rat and was more similar in this respect to the excretion pattern seen in humans. Irradiated mice and rabbits did not increase their leakage of protein into the lumen of the intestine as a response to radiation.

The mechanism of protein leakage into the intestine thus presents a dilemma. The direct experimental evidence, in some species but not in others, suggests an increased permeability, which allows macromolecules

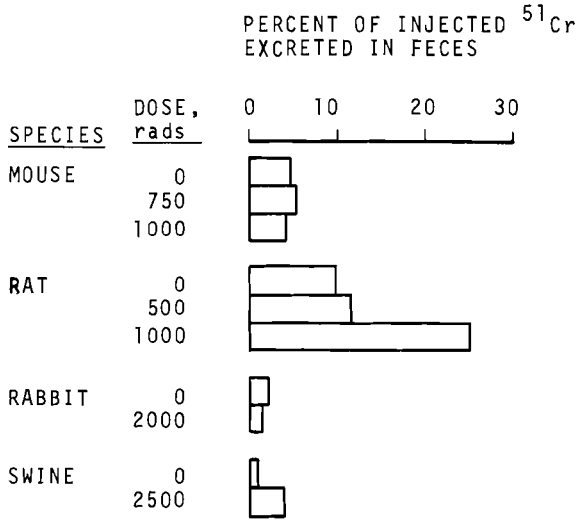


FIGURE 1. Species Comparison of ^{51}Cr-Labeled Protein Leakage from the Irradiated Intestinal Tract [$^{51}CrCl_3$ injected intravenously, 2 days (3 days in the case of swine) following lower-body irradiation; cumulative fecal excretion of ^{51}Cr measured during ensuing 4 days.]

to leak into the lumen of the intestine; whereas, it has generally been found both in this laboratory and by others, that radiation decreases permeability, as exemplified by the decreased movement of fluid and electrolytes from the blood to lumen.

We are currently investigating the possible importance of the lymphatics as a route of protein leakage into the irradiated gut. The difficulties associated with maintaining irradiated animals for several days with a lymph fistula are formidable and the data obtained are still quite limited. They do suggest, however, that the increases in fecal ^{51}Cr-labeled protein that occur following irradiation may be due to leakage via the lymphatics. This increased excretion correlates with a concomitant decrease in the level of ^{51}Cr-labeled protein in the lymph.

EFFECTS OF IRRADIATING SKIN

Investigator:
D. D. Mahlum
Technical Assistance:
D. L. Catt and Joan O. Hess

This project has three major objectives:

- Determination of the response of skin to ionizing radiation as influenced by size of exposure site and radiation dose administered.
- Determination of the effect of age at time of exposure.
- Determination of the effect of combined treatment of the skin with radiation and known pharmacologic compounds with carcinogenic properties.

Observation continues on the Hanford Miniature Swine exposed as young adults to doses of 1, 2.5, 6.25, 15, or 40 krad of β-radiation from ^{204}Tl plaques of 0.01, 0.5, 1.0, or 5.0 cm^2 areas. After 18 months, only the areas of 0.5 cm^2 or larger, which were irradiated at the 6.25 krad or higher doses, are grossly visible as thin, whitened circles. Hair continues to grow even in the heavily irradiated areas. No signs of tumor development are as yet apparent.

Additional swine were exposed for serial sampling for histologic examination. A few newborn miniature swine were also exposed to ^{204}Tl plaques. The severity of the response in these young pigs exceeds that of the older animal. These animals show impaired or altered hair growth in the heavily irradiated areas, and the size of the damaged area appears to increase as the animal grows. Additional animals will be exposed at early ages and carefully observed for both early changes and eventual tumorigenic response.

Quite interesting results are beginning to appear in the experiment where newborn, weanling, or adult rats were exposed to 0.01, 0.5, or 1.0 cm^2 field sizes of ^{204}Tl β-radiation at doses of 1, 2.5, 4, or 10 krad. At 15 months postexposure, tumors had developed at only two of 192 exposed sites on adult rats. Nine tumors had developed at 162 sites on rats exposed as weanlings, and 28 tumors had developed at 165 sites on rats exposed shortly after birth (Table 1). Only one of these tumors (from a weanling) has been examined histologically; it was classified as a basal cell carcinoma. Although the majority of the tumors arose at the 0.5 and 1 cm^2 sites, a few have appeared at the 0.01 cm^2 sites. Sex plays an important role in the tumor response, as all but two of the tumors have been found in males. In the rats exposed as adults, the areas damaged by irradiation correspond closely to the size of the original exposure site. In the rats exposed as newborns, particularly to the higher doses, scar tissue often covers an area much larger than that originally irradiated. Although still very preliminary, these results suggest that a wide difference in sensitivity of skin to tumor induction exists between the immature and adult animal.

TABLE 1. Effect of Radiation Dose, Field Size, Sex, and Age at Exposure on Skin Tumor Incidence in the Fischer Rat at 15 Months Postexposure

Age at Exposure	Dose, rads	Male			Female		
		Field size, cm^2			Field size, cm^2		
		0.01	0.5	1.0	0.01	0.5	1.0
Newborn	1,000	1/5*	2/8	1/6	0/7	0/7	0/5
	2,500	2/10	4/6	1/5	0/6	0/5	0/7
	4,000	2/6	2/11	3/8	0/9	1/3	0/6
	10,000	0/9	4/8	3/12	0/5	0/6	1/4
Weanling	1,000	0/5	0/9	0/6	0/7	0/5	0/6
	2,500	1/5	2/4	1/6	0/7	0/6	0/8
	4,000	2/10	0/8	2/5	0/7	0/7	0/8
	10,000	0/7	1/5	0/9	0/6	0/9	0/9
Adult	1,000	0/15	0/16	0/17			
	2,500	0/18	0/14	0/15			
	4,000	0/14	0/16	0/18	No female adults were exposed.		
	10,000	0/16	0/17	0/15			

* *Number of sites showing tumors/number of irradiated sites.*

RADIATION AND HUMAN SPERMATOGENESIS

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

This program of cooperative research with Dr. C. A. Paulsen of the University of Washington, School of Medicine, was initiated in 1966. It has included counts, motility, vital staining, fluorescent antibody, and morphology studies of sperm in samples of seminal fluid collected from control and testicular-irradiated subjects. Major research emphasis during the past year has been concerned with the role of the autoimmune response in effecting testicular function following radiation damage to the testes. Human sperm antibodies were produced in rabbits, the antibodies were tagged with fluorescent compounds, and the sperm samples from irradiated and biopsied subjects were tested for antibody response.

Four volunteers who received 100 R of testicular X-irradiation showed a decrease in total sperm count within 1 week. Sperm counts reached less than 10% of pre-exposure level by 9 weeks postexposure. By 59 weeks postexposure, none of the counts had increased above 10% of pre-exposure values. Sperm motility began to decrease at 3 weeks postexposure, and reached zero by 15 weeks; recovery began at 39 weeks. The live sperm count dropped below 50% from 7 to 43 weeks postexposure. Positive fluorescent antibody staining of sperm was demonstrable between 3 and 39 weeks postexposure.

Four volunteers who received unilateral testicular biopsy, but no irradiation, showed an initial decrease in total sperm count at 1 week postexposure. The count decreased to less than 40% of pre-experimental values within 9 weeks posttreatment. By 19 weeks posttreatment, total sperm counts had increased to greater than

50% of the pre-experimental values. Sperm motility decreased slightly between 5 and 27 weeks posttreatment.

It appears that spermatogenic effects of testicular biopsy are similar to those of X-irradiation of the testes, but to a less severe degree. Sperm samples from these subjects will continue to be monitored until some recovery is evident.

RADIATION EFFECTS ON CHYLOMICRON SYNTHESIS AND CAPILLARY PERMEABILITY IN MICE

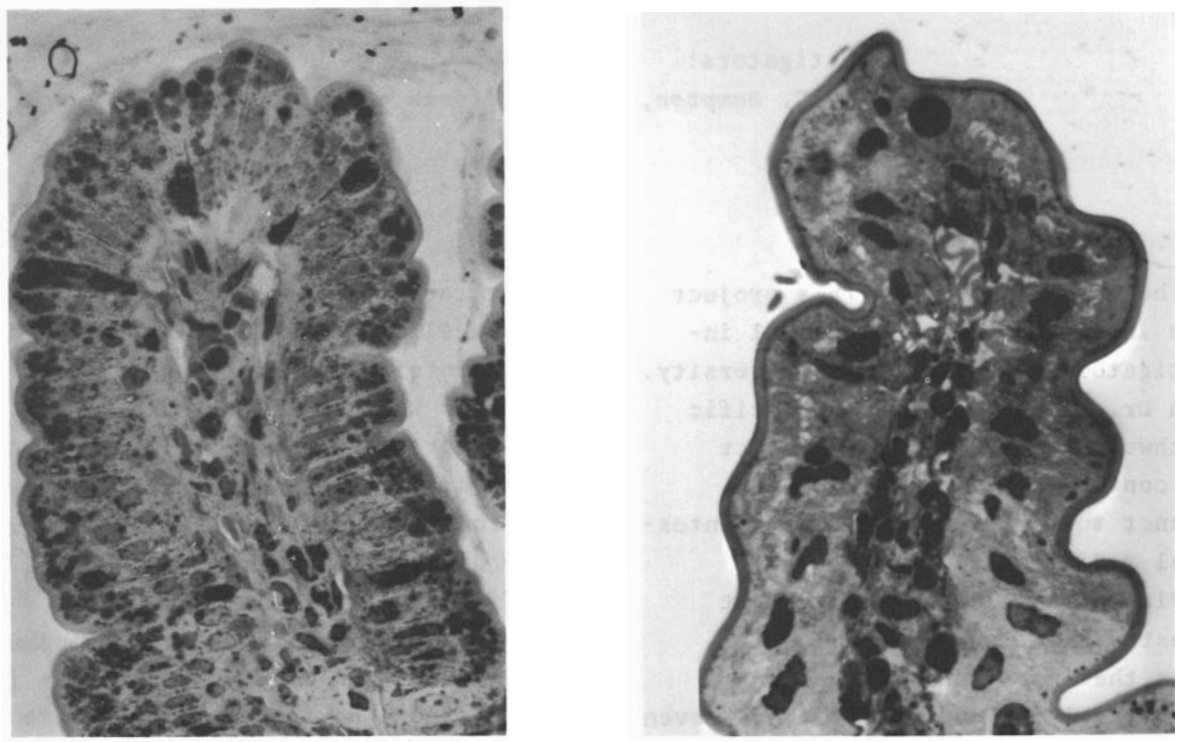
Investigators:
J. C. Hampton, M. F. Sullivan and B. Rosario

The main features of this project were initiated by the principal investigator at Northwestern University. When Dr. Hampton joined the Pacific Northwest Laboratory, the project was continued as a significant adjunct to our studies of gastrointestinal radiation effects.

Plasma sampling has shown that ingested, labeled fatty acids cross the intestinal epithelium to enter the lymph and blood stream, even after X-ray doses which are known to strip epithelial cells of ribosomes and to appreciably alter other cell organelles. Protein synthesis is required in the production of chylomicrons, the normal transport form of lipids. This must be reconciled with the fact that radiation destroys or alters the intracellular organelles responsible for protein synthesis. It is important to know whether labeled fatty acids present in plasma after irradiation are actually incorporated into chylomicrons or whether they may cross the damaged epithelium, unchanged, by diffusion.

Ileum, jejunum and liver specimens from C57 brown mice sacrificed at 1, 2, 3, and 4 days after exposure to 3kR of total body X-rays are being examined with respect to absorption of H^3-palmitic acid in safflower oil, administered by stomach tube 30 min prior to sacrifice. Preliminary observations, based upon light microscopic techniques, show that copious amounts

of lipid were absorbed by jejunal epithelial cells, and a lesser amount was absorbed by ileal cells, in controls, and at 1 and 2 days postirradiation. Liver samples from these animals showed an accumulation of lipid in hepatocytes well above that observed in mice not receiving H^3-palmitic acid and oil. Widely dilated lacteals were consistently found in the intestines of these animals. The amount of lipid in epithelial cells diminished rapidly at 3 and 4 days postirradiation (Figure 1) and this was reflected in diminished accumulations in hepatocytes and lack of dilation of lacteals. It appears that epithelial cells in the 3- and



A B

FIGURE 1. Villi from Mouse Intestine, Fixed 30 min After Feeding ^{3}H-Palmitic Acid in Safflower Oil [(A) Control - large lipid droplets are clearly visible in nearly every cell. (B) 3-1/2 days after exposure to 3000 R - mucous droplets (large black particles) are still present in goblet cells, but there is little evidence of lipid absorption.]

4-day postirradiated intestine are capable of absorbing lipid in fairly large amounts but that transport across the cells into the lamina propria is greatly impaired. Electron microscopic and autoradiographic data from this experiment are not yet available. It should establish whether the absorbed lipid is complexed with protein (chylomicrons) in irradiated animals or is present as triglyceride only.

Studies on capillaries have demonstrated that the presence or absence of bile in the intestine has little, if anything, to do with permeability changes and vascular damage, as judged by morphologic appearance. It is clear, however, that the fenestrated type of capillary normally present in intestinal villi promptly disappears as epithelial damage becomes more severe at the 3- and 4-day postirradiation interval, and that thrombi form in larger capillaries of the lamina propria. Thus a morphologically, and probably functionally, distinct vascular component is lost as a result of irradiation, and circulation is either impaired or perhaps hemorrhage prevented, by thrombi in many of the larger capillaries. In further studies, it is expected that a dose-response relationship can be established with respect to damage to the delicate, fenestrated capillaries in intestinal villi.

CELLULAR REGULATORY MECHANISMS

Studies on the nature and mechanism of cellular regulation are necessary to an understanding of the physiological effects of radiation, temperature, and other environmental stresses. We are concerned in this project with the regulation and molecular basis of three interrelated types of biological phenomena:

- The role of metabolites as effectors of specific RNA synthesis.
- Transport of nutrilites across the cell membrane.
- Secretion of exocellular enzymes involved in the degradation of macromolecules.

KYNURENINASE STUDIES

Investigator:
J. R. Turner
Technical Assistance:
Carla J. Farrell

Synthesis of messenger-RNA specific for the inducible enzyme kynureninase (i.e., transcription DNA→RNA) proceeds in cultures of Neurospora in the presence of inducer plus cycloheximide, an inhibitor of protein synthesis. Subsequent removal of inducer and cycloheximide results in the appearance of kynureninase (i.e., translation, RNA→protein) in these cells. The mRNA formed in the absence of protein synthesis is stable and as a result accumulates in the cells to a concentration (after 70 min) sufficient to allow synthesis of kynureninase at a rate seven-fold higher than the rate in maximally induced cells. Accumulation of stable messenger-RNA is linear in the absence of protein synthesis; however, the rate of decay of this messenger-RNA during subsequent translation appears to be proportional to the rate of translation.

PUBLICATIONS: CELLULAR REGULATORY MECHANISMS

TURNER, J. R. and W. A. SORSOLI. "Induction of Kynureninase in Neurospora crassa," Bacteriol. Proc., p. 50. 1969. (Abstract).

VUKOSAVOVICH, MARY J., J. R. TURNER, and W. H. MATCHETT. "A Direct Method of Assaying for Kynureninase," Neurospora Newsletter, vol. 14, pp. 19-20. 1969.

WILEY, W. R. "An Amino Acid Binding Protein Released from Neurospora crassa by Cold Osmotic Shock," Bacterol. Proc., p. 124. 1969. (Abstract).

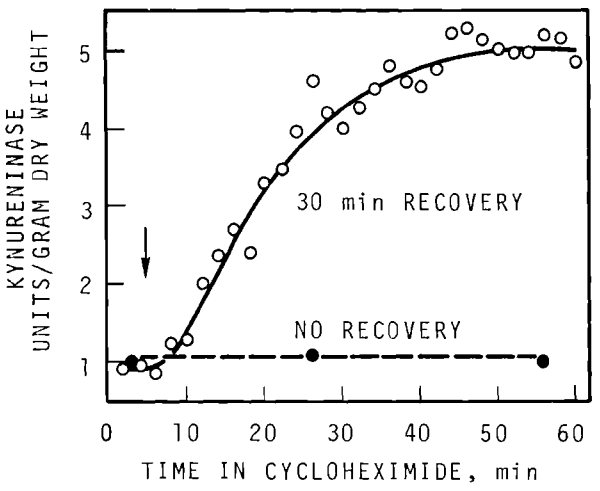


FIGURE 1. Appearance of Kynureninase in Neurospora During Recovery Following Exposure to Inducer in Presence of Cycloheximide. [Cycloheximide (4 μg/ml) was added to the culture at zero time. Kynurenine (0.05 μmole/ml) was added at 5 min, as indicated by arrow. Samples of Neurospora cells were removed at frequent intervals, washed, and either frozen immediately for later assay or reincubated for 30 min in minimal medium before freezing for later assay. The 30-min recovery period, with no inhibition of protein synthesis, allowed translation of the RNA message to an extent proportional to its accumulation during initial incubation with the inducer, kynurenine.]

Accumulation of messenger-RNA was shown to require inducer and was sensitive to actinomycin-D, whereas the expression of the messenger-RNA did not require inducer and was insensitive to actinomycin-D. These observations demonstrate a temporal separation of the processes of transcription and translation in this organism, and also indicate that the rate of synthesis of kynureninase is proportional to the concentration of kynureninase specific mRNA.

Attempts to purify kynureninase have yielded a preparation with a specific activity 30 times that of the crude extract. Measurement of some kinetic properties of kynureninase show that the Km is 1.55×10^{-5}M. The enzyme is inhibited by kynurenine at concentrations greater than 1×10^{-4}M and by N-formyl-kynurenine at concentrations greater than 8×10^{-4}M. This substrate inhibition may have significance in the mechanism of regulation of tryptophan degradation in this organism.

MEMBRANE TRANSPORT OF NUTRILITES IN NEUROSPORA

Investigators:

W. R. Wiley, R. P. Schneider and Laura S. Winn

Technical Assistance:

Clotis White

Our concept of a simplified model for nutrilite transport in Neurospora is illustrated in Figure 2.

This model proposes that a component "C" present in the cell membrane catalyses the transport process by binding the substrate "S." Component "C" is stereospecific which implies a need for different catalytic systems or carriers for different solutes. In the case of the tryptophan transport system, we have isolated and partially purified a protein component with

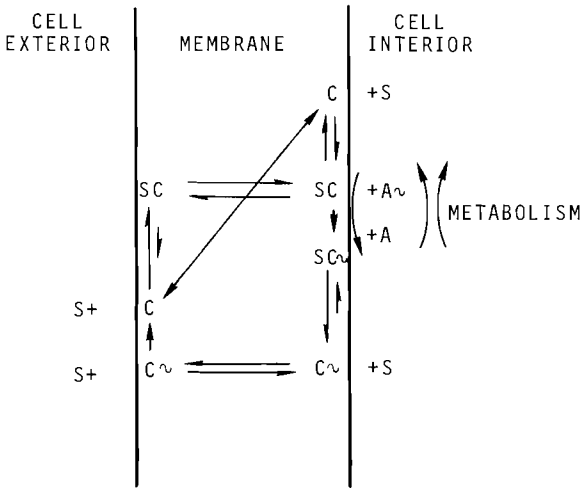


FIGURE 2. Model for Nutrilite Transport Across Cell Membrane of Neurospora. (Symbols have the following significance: S, substrate; C, carrier; SC, substrate-carrier complex; A, energy donor; ∿, metabolic energy.)

many of the properties of "C." Transport of the substrate is concomitant with the binding of the substrate (S) to the carrier (C). The affinity of the carrier for its substrate is decreased on the intracellular side of the membrane by the input of metabolic energy, which accounts for the fact that both amino acids and sugars are concentrated against a 200- to 350-fold concentration gradient.

Exchange diffusion, a nonenergy-requiring process, occurs in the case of sugar transport systems but is absent in amino acid transport processes in Neurospora. This process is represented in Figure 2 by the single diagonal line. In support of the exchange diffusion mechanism, we have observed a rapid efflux of intracellular labeled 3-0-methyl glucose when exogenously supplied 3-0-methyl glucose was added to the culture medium. This rate of efflux was not enhanced by sodium azide which suggests that energy is not required for exchange.

We showed previously that the tryptophan transport system in Neurospora was repressed by growth in high levels of tryptophan. All sugar permeases studied thus far in Neurospora are repressed by growth in glucose in much the same manner as the tryptophan transport system. The glucose permease was derepressed 1000- to 2000-fold by growth of the cells in glycerol or in the absence of an oxidizable carbon source. The site of glucose or catabolite repression appears to be at the level of transcription.

The formation of messenger-RNA during derepression for the glucose permeases, was investigated in the manner just described for specific kynureninase message accumulation. Cells were derepressed for glucose permease in cycloheximide, a condition which permits the accumulation of specific messenger-RNA in the absence of protein synthesis. Using glucose as a co-repressor for the synthesis of new message, the half-time of mRNA for the glucose permease was shown to be 7 min; under conditions where translation of the message was inhibited the half-time was increased to approximately 40 min. These results suggest that mRNA turnover is coupled to translation.

Amino acids and sugars appear to be transported by similar but distinct mechanisms in Neurospora; both systems

seem to be regulated in much the same manner. The following questions remain to be answered:

- What is the nature of the energy donor to the transport processes?
- What is the precise mechanism by which the "carriers" or permeases mediate translocation?
- What is the special arrangement of these proteins in the membrane?

Purification and determination of the physical properties of the transport proteins are expected to provide some answers to these questions. Studies on the in vitro aggregation of isolated membranes will hopefully provide information on the precise mechanism of translocation.

SECRETION OF EXOCELLULAR ENZYMES

Investigator:
H. Drucker
Technical Assistance:
Louise C. Neil

Cultures of Neurospora were found to secrete a proteolytic activity when grown in medium containing calcium, bovine serum albumin, and a trace of sucrose. The secretion or synthesis of the proteolytic activity is catabolically repressed when substrate quantities of sucrose are present in the growth medium. Calcium appears to be required for either the synthesis or secretion of proteolytic activity in Neurospora as well as in a comparative bacterial system, Streptomyces fradiae.

Examination of the effect of calcium on the activity of a representative bacterial protease, thermolysin, (Figure 3) from Bacillus thermoproteolyticus, has shown that the kinetic parameters K_m and V_{max} determined for the substrate furylacryloylglycine leucinamide, vary with calcium concentration in a complex fashion. Both K_m and V_{max} peak at a calcium concentration of 3×10^{-4}M to 7×10^{-5}M, at all temperatures investigated. It seems clear that Ca^{++} does not behave as a simple co-factor. As a possible explanation, we would suggest that there is a Ca^{++} dependent structural change occurring at a Ca^{++} concentration of about 7×10^{-5}M, which alters the kinetic properties of the molecule and results in a product which is autodigested, thus resulting in an irreversible reaction.

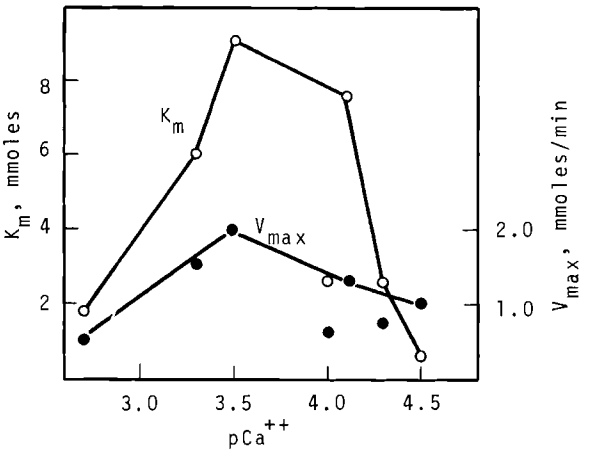


FIGURE 3. Effect of Calcium on the Kinetic Parameters (K_m and V_{max}) of the Bacterial Protease Thermolysin Acting on the Substrate Furylacryloylglycine Leucinamide

FINE STRUCTURAL EFFECTS OF TEMPERATURE IN FISH

Investigators:

J. C. Hampton, J. M. Dean and R. R. Adee

Our previous work on temperature effects demonstrated a shift in metabolism during acclimation to changes in water temperature. Liver and muscle were used as model organs, and some fine structural changes in liver were correlated with the biochemical data.

A comparative study of red and white muscle fine structure is now being completed on tissues from trout acclimated to 7.5 °C. This work will provide background for future studies on temperature effects on the size of actin and myosin filaments and for autoradiographic studies on protein (myosin) synthesis in muscle. The results of the present studies show only minor differences, if any, in sarcomere length and dimensions of filaments but a significant increase in the number of mitochondria and glycogen in red muscle as compared to white muscle. Figure 1 is an electron micrograph of red muscle and shows numerous large mitochondria, large numbers of glycogen particles, and myofibrils having a diameter at least twice those of the white muscle shown in Figure 2. White muscle is low in glycogen content, has few mitochondria and frequently shows Z lines and adjacent sarcomeres out of register. This is rarely seen in red muscle. The two micrographs are printed at the same magnification.

Further work is planned on fine structural changes in red and white muscle. However, emphasis in this project will shift from a concern with metabolic adaptations to temperature changes, to a concern with early changes in organs which are called into play immediately at the onset of temperature change. In this way, a more direct understanding of the interplay between biological effects and the environment may be forthcoming. The sensing mechanism or pathway which mediates acclimation is unknown, but certain organs, for example the

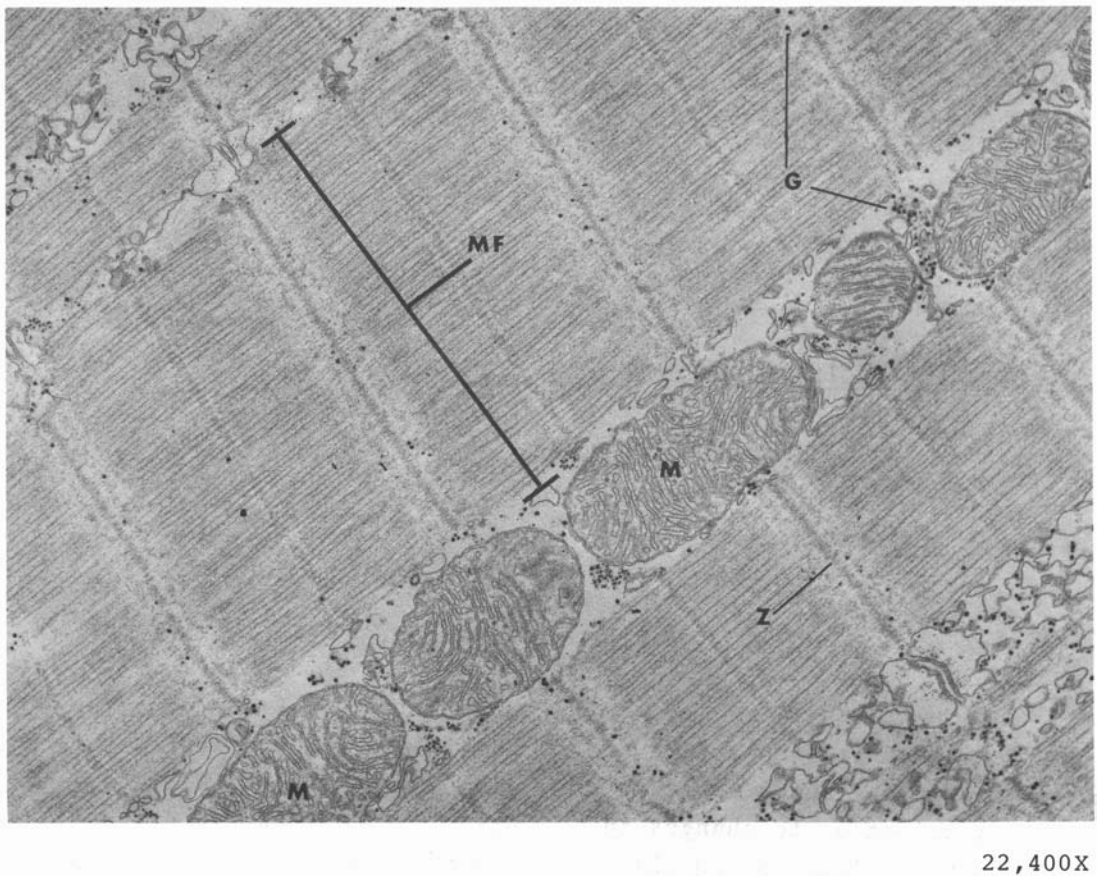
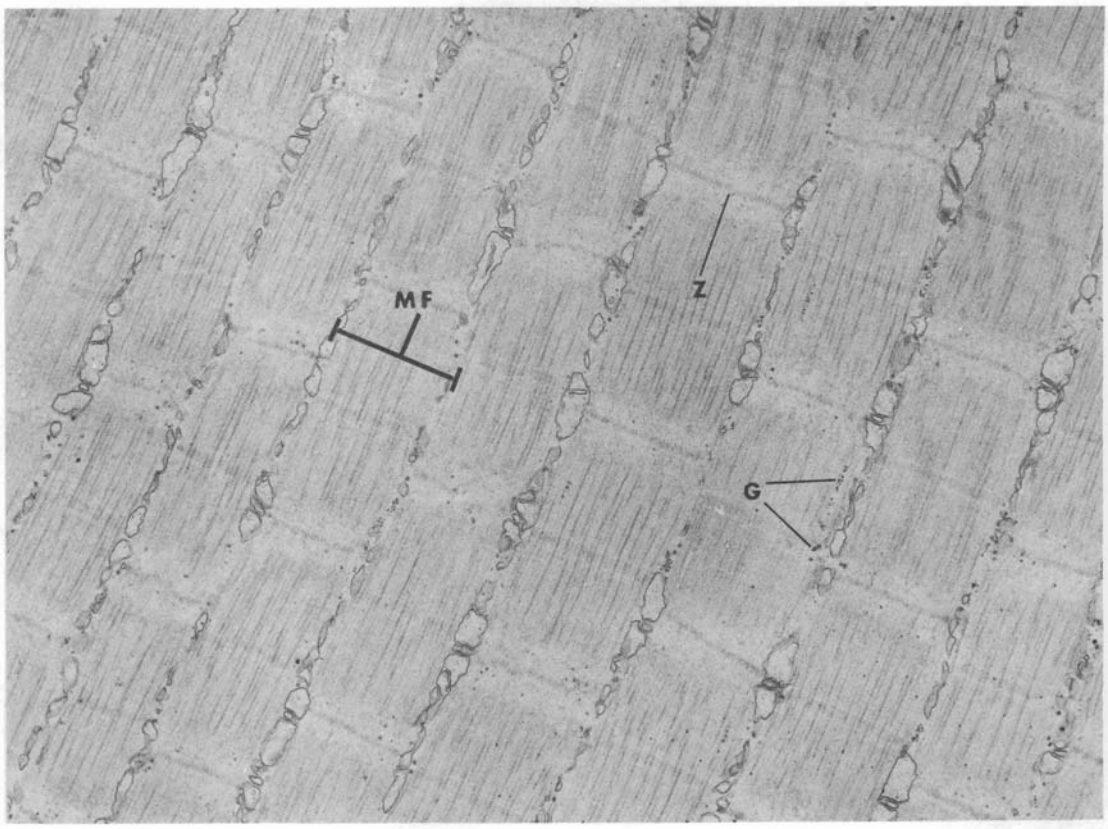


FIGURE 1. Electron Micrograph of Red Muscle from Trout Acclimated to 7.5 °C [Large mitochondria (M) occur between myofibrils (MF). Z-lines (Z) are in register. Numerous dense glycogen particles (G) are seen.]

hypothalamus, lateral line system, swim bladder, and gills, are suspect because they function in sensing and initiating responses to environmental changes such as temperature, dissolved gases, and pressure. Accordingly, these organs in the trout will be studied for possible fine structural changes at temperatures ranging from 7.5 to 30 °C. Autoradiographic studies on cell turnover and protein synthesis in these organs and in liver during temperature acclimation should also provide useful information.



22,400X

FIGURE 2. Electron Micrograph of White Muscle from Trout Acclimated to 7.5 °C. [There are no mitochondria myofibrils (MF) are small, Z-lines (Z) are out of register, and glycogen particles (G) are few.]

METABOLISM AND EFFECTS OF RADIONUCLIDES IN THE DEVELOPING FETUS AND THE YOUNG

Many of the biological parameters used to calculate permissible levels of exposure of adults to radioactive materials are inappropriate for the rapidly growing infant and child. These differences, when considered with the greater intrinsic radiosensitivity of the immature organism, emphasize the need for more detailed information on the metabolism and toxicity of radionuclides in the juvenile animal. This project has been principally concerned with the effects of age on the metabolism and toxicity of several radionuclides of current interest. These include ^{131}I, ^{137}Cs, ^{144}Ce, ^{32}P, ^{65}Zn, and the monomeric and polymeric forms of $^{238,239}Pu$.

PLUTONIUM IN THE PLACENTA

Investigators:

M. R. Sikov and D. D. Mahlum

Technical Assistance:

Joan O. Hess and D. L. Catt

There has been speculation regarding the radiosensitivity of the placenta and the role of placental damage in producing prenatal death. Monomeric plutonium localizes in the placenta and in the fetal membranes and may provide a system for studying the radiosensitivity of the placenta.

Monomeric ^{239}Pu was intravenously administered to pregnant rats at a single time between 14 and 19 days of gestation; fetuses and placentas were collected at intervals thereafter. Autoradiographs (Figure 1) demonstrated a pronounced localization of plutonium in the villus visceral splanchnopleure portion of the yolk sac. Significant amounts were also seen in the decidua and labyrinth layers of the placenta. The concentration was greatest in the decidua following injection at 15 days of gestation, and in the labyrinth after injection at 19 days. The concentration was lower in the spongiotrophoblast, the activity being primarily localized in the giant cells. Although high radiation doses were received by these localized areas, no intrauterine mortality was observed at doses of up to 50 μCi to the dam. The cross-placental

PUBLICATIONS: METABOLISM AND EFFECTS OF RADIONUCLIDES IN THE DEVELOPING FETUS AND THE YOUNG

ERDMAN, H. E., M. R. SIKOV, and D. D. MAHLUM. "Age-Related Differences in Zinc Metabolism in the Rat." In: M. R. Sikov and D. D. Mahlum (ed.), Radiation Biology of the Fetal and Juvenile Mammal, USAEC CONF-690501, pp. 207-216. 1969.

MAHLUM, D. D. and M. R. SIKOV. "Comparative Metabolism of Cesium-137 by Adult, Suckling, and Prenatal Rats," Comparative Biochemistry and Physiology, vol. 30, pp. 169-175. 1969.

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MAHLUM, D. D. and M. R. SIKOV. "Skeletal Changes Induced in Weanling Rats by Plutonium-239 and Cerium-144." In: M. F. Sikov and D. D. Mahlum (ed.), Radiation Biology of the Fetal and Juvenile Mammal, USAEC CONF-690501, pp. 567-576. 1969.

MAHLUM, D. D. and J. D. BERLIN. "Ultrastructural Alterations Associated with Neptunium-Induced Fatty Livers," Federation Proc., vol. 28, p. 424. 1969 (Abstract)

SIKOV, M. R. "Effect of Age on the Iodine-131 Metabolism and the Radiation Sensitivity of the Rat Thyroid," Radiation Res., vol. 38, pp. 449-459. 1969.

SIKOV, M. R. and D. D. MAHLUM (ed.), Radiation Biology of the Fetal and Juvenile Mammal, USAEC CONF-690501. 1969.

SIKOV, M. R., J. M. THOMAS, and D. D. MAHLUM. "Comparison Passage of a Tracer Through the Gastrointestinal Tract of Neonatal and Adult Rats," Growth, vol. 33, pp. 57-68. 1969.

FIGURE 1. Autoradiograph of Sections of Placenta and Associated Structures from 19-Day Rat Fetus Injected 24 hr Previously with Monomeric ^{239}Pu (The highest concentration of plutonium is in the villus visceral splanchnopleure portion of the yolk sac; there is less in the labyrinth layer, and least in the decidua or maternal placenta.)

movement of ^{125}I-labeled albumin was determined at 3 or 5 days after exposure of the dam to plutonium to measure placental damage; there was no indication of altered function. Only a small amount of plutonium crossed the placenta, in accord with our earlier findings; autoradiographically, this appeared to be entirely in the monomeric form and was primarily located in the fetal bone.

Studies in progress, with plutonium injected after 9 days of gestation, show similar localization in the placenta and fetal membranes, but doses as low as 6.25 μCi to the dam have produced extensive prenatal deaths (Table 1).

TABLE 1. Fetal Mortality in Rats Following Injection of Dam with ^{239}Pu (number dead/total number at risk)

Dose, μCi	Gestation Day Injected	Gestation Day Examined					
		10	12	14	17	18	20
0	--	3/22	0/6	1/23	0/14	0/10	0/45
6.25	9			23/38			
12.5	9	17/20	15/25	29/29		16/38	
25	9	9/15	14/24	29/29			
50	15				0/30		0/11

CARCINOGENICITY OF ^{131}I RELATIVE TO AGE AT EXPOSURE

Investigators:

M. R. Sikov and D. D. Mahlum

Technical Assistance:

Joan O. Hess and D. L. Catt

An experiment was started about 30 months ago to determine the carcinogenicity of ^{131}I relative to the age of the rat at the time of exposure. Prenatal, newborn, weanling, and adult rats were exposed on five consecutive days to three levels of ^{131}I. The adult and weanling animals were injected intraperitoneally; the newborn animals were exposed via the milk by injection of the dam on the day of parturition and on the next 4 days; and the prenatal animals were exposed by injection of the pregnant animal during the last 5 days of gestation. Exposures were contrived to produce the same range of radiation doses to the thyroids of all age groups.

All surviving animals are being killed as they reach 30 months of age; approximately 50% of the animals of each age group have died by that age. Thyroid function is measured prior to sacrifice by injection of a tracer dose of ^{125}I and measurement of thyroid incorporation at 24 hr postinjection. Thyroid function, as measured in this manner, is slightly impaired in the animals of all age groups which received the highest exposure level of radioiodine. Effects on thyroid function at lower exposure levels are not apparent at this time. A limited study of plasma levels of tri- and tetraiodothyronine did not show impairment at any dose level.

Thyroid pathology, including neoplasia, has been noted in all age and dose groups. Analysis of these data must be deferred until all animals are dead. There is some indication that the incidence of mammary tumors in the rats exposed while immature is elevated in comparison to controls or animals exposed to the same radiation dose as adults.

MECHANISM OF ^{131}I EFFECTS AS RELATED TO AGE AT EXPOSURE

Investigators:

D. D. Mahlum and M. R. Sikov

Technical Assistance:

Joan O. Hess and D. L. Catt

Several experiments have been performed to determine whether effects noted following the administration of relatively large doses of ^{131}I are due to direct radiation damage to the thyroid, to indirect effects of the induced hypothyroid state, or to radiation effects on the surrounding tissues and the whole-body due to radioiodine in the thyroid gland or circu-

lating throughout the body. In initial experiments, the effects of X-irradiation of the thyroid were compared with effects produced by radioiodine in the neonatal rat. Unfortunately, due to technical difficulties, the X-rays were not very well localized to the thyroid gland. Assay for thyroid stimulating hormone (TSH) in the pituitary gland at 4 months postexposure showed a much greater decrease in the X-rayed animals, which suggested a direct effect on the pituitary and a marked radiosensitivity of the neonatal hypophysis.

In subsequent experiments, head-only exposure of neonatal rats to 500 R resulted in no decrease in thyroid uptake of a tracer dose of ^{125}I at 8 weeks postexposure; but 750 R to the head consistently decreased uptake. Irradiation of the newborn rats with 3000 R failed to decrease thyroidal uptake of subsequently administered ^{131}I. Analysis of these results suggested that X-radiation is histologically less effective than ^{131}I β-radiation in damaging the thyroid. This is in contrast to reported results in older animals.

To extend this study, newborn rats were X-irradiated in the neck region, given graded doses of ^{131}I, or surgically thyroidectomized. A series of adult animals were treated in the same manner. These animals will be subjected to various physiologic tests of thyroid function and eventually sacrificed for histopathologic study.

CARCINOGENICITY OF ^{144}Ce RELATIVE TO AGE AT EXPOSURE

Investigators:

D. D. Mahlum and M. R. Sikov

Technical Assistance:

Joan O. Hess and D. L. Catt

A limited number of rats were exposed to 0.25, 0.50, or 1.0 μCi/g of ^{144}Ce. The radionuclide was injected into the tail vein of weanling or adult rats or into the heart of newborn rats. Approximately 35 rats of each age group were injected at each dose level. Radioanalyses indicated similar concentrations of ^{144}Ce in the skeletons of animals injected as weanlings or adults; the concentrations in the skeleton of animals injected neonatally, however, were substantially lower. The relative concentrations in the livers of the weanling and adult animals were diminished in those which received the highest dose. A lesser effect of dose was seen in the newborn animals.

Nine months postexposure osteogenic sarcomas have developed in three-quarters of the animals exposed as weanlings to 1 μCi/g. A lesser tumor incidence was observed among the animals injected as weanlings at the lower dose levels. A year postexposure, no tumors have been observed in animals exposed to 1.0 or 0.25 μCi/g as adults, but 50%

of those which received 0.5 μCi/g as adults have developed bone tumors. No bone tumors have been observed in the rats exposed neonatally.

Larger groups of weanling rats have been exposed to 0.5 or 1.0 μCi/g of ^{144}Ce to obtain additional data on tumor incidence and to evaluate the intermediate effects preceding frank neoplasia. Liver function was determined in some of these animals at 1 month postexposure; no effects were noted in the function of either the reticuloendothelial or parenchymal elements of the liver. Radiographs obtained at 1 month postexposure, showed evidence of osteoporosis and a low incidence of spontaneous hairline fractures in rats exposed to the highest dose level. Breaking strength of excised femurs was markedly reduced as early as 1 month after exposure (Table 2).

EFFECT OF AGE ON THE BINDING OF ZINC

Investigators:

H. E. Erdman, D. D. Mahlum and M. R. Sikov

Technical Assistance:

D. L. Catt and Joan O. Hess

We had previously observed that ^{65}Zn is retained more tenaciously by newborn rats than by adults or weanlings. To determine whether these differences were correlated with the ability of selected tissues to bind zinc, homogenates of liver, muscle, or kidney were dialyzed against 0.001 M EDTA (ethylenediaminetetraacetic acid) in tris buffer, pH 7.4. There was a progressive removal of ^{65}Zn during the 4-day dialysis period.

TABLE 2. Effect of ^{144}Ce Administered to Weanling Rats on Breaking Strength and Chemical Composition of the Femur at One Month Postadministration

Measure	Treatment Level (μCi ^{144}Ce/g) 0	0.5	1.0
Calcium, % wet wt	11.1	11.1	10.6
Phosphorus, % wet wt	6.5	6.2	5.6
Magnesium, % wet wt	0.2	0.2	0.2
Zinc, % wet wt	0.01	0.01	0.01
Dry wt, % wet wt	58.5	59.3	56.8
Ash wt, % wet wt	35.2	33.8	34.0
Breaking Strength of Femur (S)[(a)]	13.2	11.9	5.9

(a) S = grams × length/2 ÷ cross sectional area.

The fraction removed, of that present, was highest from the liver samples obtained from newborn rats. The ease of removal decreased with the age of the animal at time of ^{65}Zn injection, and with time elapsed since injection. Qualitatively similar patterns were found for muscle and kidney.

These results failed to provide a simple explanation for the observed differences in retention of ^{65}Zn by neonatal rats as compared to older animals. Further experiments were performed using chelating agents which exhibit a graded series of binding strengths for zinc in an attempt to resolve differential binding pools. Dialysis against glycine did not remove as much ^{65}Zn from livers of newborn rats as did dialysis against histidine or EDTA (Figure 2). This distinction was even more apparent with livers of weanlings and adults. Age-related differences in the sizes of zinc pools were apparent with dialysis against glycine and water that were not apparent with dialysis against the stronger chelators, histidine and EDTA. The significance of these observations remains obscure.

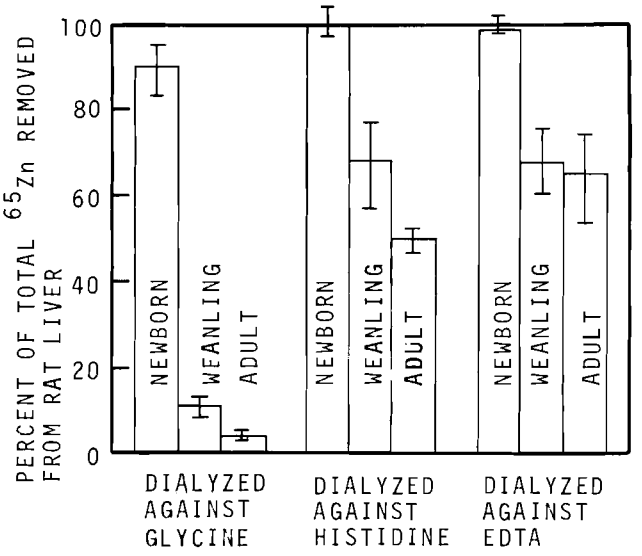

FIGURE 2. Removal of ^{65}Zn from Liver Homogenates During 4-Day Dialysis Against Solutions of Varying Complexing Strength

EFFECTS OF RADIOIODINE IN SHEEP AND SWINE

Investigators:
R. F. Palmer and H. A. Ragan

Since 1949, we have conducted radioiodine studies in sheep to define the lowest daily feeding level which will cause detectable damage during the lifespan of these animals. The major aspects of this work have been completed and reported. Fifteen offspring sheep, exposed to radioiodine in utero and by suckling only, remain on experiment and will be sacrificed during the coming year. Five ewes, 6 to 9 years of age, whose only ^{131}I exposure occurred in utero and during suckling of their 1.5- or 5.0-μCi/day dams, and 20 controls, were sacrificed during the past year. No gross or histopathologic lesions attributable to radiation exposure were found.

EFFECTS OF RADIOSTRONTIUM IN MINIATURE SWINE

Initiated in 1958, this study has as its general goal the development of a better understanding of the potential hazard of radiostrontium to large animals and man. This is being accomplished by defining the biological effects of daily ingestion of ^{90}Sr in miniature swine, a large animal species more comparable to man than the smaller laboratory animals. Nearly 800 female Pitman-Moore miniature swine, representing three generations, have been exposed to ^{90}Sr at feeding levels ranging from 1 to 3100 μCi/day. There have been 194 untreated littermate controls. Currently, 173 experimental swine with 8 to 11 years of exposure, and 67 controls, are being maintained for lifetime observation and study. At the present time, the study consists of three distinct areas of research which are separately discussed below.

CHRONIC TOXICITY STUDY

Investigators:

W. J. Clarke, E. B. Howard, H. A. Ragan, Patricia L. Hackett, Glenda S. Vogt, J. L. Beamer, M. T. Karagianes and R. F. Palmer

Original animals were started on experiment at 9 months of age, fed ^{90}Sr daily, bred at apparent ^{90}Sr skeletal equilibrium, and their offspring gradually raised to the same feeding level as the dam. These F_1 animals were then bred to provide the F_2 generation, which was exposed in the same manner as the F_1. Animals were fed at 1, 5, 25, 125, 625, and 3100 µCi/day. The past year has seen no dramatic new developments in toxic effects. It has been of interest, however, to collect the data from this experiment on farrowing performance, in the light of allegations concerning possible effects of fallout ^{90}Sr on human fetal and infant mortality (Tables 1 and 2).

Litters from the parent generation of swine, which ingested ^{90}Sr from 9 months of age, showed no significant differences in litter size, percent stillborn, or birth weight between controls and animals ingesting up to 625 µCi/day. Animals ingesting 3100 uCi/day did not survive the gestation period. Among litters from swine exposed to ^{90}Sr from conception (F_1 generation), there also were no significant differences in percent stillborn, birth weight, or weaning weight between controls and animals at all levels of ^{90}Sr feeding through 125 µCi/day. F_1 animals receiving in excess of 125 µCi/day did not survive to produce offspring.

To place these swine data in some perspective relative to human exposures, the feeding level of 625 µCi/day is more than a million times the peak dietary level of ^{90}Sr ever reported in American diets (about 30 pCi/g dietary calcium), and more than half-a-million times higher than the permissible daily intake limit of 10^{-3} µCi established for occupational exposure by the International Commission on Radiological Protection.

While it cannot be considered proved (nor can it ever be proved with

PUBLICATIONS: EFFECTS OF RADIOSTRONTIUM IN MINIATURE SWINE

CLARKE, W. J., E. B. HOWARD, and P. L. HACKETT. "Strontium-90 Induced Neoplasia in Swine." In: C. W. Mays, et al. (ed.) Delayed Effect of Bone-Seeking Radionuclides. University of Utah Press, pp. 263-277. 1969.

HOWARD, E. B., W. J. CLARKE, M. T. KARAGIANES, and R. F. PALMER. "Strontium-90 Induced Bone Tumors in Miniature Swine." Radiation Res. vol. 39, pp. 594-607. 1969.

PALMER, R. F., C. R. WATSON, and J. L. BEAMER. "Radiation Dose to Fetuses of Miniature Swine Ingesting Strontium-90." In: M. R. Sikov and D. D. Mahlum (eds.) Radiation Biology of the Fetal and Juvenile Mammal. U. S. Atomic Energy Commission, CONF-69051, pp. 89-96. 1969.

TABLE 1. Farrowing Performance of Swine Ingesting ^{90}Sr from Age 9 Months[a]

Feeding Level (μCi/day)	No. of Litters	Litter Size	Percent Stillborn	Birth wt, g	Percent Weaned	Weaning wt, kg
625	6	6.0 ± 1.5	5.5	615 ± 143	89 ± 19	4.7 ± 1.1[b]
125	12	5.8 ± 1.4	0	675 ± 28	95 ± 6	5.8 ± 0.3
25	17	6.4 ± 1.0	0.9	729 ± 56	90 ± 9	5.3 ± 0.6
5	8	5.6 ± 1.2	4.8	799 ± 76	84 ± 18	6.1 ± 0.8
1	22	5.8 ± 0.8	1.6	696 ± 48	81 ± 13	5.1 ± 0.5
0	49	5.3 ± 0.5	1.9	707 ± 43	87 ± 6	6.0 ± 0.4

(a) All values expressed with 95% confidence intervals, except percent stillborn where, using binomial distribution tables, no significant differences were noted from control values.

(b) Significantly different (P <0.05) from controls. (Attributed to radiation effects on milk output of dam)

TABLE 2. Farrowing Performance of Swine Exposed to ^{90}Sr from Conception[a]

Feeding Level, μCi/day	No. of Litters	Litter Size	Percent Stillborn	Birth wt, g	Percent Weaned	Weaning wt, kg
125	3	4.0 ± 2.5	0	718 ± 260	100	4.7 ± 1.4
25	13	6.7 ± 0.7[b]	0	711 ± 64	92 ± 6	5.5 ± 0.8
5	11	4.8 ± 0.4	1.9	725 ± 69	89 ± 9	5.4 ± 0.4
1	14	4.9 ± 0.6	0	719 ± 53	95 ± 7	5.4 ± 0.6
0	49	5.3 ± 0.5	1.9	707 ± 43	87 ± 6	6.0 ± 0.4

(a) All values expressed with 95% confidence intervals, except percent stillborn where, using binomial distribution tables, no significant differences were noted from control values.

(b) Significantly different (P <0.05) from controls.

any amount of data) that no fetal or neonatal effects occurred in these animals, it can be most confidently stated that any such effects were of very minor significance compared to other effects observed. None of the second generation swine on 625 μCi/day even survived to produce a third generation. There have been 73 cases of hematopoietic disorders in swine ingesting ^{90}Sr, and two in controls. Significant increases in these disorders have occurred only in the 125- and 625-μCi/day groups. Bone tumors have been seen in seven animals of the 125- and 625-μCi/day groups. We would conclude from this study that ^{90}Sr possesses no uniquely hazardous characteristics not attributable to its radiation quality and metabolic behavior. As a predominantly bone-seeking radionuclide, its effects will be manifest in bone and hematopoietic tissue and not in effects on fetal or neonatal mortality.

In an effort to optimize the production of hematopoietic neoplasia in ^{90}Sr-fed swine, four sows were started on a regimen of 313-μCi/day. Platelet levels in these animals lie between those previously observed in the 125- and 625-μCi groups; neutrophil levels approach those of the 625-μCi group. Hematopoietic neoplasia may be expected to develop in these animals within the next year. Of the 15 offspring from these 313-μCi/day sows, only two have survived for 7 months. The 13 that died had clinical symptoms of the hemorrhagic syndrome similar to those encountered in 625-μCi/day offspring. On the basis of these results, six sows were started on 250 μCi ^{90}Sr/day, a level that hopefully will permit survival of their offspring long enough to produce myeloproliferative disorders with a shorter induction period than encountered with 125-μCi/day offspring.

A significant effort this past year was placed on the study of leukocyte alkaline phosphatase (LAP) values in control swine and in those exhibiting hematopoietic disorders. The average for 57 normal adult swine was 200, compared to the reported human value of 46. Ninety percent of segmented neutrophils showed alkaline phosphatase activity, while only 35% of the band neutrophils showed activity. Age effects were not observed in healthy control animals 9 to 114 months of age. One case of chronic myelogenous leukemia in a 25-μCi/day offspring was thoroughly studied for 18 months and demonstrated a marked depression in enzyme activity. In other myeloproliferative disorders, the two lowest values were observed in myeloid metaplasia. In myeloid leukemia the granulocytic variants were lowest, eosinophilic next, and the erythroid and myelomonocytic variants overlapped the control range. "Aleukemic" leukemia cannot be distinguished from myeloid metaplasia on the basis of LAP scores. In myelomonocytic leukemia alkaline phosphatase activity is observed in granulocytes as immature as the myelocyte. It was concluded from this work that LAP scores are a valuable diagnostic aid if the following precautions are observed:

- Single terminal scores are invalid if hemolysis occurs, as

this may interfere with the reaction.

- Pregnancy increases the LAP score.
- Infections and abscesses increase the LAP score.
- The presence of increased numbers of basophils in peripheral blood decreases the LAP score.
- Differentials on alkaline phosphatase smears are important, but calculated scores must be based on segmented neutrophils only.

Chromosome preparations were obtained from all ^{90}Sr swine that died or were sacrificed during the past year; metaphase spreads were examined from those animals that received complete histopathologic examination. Except for nonspecific chromatid gaps and breaks, no consistent aberrations were observed that could be correlated with either the disease state or the ^{90}Sr exposure of the animal. One control animal that died of a spontaneously occurring chronic granulocytic leukemia, eosinophilic type, showed an abnormality in both leukocyte cultures and in bone marrow preparations that was similar to the Philadelphia chromosome found in many humans with chronic granulocytic leukemia. This abnormal chromosome appeared to be a metacentric with a missing portion of either the short or long arms, giving rise to an apparent acrocentric.

Preliminary results of an in vitro experiment to compare the responses of human and porcine chromosomes to either X-rays or β-particles from ^{90}Sr-^{90}Y suggest that the behavior of the two species is qualitatively similar, but that fewer "one-hit" and "two-hit" aberrations are produced in porcine chromosomes exposed to X-ray doses of 50 to 200 rad.

DOSIMETRY STUDIES

Investigators:

R. F. Palmer, J. M. Thomas, C. R. Watson** and J. L. Beamer*

Miniature swine, ingesting ^{90}Sr daily, were sacrificed at intervals during the second half of gestation, and thermoluminescent dosimeters were implanted in critical tissues of the fetuses. The dosimeters were exposed with the fetuses in place, in utero, and with the fetuses exteriorized, and from such measurements it was determined that the fetuses are irradiated from their own ^{90}Sr-^{90}Y and not significantly from the ^{90}Sr-^{90}Y of their dam. Applying these findings to the animals in the long-term ^{90}Sr toxicity study, cumulative skeletal radiation doses of about 50 rad were calculated for the fetuses of animals receiving 125-μCi/day, and 400 mrad for fetuses of the 1-μCi/day animals.

Thermoluminescence dosimeter measurements on periosteal and endosteal surfaces of femur diaphyses from two 125- and two 25-μCi/day offspring animals averaged 67 ± 5 (± S.E.)

* *Applied Mathematics Department*

** *Radiological Sciences Department*

and 103 ± 11%, respectively, of the average bone dose rates calculated for each bone from measured ^{90}Sr concentrations. Extension of these types of measurements to bones other than femurs is in progress.

UTERINE-MILK EXPOSURE STUDY

Investigators:

Beatrice J. McClanahan, Patricia L. Hackett and J. L. Beamer

This study was designed to determine the effect of ^{90}Sr exposure at different stages of pre- and postnatal development on subsequent production of hematopoietic disorders. The heat periods of 20 sows were synchronized. Part of the animals were fed 125 μCi ^{90}Sr/day until apparent equilibrium body burdens were attained. All were bred and the litters switched at birth to provide a total of 47 animals in the following ^{90}Sr exposure groups: uterine exposure only, uterine + milk exposure, uterine + milk + subsequent radioisotope feeding exposure, milk exposure only, and milk + subsequent radioisotope feeding exposure. Except for these alterations, the same protocol was employed as was used in the 125-μCi/day chronic toxicity study.

The animals in the two treatment groups receiving 125 μCi ^{90}Sr/day subsequent to weaning are showing small but significant depressions in peripheral blood platelet levels. Clinically, all animals appear to be in good health at age 1 year. However, in the group that received ^{90}Sr in milk only, four animals have maintained high serum alkaline phosphatase levels as compared to their controls. Lack of correlation of these values with the glutamic-oxalacetic transaminase (SGOT), and glutamic-pyruvic transaminase (SGPT) values would seem to indicate that this alkaline phosphatase elevation is not of hepatic origin. It will be of interest to study alkaline phosphatase isoenzymes and acid phosphatase concentrations in these animals.

RADIATION-INDUCED LEUKEMOGENESIS IN MINIATURE SWINE

Thus far, we have observed 72 cases of hematopoietic disorders among the swine on our chronic ^{90}Sr ingestion study: 70 cases in irradiated pigs and two in control pigs. The spectrum of these disorders has ranged from myeloid metaplasia to granulocytic leukemia to lymphocytic leukemia, with hematologic variables in each group. These cases represent the largest number of experimentally induced hematopoietic disorders ever produced in large experimental animals and afford an excellent model system for basic studies, not only on radiation-induced leukemia but also on the myeloproliferative and lymphoproliferative disorders of man, and leukemogenesis in general.

VIRAL STUDIES IN MINIATURE SWINE

Investigators:

E. B. Howard, M. E. Frazier and C. C. Jannke

Technical Assistance:

Marilyn J. Bottorff

Our objectives in this study are to isolate viruses from pigs with ^{90}Sr-induced leukemia, identify the viruses, adapt them to tissue culture systems, and eventually to pass the viruses to immunologically depressed unirradiated animals in an attempt to transmit the leukemias. The study is also concerned with the detection of humoral factors in plasma and tissues from these leukemic animals, responsible for the induction or regulation of hemopoiesis.

PUBLICATIONS: RADIATION-INDUCED LEUKEMOGENESIS IN MINIATURE SWINE

CLARKE, W. J., E. B. HOWARD, and P. L. HACKETT. "Strontium-90 Induced Neoplasia in Swine." In: C. W. Mays, et al. (ed.) Delayed Effects of Bone-Seeking Radionculides. University of Utah, pp. 263-277. 1969.

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Viruses have been isolated from three miniature swine with myelogenous leukemia and have been identified as adenoviruses. However, in addition to these adenoviruses, we continue to see "C" type particles in preparations from these and other leukemic swine. We currently suspect that the leukemogenic agent is the "C" type particle and that the adenoviruses are contaminating or helper viruses.

Efforts to separate the adenovirus from the "C" type particle have been unsuccessful, indicating the possible role this virus is playing in the leukemogenic process.

Attempts to transmit the leukemia to newborn mice by means of tissue culture preparations, viral isolates, and fresh plasma from leukemic swine have been inconclusive. Such attempts will continue with cross transmission studies in immunologically depressed cats and swine.

VIRAL STUDIES IN CATS

Investigators:

E. B. Howard, M. E. Frazier, R. F. Palmer and J. L. Beamer

Technical Assistance:

Linda C. Smith

The feline ^{90}Sr-viral leukemogenesis project was started during the past year to provide a better system for studying the possible viral release or activation in an animal chronically exposed to ^{90}Sr. The so-called "stem-cell" leukemias occurring in some of the swine of our chronic ^{90}Sr feeding experiment are in many respects comparable to the spontaneous "reticulosis or reticulo-endotheliosis" that occurs with some frequency in cats. Bone marrow dyscrasia, genetic predisposition, and viral activity are considered to be involved in the etiology and pathogenesis of this disease. The cat has the additional advantages of a shorter gestation period and a lower maintenance cost when large experimental numbers are necessary.

Forty queens and four toms have been purchased and will be bred as they come in season. The female offspring (eventually 100 animals) will be divided into five experimental groups as follows:

1. Twenty kittens, derived from ^{90}Sr-fed queens, and placed on ^{90}Sr feeding at weaning.
2. Twenty kittens, derived from ^{90}Sr-fed queens, inoculated with feline leukemia virus at birth, and placed on ^{90}Sr at weaning.
3. Twenty kittens, derived from ^{90}Sr-fed queens that were inoculated with feline leukemia virus at mid-pregnancy. The kittens will be placed on ^{90}Sr at weaning.
4. Twenty kittens from queens not fed ^{90}Sr; the kittens will be injected with feline leukemia virus at birth and will not be fed ^{90}Sr.
5. Ten kittens from queens not fed ^{90}Sr; the kittens will be kept as controls for hematology, chemistry, and histology.

In a pilot study, seven kittens were placed on a feeding regimen of 1 μCi ^{90}Sr/day at 4 or 15 days postweaning. The animals were sacrificed at 28, 63, 93, 139, and 180 days of ^{90}Sr feeding. Unlike swine of comparable maturity,

none of the cats attained an equilibrium level of ^{90}Sr in their skeletons. These data were used to estimate the radiation dose that will be delivered to the skeleton of kittens born to a queen ingesting 2 μCi ^{90}Sr/day, and who will receive 0.5 μCi ^{90}Sr/day from weaning to 90 days of age, 1 μCi/day from 90 to 180 days of age, and then 2 μCi/day for the rest of their lives. By 180 days of age, the cat's skeletons will have accumulated a dose of about 800 rad, with a dose rate at that age of 13 rad/day. At 300 days of age, these values will approximate 3500 rad and 32 rad/day; by 450 days, 9500 rad and 50 rad/day.

INHALATION STUDIES

This project emphasizes the biology of inhaled plutonium and other alpha emitters. It is concerned with answering several major questions:

- What are the consequences of inhaling plutonium or other alpha emitters, and what are the biological responses and the dose relationships?
- By what parameters are potential human exposures recognized and evaluated in terms of the hazard to the individual?
- What are effective therapy procedures for removing inhaled radionuclides from the body?

To answer these questions, a variety of investigations are included in this project, some at a quite basic level. Progress on these investigations is summarized in the reports which follow. Several major long-term studies of biological effects that have grown out of this project are covered in separate projects: "Low-Level Plutonium Inhalation Studies in Beagle Dogs," "Inhalation Hazards to Uranium Miners - Biological Studies," and "Space Nuclear Systems Studies."

CHRONIC EFFECTS OF INHALED $^{239}PuO_2$ IN BEAGLES

Investigators:

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

Technical Assistance:

L. R. Richardson

The long-term study of the biological effects of inhaled $^{239}PuO_2$ in beagle dogs is in its eleventh year. Of 40 exposed dogs, 30 have died and five were sacrificed for tissue distribution data. Twenty-two dogs which have come to autopsy had multiple primary pulmonary tumors. All 15 of these dogs that survived as long as 54 months postexposure had lung tumors. The lowest plutonium lung burden associated with a tumor was 0.05 μCi at death 9 years after exposure.

The tumors in the lungs of all the dogs were classified as bronchiolar adenocarcinomas. In addition to bronchiolar carcinomas, one dog had bronchial carcinoma, five dogs epidermoid carcinoma, two dogs lymphangiosarcomas and one dog a capillary hemangioma. Two of the dogs that died developed secondary pulmonary osteoarthropathy related to the pulmonary lesions.

In addition to pulmonary lesions, the tracheobronchial and mediastinal lymph nodes were severely damaged with nearly complete replacement by

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scar tissue and metastatic tumors. One dog had a lymphagiosarcoma and two dogs capillary hemangiomas in the mediastinal lymph nodes. One dog showed lymphoma in the mesenteric and mandibular lymph nodes. The hepatic lymph nodes were also fibrotic, with lymphoid necrosis.

Several aging control dogs were euthanized for histopathological comparison to the exposed dogs. Analysis of hematology data indicated that dogs surviving for 93 months after exposure to $^{239}PuO_2$, with body burdens of 0.2 to 1.0 μCi, continue to show lymphopenia compared to controls of similar age. The absolute lymphocyte count of the exposed dogs was 1.5 ± 0.3 × $10^3/mm^3$ (95% confidence interval) compared to 2.5 ± 0.2 × $10^3/mm^3$ for the controls.

Since the last report, plutonium analyses have been completed on four dogs euthanized when death was imminent due to pulmonary neoplasia, 85 to 110 months after plutonium inhalation (Tables 1 and 2). The estimated initial alveolar deposition ranged from 0.6 to 1.8 μCi. The terminal body burdens were 0.4 to 1.4 μCi with 7 to 21% retained in the lungs, 41 to 56% in the tracheobronchial and mediastinal lymph nodes, 16 to 23% in the liver, 5 to 10% in the skeleton, 6 to 10% in the abdominal lymph nodes and 1.4 to 1.6% in the spleen. The lymphatic system contained 52 to 66% of the total plutonium retained in these animals. The hepatic lymph nodes contained nearly all of the plutonium in the abdominal lymph nodes. The highest concentration occurred in the tracheobronchial or mediastinal lymph nodes followed in descending order by hepatic lymph nodes, lungs, liver, other lymph nodes, spleen and skeleton. These results emphasize

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TABLE 1. Plutonium Distribution in Dogs 85 to 110 Months After Inhalation of $^{239}PuO_2$

Dog Number	Survival, Months After Exposure	Terminal Body Burden, μCi	Plutonium Distribution (Percent of Terminal Body Burden)					
			Lungs	Liver	Skeleton	Thoracic Lymph Nodes	Abdominal Lymph Nodes	Spleen
273	85	1.4	7	21	5	56	9	0.6
278	93	0.8	14	23	10	41	10	1.4
254	94	0.6	21	21	5	45	6	0.7
109	110	0.4	13	16	6	56	7	0.6

TABLE 2. Plutonium Concentration in Dog Tissues 85 to 110 Months After Inhalation of $^{239}PuO_2$

Dog Number	Survival, Months After Exposure	Terminal Body Burden, μCi	Plutonium Concentration (μCi Per Gram Wet Tissue)						
			Thoracic Lymph Nodes	Abdominal Lymph Nodes	Lungs	Liver	Other Lymph Nodes	Spleen	Skeleton
273	85	1.4	3654	157	1.1	1.1	0.6	0.3	0.11
278	93	0.8	1201	214	1.2	0.5	0.1	0.4	0.11
254	94	0.6	2635	92	1.5	0.4	0.2	0.2	0.03
109	110	0.4	424	90	0.7	0.3	0.1	0.1	0.04

the increasing importance, with the passage of time, of the lymphatic system as a possible critical tissue.

The livers of several dogs were divided along their anatomical fissures and each sample analyzed to determine the gross distribution of plutonium in the liver. Concentrations varied by about a factor of two between the highest and lowest lobe in a dog. This information is of significance to the interpretation of analyses on human liver tissue collected at postmortem.

Of the five surviving dogs in this experiment, two show radiographic evidence of pulmonary neoplasia.

COMPUTER SIMULATION OF THE KINETICS AND DOSIMETRY OF INHALED PLUTONIUM

Investigators:

*B. O. Stuart, C. L. Sanders, W. J. Bair and P. J. Dionne**

Additional data on the distribution of plutonium in beagle dogs sacrificed up to 9 years after exposure were incorporated into the simulation model for inhaled $^{239}PuO_2$. This model, to date, suggests a biphasic pattern of slow clearance from the pulmonary lung, with a long-term component of 4-year half-time. The model indicates that tracheobronchial lymph nodes may attain 60% of the initial slow pulmonary component; liver burdens may reach 15 to 20% at times up to 15 years after exposure; skeletal burdens rise to 5 or 6%. The model is being modified to incorporate cumulative doses in pulmonary and systemic organs based upon daily low-level exposures, to provide a more realistic evaluation of current MPC levels and to provide insight into reactor siting problems. Extrapolation of the accumulation of chronically inhaled ^{239}Pu in various tissues over 20 or more years suggests a plateauing of lung burdens at about 12 times the yearly pulmonary deposition. Tracheobronchial lymph nodes, liver and skeletal burdens continue to rise almost linearly, reaching 10 times, 2 times and 0.6 times the yearly pulmonary deposition by the end of 20 years.

Further studies on the distribution of radiation dose around an inhaled plutonium particle were carried out using a computer simulation technique. Over 50% of the available α energy from one plutonium particle is absorbed within the tissue equivalent of one alveolus. The average penetration distance of an α particle in the alveolar region is 100 μm, with ranges of 48 to 270 μm observed. About 45 alpha emissions are required to result in a "reproductive death" of 63% of the nuclei in a two-dimensional section of a "typical alveolus." The number of α emissions required for a D_{37} dose is much higher in a three-dimensional array with over a thousand cells at risk. Significant alveolar damage around a particle would only be expected if a high enough alpha flux was produced to overwhelm the normal reparative and cellular renewal processes of alveolar epithelium and endothelium.

* *Control and Instrumentation Department*

UNILATERAL PULMONARY DEPOSITION STUDIES

Investigators:

J. F. Park and R. L. Amster

Technical Assistance:

M. D. Snyder

Six beagle dogs were exposed to aerosols of $^{239}PuO_2$ through a bronchial catheter which deposited plutonium in only one side of the lung. The

total quantity deposited, as estimated from in vivo counting, ranged from 50 to 100 μCi. Lymphopenia developed promptly in all animals.

Thirty days postexposure, the portion of the lung with the plutonium burden was surgically removed. One year after this surgery there was no indication that the lymphocyte count was returning toward normal. Lymphocyte count in the dogs surgically relieved of their plutonium burdens was $0.45 \pm 0.3 \times 10^3/mm^3$, and in the nonrelieved dogs was $0.42 \pm 0.3 \times 10^3/mm^3$, as compared to $2.26 \pm 3 \times 10^3/mm^3$ in surgically relieved controls. In vivo counting indicated thorax burdens of 3 to 4 μCi in the surgically relieved dogs and 20 to 50 μCi in the nonrelieved dogs. The pathological response in the exposed lung was observed radiographically. One year postexposure, the exposed lung shows severe fibrosis compared to the contralateral control lung in the same animal (Figure 1).

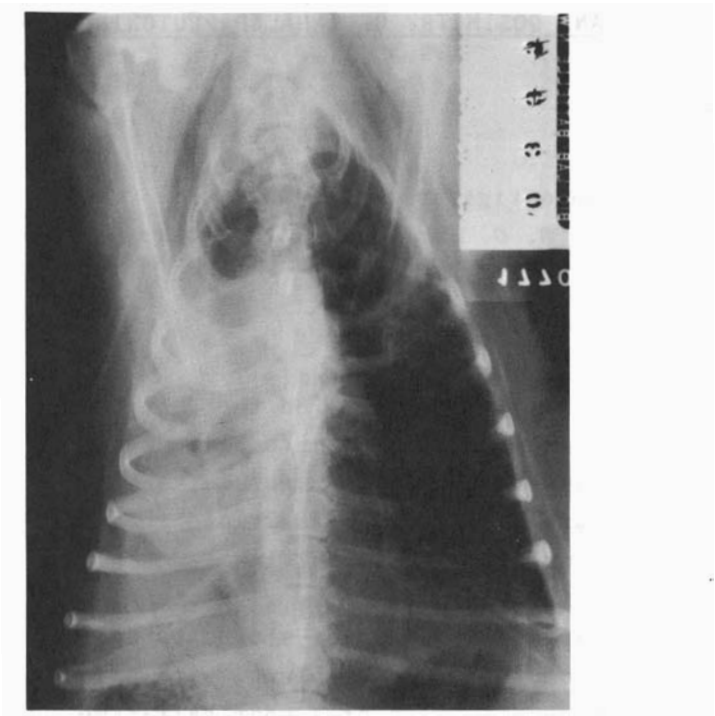

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FIGURE 1. Thoracic Radiograph of a Dog Showing Severe Unilateral Pulmonary Fibrosis 1 Year After Deposition of Approximately 100 μCi $^{239}PuO_2$ on the Right Lung

One dog was euthanized 6 months after exposure, 5 months after surgical removal of the left lung, which contained 42 μCi of plutonium. The final body burden was 3.9 μCi with 51% in the thoracic lymph nodes, 46% in the remaining lung, 1% in the liver, 1% in all other tissues. Since some plutonium was retained in the lung, it is not clear whether the continuing lymphopenia is due to the lung or lymph node burden. The thoracic lymph nodes in the remaining animals will be removed and lymphocyte values compared prior to sacrifice.

This technique should prove valuable in future studies. Exposures at lower dose levels should help to elucidate the mechanism of lymphopenia production. Use of this technique will make possible the study of tumor production at dose levels that would normally kill the dog in a few weeks due to radiation pneumonitis.

PULMONARY CARCINOGENESIS STUDIES IN RATS

Investigator:
C. L. Sanders
Technical Assistance:
Thelma Jackson

This is a report of very preliminary observations on the effect of dose distribution on the carcinogenicity of plutonium in the lung of rats. Sixty-nine rats were injected through the thoracic wall, into the lung, with 0.7 μCi $^{239}PuO_2$ (0.25 μm, CMD). About 0.2 μCi of this plutonium was retained in the lung during the interval from 27 to 400 days after injection. During this interval, about half of the animals were sacrificed for study of plutonium distribution and histopathology. Injected particles initially form a pocket of intense radioactivity in the lung. Later, these particles may spread peripherally near the surface of the lung and into adjacent lobes. Hyperplasia and fibrosis were observed in areas of highest plutonium concentration.

Twenty-seven rats were sacrificed at 400 days postinjection. In 11 of these animals, the plutonium was fairly evenly dispersed throughout the lung. None of these animals showed metaplasia or neoplasia. Sixteen of the animals showed localization of greater than 90% of the plutonium in less than 10% of the lung volume. These animals showed severe fibrosis in the areas of plutonium deposition. Metaplasia was observed in these fibrotic areas in 10 of the rats. Neoplastic lesions, including an endothelioma, adenocarcinomas, and epidermoid carcinomas, were seen in 5 of the rats with localized plutonium deposits. The tumors arose from the margins of the fibrotic zones and extended into unirradiated areas of the lung. It was estimated that from 1 to 6 x 10^5 rads were delivered to the isolated pockets of lung tissue from which the cancers arose. These results suggest that concentration of the dose in a functionally insignificant fraction of the lung is considerably more hazardous than spreading the same total amount of activity throughout the lung.

ULTRASTRUCTURAL EFFECTS OF INHALED $^{239}PuO_2$

Investigators:
C. L. Sanders and R. R. Adee
Technical Assistance:
Thelma Jackson

The ultrastructural response of the lung to inhalation of 1 to 2 μCi $^{239}PuO_2$ was investigated. Light and electron microscopic autoradiography were used to identify plutonium particles. Particles were engulfed by Type I alveolar epithelium within a

few hours after exposure (Figure 2). Many similarities were observed between previous electron microscope studies on radiation pneumonitis from external radiation and this study with plutonium. The number of Type II alveolar epithelium cells was initially increased and there was a change in the structure and type of inclusions found in these cells. There was a marked accumulation of an exudate in the air space, comprised

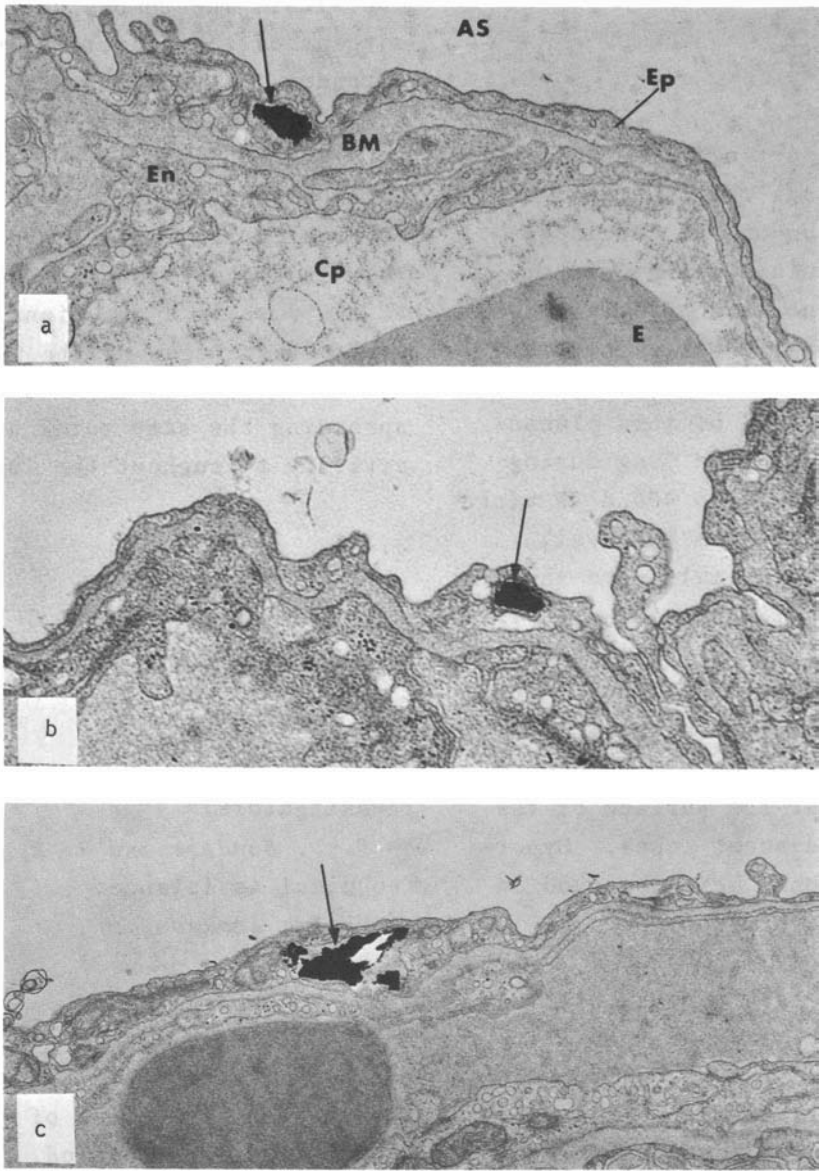

FIGURE 2. Electron Micrographs Showing Inhaled $^{239}PuO_2$ Particles Engulfed by Type I Alveolar Epithelial Cells [(a) 60 min postexposure (b) 90 min postexposure, (c) 7 days postexposure; AS = air space, Ep = epithelium, BM = basement membrane, En = endothelium, Cp = capillary, E = erythrocyte, arrow indicates plutonium particle]

of phospholipid residual material, surfactant-like material and protein. This coincided with a large increase in alveolar macrophages of histocytic appearance, often occluding the entire air space of some alveoli. Loss of endothelium and replacement with fibrin and collagen was seen along with greatly increased numbers of septal cells. Edema, cytoplasmic fragmentation, mitochondria damage, loss of endoplasmic reticulum, were usual findings in pulmonary cells. In the latter stages, a large increase in tissue mast cells and plasma cells was observed, with the mast cells being associated with septal cells and collagen fibrils in the walls. The radiation pneumonitis was characterized by extensive loss of endothelium, fibrosis, edema with fluid and protein accumulation in the alveoli.

THERAPEUTIC REMOVAL OF INHALED PLUTONIUM

Investigator:
J. V. Dilley
Technical Assistance:
K. E. McDonald

Forty therapeutic agents have been tested in continuing studies of the removal of inhaled $^{239}PuO_2$ from the lungs of rats. Promazine HCL, Halotestin, Estradiol, Diuril, Phenergan, Progesterone, Diamox and Miltown, appeared to show some effectiveness. Diamox, Diuril and Miltown decreased the rat lung burden but increased the translocation to other tissues. When the animals were treated with these drugs in combination with DTPA the systemic burden was reduced 45 to 90% in each group and the lung burden was decreased as much as 25 to 50% in the Diamox+DTPA and Diuril+DTPA groups. Miltown+DTPA did not decrease the lung burden. These studies suggest that the primary drug treatment is somehow responsible for making the inhaled $^{239}PuO_2$ available for chelation by DTPA.

PROMETHIUM OXIDE INHALATION STUDIES

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

Some long-term results have become available from three dogs exposed to aerosols of $^{147}Pm_2O_3$ calcined at 750 °C and from one dog exposed to $^{147}Pm_2O_3$ dissolved and recalcined at 750 °C (Table 3). Four years after initial alveolar deposition of 1 to 2 μCi of $^{147}Pm_2O_3$, all dogs retained about 20%. The dogs exposed to calcined $^{147}Pm_2O_3$ retained only 2 to 3% of the final burden in the lungs, and less than 1% in the tracheobronchial

TABLE 3. Promethium Distribution in Beagle Dogs After Inhalation of $^{147}Pm_2O_3$

Dog No.	Material	Years After Exposure	Initial Alveolar Burden, μCi	Terminal Body Burden, μCi	Percent Total Body Burden at Sacrifice						
					Lungs	TBLN*	Liver	Skeleton	Muscle	Kidneys	Spleen
364	Calcined Oxide	4	1060	240	2	0.08	34	56	4	1	0.04
366	Calcined Oxide	4	1220	260	3	0.06	39	49	3	1	0.03
382	Calcined Oxide	4	1710	380	2	0.03	29	59	5	1	0.05
374	Recalcined Oxide	3.7	1190	220	30	13	18	29	3	1	0.04

* *Tracheobronchial Lymph Nodes*

and mediastinal lymph nodes; 50 to 60% was in the skeleton, 30 to 40% in the liver and 3 to 5% in the muscle. The dog exposed to recalcined $^{147}Pm_2O_3$ retained 30% of the plutonium in the lungs, 13% in the tracheobronchial and mediastinal lymph nodes, 29% in the skeleton, 18% in the liver and 3% in the muscle. A greatly increased pulmonary retention of the recalcined material is evident together with a much greater translocation to, and retention in, the tracheobronchial lymph nodes. Chemically identical substances may differ markedly in their pulmonary behavior, dependent on minor changes in physical composition.

Tracheobronchial lymph nodes of the dog exposed to recalcined $^{147}Pm_2O_3$ showed proliferation of basophilic plasma cells, total lymph node necrosis in some nodes, and much replacement by fibrous connective tissue. Four years after alveolar deposition of 1200 μCi the lung showed slight to moderate hypertrophy of small bronchiolar muscle, foci of fibrous proliferation, and a slight proliferation of the epithelium from several small bronchiols. The lungs and lymph nodes from dogs that inhaled the non-recalcined oxide at similar doses and retained less than 3% after 4 years, showed no significant lesions. Histopathology of liver and skeleton is not completed.

BIOCHEMISTRY OF PULMONARY TISSUES

Investigator:

E. G. Tombropoulos

Technical Assistance:

A. Jacqueline Clary

Lung lipid content has been shown to increase 24 hr after X-irradiation. Lung mitrochondria derived from thoracic X-irradiated animals incorporated more ^{14}C from palmitate into lecithin, triglycerides, monoglycerides, and

diglycerides, between 14 and 24 hr postexposure, than did mitochondria derived from control animals. The microsomal fraction did not show this stimulation.

In experiments designed to elucidate the pathway of lung lecithin synthesis, lung mitochondria, lung microsomes, and slices of lung tissue were incubated with (Me-^{14}C) choline, (1, 2-^{14}C) phosphorylcholine, (Me-^{14}C) methionine, and (U-^{14}C) ethanolamine (Table 4). The optimal conditions for incorporation of the above components by the subcellular fraction were determined. These studies showed that lung subcellular fractions incorporate ^{14}C from choline into lecithin mostly by exchange reactions and to a lesser degree by the CDP-choline pathway. The ethanolamine pathway is also operative in lung tissue, but is of only minor importance.

Experiments using gas chromotography indicate that palmitate is incorporated into lecithin almost exclusively as a whole molecule without prior elongation or unsaturation.

Lung washings collected immediately prior to euthanasia, from dogs with radiographic evidence of plutonium-induced pulmonary neoplasia, showed no significant difference in surface tension measured with a surface tension balance. Methods were outlined to measure lipid composition of lung washings and to study the relationship of different classes of lipids, their fatty acid composition and surface tension activity.

TABLE 4. Incorporation into Lecithin of ^{14}C from Nitrogenous Bases and Methyl Donors by Lung Subcellular Fractions

	Nanomoles of ^{14}C compounds incorporated per mg of incubated protein by:		
	Mitochondria	Microsomes	Slices
Choline (Me-^{14}C)	0.75	1.2	1.6
Phosphorylcholine (1,2-^{14}C)	0.38	0.42	0.30
Ethanolamine (UL-^{14}C)	0.036	0.019	0.12
Methionine (Me-^{14}C)	0.14	0.22	0.20

AEROSOL TECHNOLOGY STUDIES

Investigator:
D. K. Craig

An examination of the characteristics of an aerosol as determined by several types of sampling instruments was initiated. It was shown, for example, that the particle size distribution obtained from a thermal precipitator sampling at 20 ml/min was significantly different from that obtained from a point-to-plane electrostatic precipitator sampling at 100 ml/min if the aerosol contained a significant number of particles

greater than 1 μm in diameter. Calibration work on a range of sampling devices, including the above two types of precipitators, and the Mercer, Casella and Andersen cascade impactors was initiated. Rotameters used to measure the volume of air drawn through aerosol samplers can be in error by several hundred percent if there is a significant pressure drop across the sampling device. A method was derived for correcting the indicated flow.

Techniques for the generation of monodispersed aerosols were surveyed, and it was concluded that the most promising methods for plutonium aerosols would be: (a) a spinning disk generator, for the size range 0.5 μm to 10 μm, using a plutonium dioxide or a plutonium nitrate colloid as feed material; and (b) a vaporization-condensation technique for the size range 0.01 to 0.5 μm, using plutonium metal as the source material in an atmosphere of helium. The generation of constant concentrations of aerosols, suitable for chronic inhalation experiments, from dry powders of various materials was investigated in some detail. A cyclone (Figure 3) was designed for insertion between the aerosol generator and the exposure chamber. It was shown to be effective in removing non-respirable particles from the aerosol, eliminating all aerodynamic equivalent spheres greater than the sizes shown in Table 5 for various flow rates.

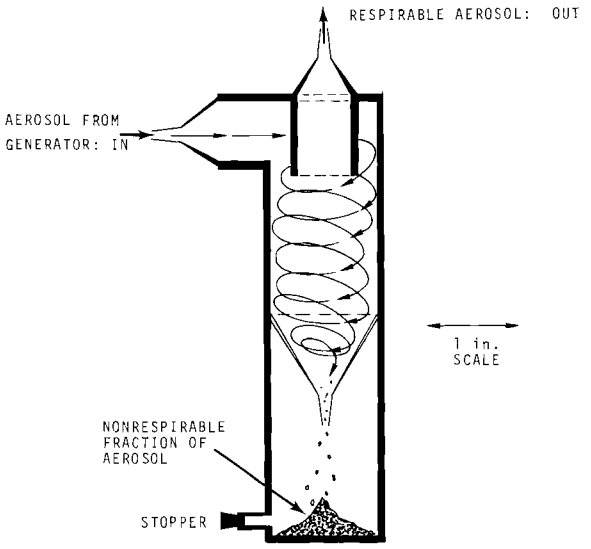

FIGURE 3. Cyclone Used as an Elutriator to Remove Non-respirable Particles from the Air

TABLE 5. Diameter of Smallest Aerodynamic Equivalent Sphere that Is Completely Trapped in Cyclone Shown in Figure 3, as a Function of Flow Rate

Air Flow Rate		Diameter
l/min	cm^3/sec	μm
6	100	16.4
12	200	11.6
18	300	9.47
24	400	8.21
30	500	7.34
36	600	6.70
42	700	6.20
48	800	5.80
54	900	5.47
60	1000	5.19

LOW LEVEL PLUTONIUM INHALATION STUDIES IN BEAGLES

Investigators:

J. F. Park, P. L. Clary,
D. K. Craig and M. G. Brown

Technical Assistance:

L. R. Richardson and M. D. Snyder

In studies that were initiated 7 to 10 years ago, 35 beagle dogs inhaled $^{239}PuO_2$ aerosols and were held for duration of life observation. Of 18 of these dogs that died after surviving for at least 4-1/2 years post-exposure, all had primary pulmonary tumors. Their plutonium burdens at autopsy ranged from 0.4 to 2.7 μCi. It is apparent from this data that studies must be conducted at much lower exposure levels if information is to be obtained concerning dose-effect relationships.

Dogs are being raised for this low-level plutonium inhalation study and exposures will commence during the coming year. Dose levels to be employed and numbers of animals to be assigned to each group are shown in Table 1. The two highest exposure levels overlap those in the earlier studies and should, predictably, lead to a high incidence of lung tumors. The lowest level corresponds to the presently established permissible body burden; i.e., it should result in an average dose (as calculated by ICRP) of 15 rem/year to the lung. Both $^{238}PuO_2$ and $^{239}PuO_2$ will be studied, since both are of primary hazard concern and because comparison of effects will provide data of basic interest on the influence of specific activity. These two isotopes of plutonium have very similar radiation characteristics, but ^{238}Pu has a specific activity 280 times greater than ^{239}Pu.

TABLE 1. Experimental Design For Low Level Plutonium Inhalation Study in Beagles

Initial Alveolar Deposition Level, μCi	Number of Dogs Exposed to $^{238}PuO_2$	Number of Dogs Exposed to $^{239}PuO_2$
3.0	10	10
1.25	10	10
0.25	20	20
0.05	20	20
0.01	20	20
0.002	20	20
Controls	-20-	

Studies are in progress on the generation of aerosols of reproducible particle size distribution over the wide range of concentrations required. Preliminary studies are also being initiated to determine whether deposition and early retention behavior are influenced by dose level at the very low levels to be employed in this experiment.

INHALATION HAZARDS TO URANIUM MINERS - BIOLOGICAL STUDIES

Etiological studies of the relatively high incidence of lung cancer and other pulmonary diseases in the miners have shown clearly increasing effects above 840 cumulative working level months (CWLM).* Many recent reports have discussed the difficulty of establishing a cause and effect relationship between increased incidence of lung cancer and exposure to 100 to 400 CWLM. In addition to the hazard of inhaled radon daughters, ore dust concentrations in the mine atmosphere, diesel engine exhaust fumes, and cigarette smoking are possibly synergistic factors which must be considered.

The continuing experiments in this research project involve daily exposures of both hamsters and dogs to controlled levels of radon daughters and uranium ore dust for the lifespan of the animals, at levels which should cause minimal lifespan shortening. A closely related project supported by the National Institute for Environmental Health Sciences, HEW, through an interagency agreement with the AEC, supplements these studies to include exposures to diesel exhaust fumes and cigarette smoke. The HEW and AEC projects were integrated into one experimental design at the request of the two funding agencies to ensure maximum effort and efficiency. Results of the combined projects are described in this report.

Two other AEC-sponsored projects are closely coordinated with these animal experiments. Under the project entitled "Inhalation Hazards to Uranium Miners - Radiochemical Studies," the radionuclide and trace element contents of aerosol samples from uranium mines and the contents of biopsy and autopsy samples from uranium miners will be analyzed for comparison with the results from the animal experiments. Special analytical procedures required for the animal experiments were developed under the "Radiochemical Studies" project. Also closely related is the project "Uranium Miner Exfoliative Pathology" in which lung washing samples from dogs exposed to laboratory-produced uranium mine atmospheres will be compared with similar samples from uranium miners for study of preneoplastic and neoplastic cells.

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* *170 working hours with exposure at one Working Level; defined as 1.3×10^5 potential MeV of alpha energy, from radon daughter decay, per liter of air.*

CHRONIC EXPOSURE OF HAMSTERS TO SIMULATED URANIUM MINE ATMOSPHERES

Investigators:

B. O. Stuart, E. B. Howard, P. L. Clary and D. K. Craig

Technical Assistance:

J. C. Gavin and H. G. Steele

PUBLICATIONS: INHALATION HAZARDS TO URANIUM MINERS - BIOLOGICAL STUDIES

BAIR, W. J., N. S. PORTER, D. P. BROWN, and A. P. WEHNER. "Apparatus for Direct Inhalation of Cigarette Smoke by Dogs," J. Appl. Physiol., vol. 26, pp. 847-850. 1969.

BAIR, W. J. "Inhalation of Radionuclides and Carcinogenesis," Conference on Inhalation Carcinogenesis, Gatlinburg, Tennessee, October 8-11, 1969. (In Press).

Four groups of hamsters (initially 102 animals per group) are receiving lifetime, daily, 6-hr exposures to 30 WL and 600 WL radon daughters with and without simultaneous exposure to aerosols of carnotite ore dust (15 mg/m^3). Bimonthly hematological sampling of selected hamsters from each group has shown no significant differences in peripheral blood parameters. Biweekly weighing of all animals has shown no significant differences in mean body weight. Animals are sacrificed when moribund. Death is usually preceded by a sharp loss of body weight. At nearly 1-1/2 years of age, these hamsters are approaching their mean life-span. About half of the animals remain alive in each of the study groups, and there is no evidence of life-shortening in the experimental groups (Figure 1).

After the first 6 months of daily exposures to 30 WL, the lungs of sacrificed animals showed some congestion and edema, with slight thickening and early hyalinization of the interalveolar septa. A slight degree of emphysema was also found. After 6 months and longer of daily exposures to 600 WL, edema was associated with the alveolar septa; areas of peripheral emphysema were found, with a moderate inflammatory reaction involving mononuclear and polymorphonuclear cells. Alveolar cell proliferation and hyperplasia of the bronchiolar epithelium are occasionally seen.

Lungs of animals exposed to 600 WL plus carnotite ore dust showed areas of alveolar septal breakdown with emphysema plus dilation and congestion of the pulmonary vasculature. Several animals have shown some basal cell hyperplasia of the lining epithelium of intermediate bronchioles with an early indication of epithelial metaplasia. A severe generalized pneumonitis has been observed in many animals of this group; less severe pneumonitis has been observed in ani-

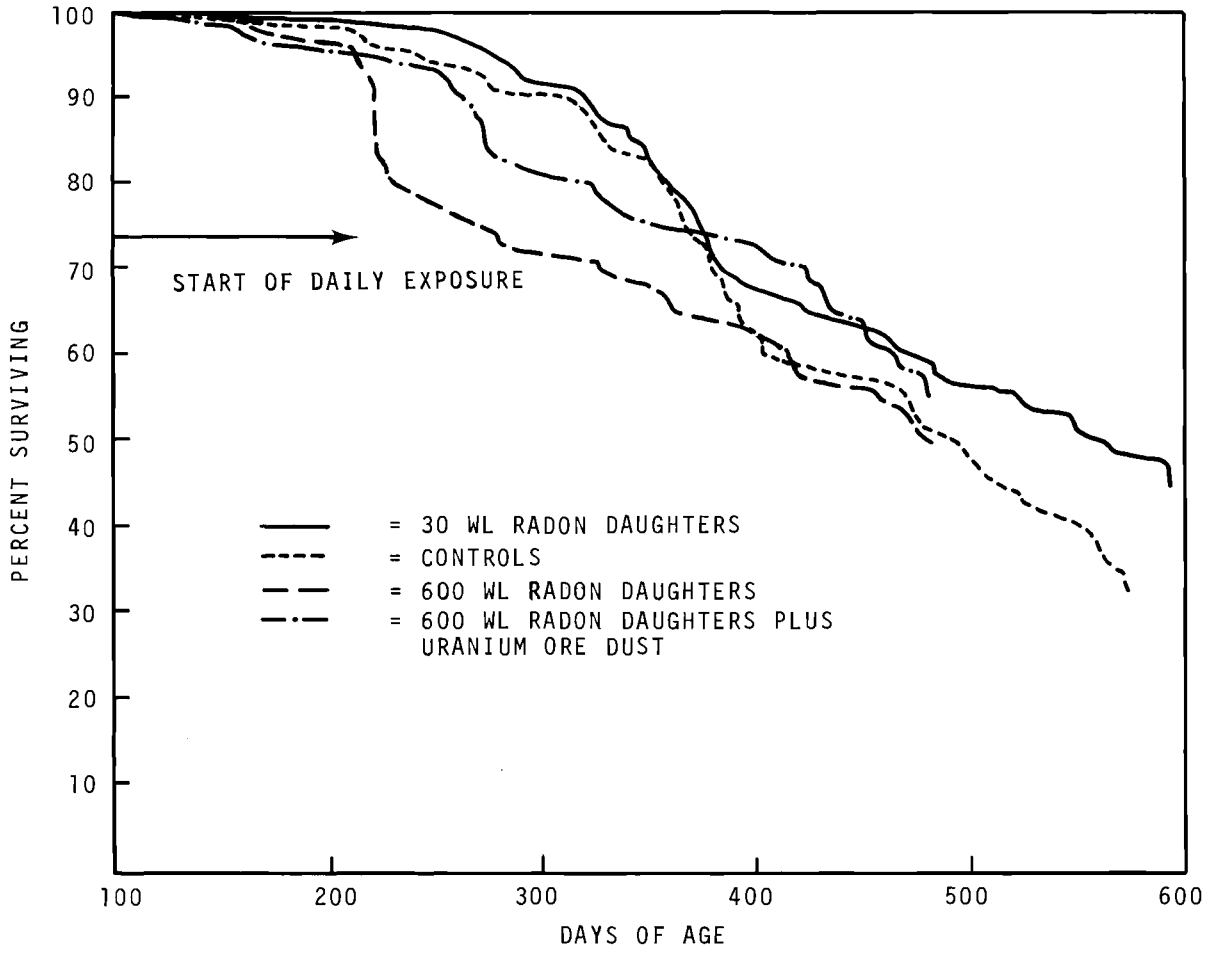

FIGURE 1. Cumulative Mortality Among Hamsters Exposed Daily to Radon Daughters and Uranium Ore Dust

mals from all groups, including controls. None of the animals examined to date showed pulmonary neoplasia. The final interpretation of these changes must await detailed histopathological examination of all animals at the conclusion of these studies.

Exposure of two additional groups of hamsters will soon be instituted. These animals (supported by the HEW project) will be exposed to diesel exhaust fumes, with and without associated radon daughters and carnotite ore dust. Facilities for these exposures are in place and tested.

During the past year, modifications were made in aerosol generators, exposure chambers, cage cleaning facilities, and automatic watering systems, resulting in improved maintenance under daily operation. An emergency electrical generator system was installed to supply animal life-support needs and personnel safety in case of electrical failure. The

4-chamber radon detector and solid-state surface barrier detector instrumentation for radon daughters provides hourly monitoring of exposure chamber aerosols. Additional instrumentation determines the fraction of radon daughter radionuclides that are free or attached to condensation nuclei or larger particles.

CHRONIC EXPOSURE OF DOGS TO SIMULATED URANIUM MINE ATMOSPHERES

Investigators:

B. O. Stuart, D. H. Willard, E. B. Howard, P. L. Clary and D. K. Craig

Technical Assistance:

J. C. Gavin and W. Skinner

Twenty beagle dogs are being exposed daily to 600 WL of radon plus daughters on ore dust (Figures 2 and 3). An additional 20 dogs are similarly exposed, but in addition smoke 10 cigarettes per day. Twenty dogs serve as smoking controls, and nine dogs are maintained as sham-exposed controls. One dog from each exposure group will be sacrificed and replaced after 6 months exposure to follow developing pathology. Periodic measurements of respiratory rates and volumes, body weights, hematology, clinical chemistry, and radiographic examinations are performed on each animal. Blood and excreta analysis should help to establish the feasibility of bioassay as a means of estimating exposure to radon daughters. External monitoring for ^{214}Pb and ^{214}Bi gamma over several regions of the respiratory tract of these animals will be evaluated to provide deposition and retention data and to aid in determining the crucial relationship between measured working levels and absorbed radiological dose. Two dogs from each exposure group will be subjected to periodic lung washing to obtain cells for cytology studies; these samples will be compared with those obtained from uranium miners.

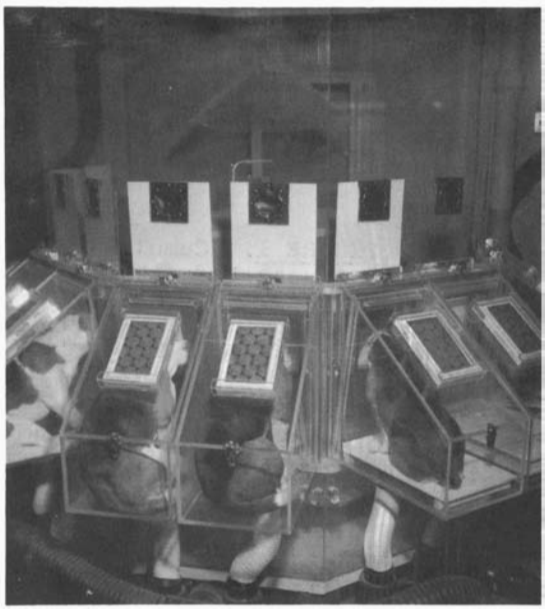

FIGURE 2. Beagle Dogs Receiving Simultaneous, Head-Only Exposure to 600 WL of Radon Daughters Plus Carnotite Ore Dust. (Each dog box has magnetically sealing doors and a separate ventilation system.)

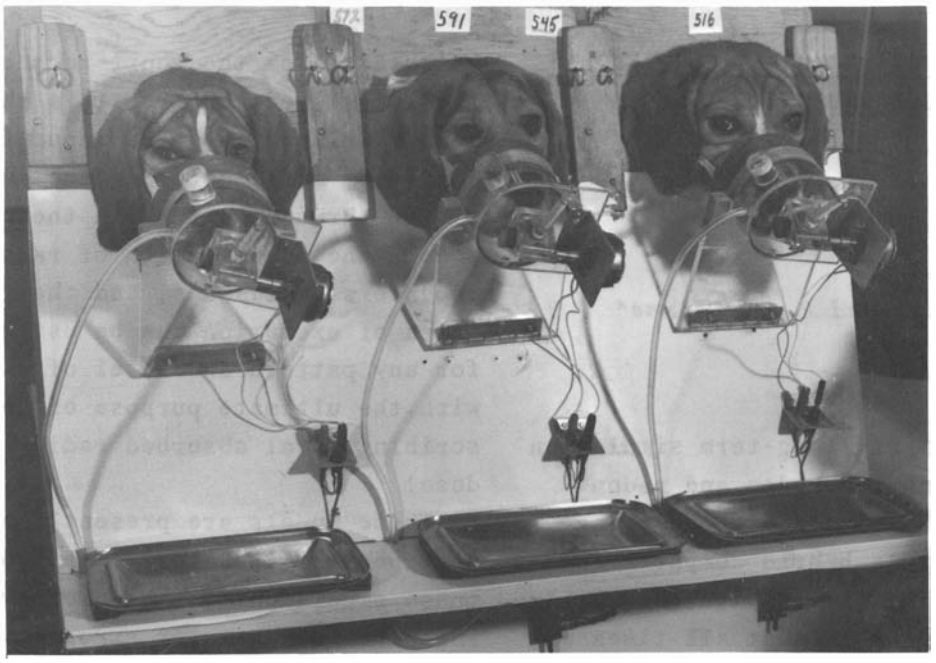

FIGURE 3. Beagle Dogs Receiving Mouth-Only Inhalation of Cigarette Smoke During Routine 5-Day/Week Exposures

No significant differences in respiratory rate, minute volume, tidal volume, thoracic radiographs, hematology, or clinical chemistry, have been observed between groups at 2 months after beginning daily exposures.

From an earlier experiment, two dogs were sacrificed 15 months after conclusion of 1/2 hr, twice daily exposures to pitchblend uranium ore dust (0.1 mg/liter). Radiochemical analysis revealed nonequilibrium ratios of ^{234}U, ^{230}Th, ^{210}Pb and ^{210}Po in the lungs, tracheobronchial lymph nodes, kidney, liver, spleen, and skeleton, with $^{230}Th/^{234}U$ ratios ranging from 2 to 10. $^{210}Po/^{234}U$ ratios in the lungs and tracheobronchial lymph nodes varied from 5 to 20. The highest concentrations of ^{230}Th were found in the lungs, skeleton, and tracheobronchial lymph nodes.

DYNAMIC SIMULATION MODELS FOR INHALED RADON AND RADON DAUGHTERS

Investigators:

*B. O. Stuart and P. J. Dionne**

Short-term and long-term simulation models for inhaled radon and radon daughters are being developed using the laboratory's hybrid computer complex. Modeling starts with the build-up of radon daughters at all times and positions in the exposure chambers, which determines the quantity of radionuclides inhaled. The dynamic organ burden of RaA through RaD is calculated for the short-term case. The long-lived RaD from this short-term model provides the input for a long-term model covering the buildup of RaE through RaG. With these models the accumulation of radon daughters in tissues, and their rates of excretion can be estimated for any pattern and level of exposure, with the ultimate purpose of describing total absorbed radiation dose.

These models are presently based on reported literature data from mouse experiments, and on early findings from our hamster exposure studies. As more hamster data and dog data become available, the models will be improved and extended.

* *Control and Instrumentation Department, Systems and Electronics Division*

SPACE NUCLEAR SYSTEMS STUDIES

Space vehicles containing nuclear reactors or radionuclide power sources present special hazards in case of early abort or upon return from orbit. These problems are principally concerned with the biological effects of intensely radioactive, small, insoluble particles. For the past several years, we have studied the deposition and retention kinetics of inhaled particles, with determinations of dose-effect relationships for individual or crushed particles passing through, or retained in contact with, various regions of the gastrointestinal tract and lungs.

FATE OF INGESTED $^{238}PuO_2$ IN MINIATURE SWINE

Investigator:
V. H. Smith
Technical Assistance:
J. L. Beamer and S. A. Hughes, Jr.

Data were reported earlier on the absorption of plutonium from intact $^{238}PuO_2$ microspheres fed to miniature swine. Fine, submicron particles often accompany these microspheres, being produced by microsphere fracture. Such fine particles might also be produced by "burn-up" during re-entry. It seemed quite possible that solubility characteristics or transport mechanisms for this finely divided material might be different from those of the larger particles previously studied.

Cleansed, $^{238}PuO_2$ microspheres from Mound Laboratory were ground under ethanol. The resulting particles were irregularly shaped, mostly <1 μm on their longest dimension with a range from 10 to 0.01 μm. These particles, in capsules, were administered by stomach tube to adult, female pigs held in metabolism cages. Urine and feces were collected daily, effective separation being achieved by catheterization of the bladder. After 14 days, the pigs were sacrificed and tissues taken for analysis.

A comparison of the crushed microsphere results with the earlier data on intact microspheres is shown in Table 1. Two differences are strikingly apparent. The very much higher deposition in the lungs following feeding of the crushed microspheres is probably due to inhalation of material resuspended from feces. This interpretation is supported by the fact that the cages became generally contaminated. The lower deposition of plutonium in the skeleton

TABLE 1. Fate of Crushed and Intact $^{238}PuO_2$ Microspheres Fed to Miniature Swine

	Crushed Microspheres			Intact Microspheres		
Quantity Fed, Ci	0.31	0.09	0.10	0.27	0.33	0.18
Tissue	Plutonium in Tissues 14 Days After Feeding (fraction of dose × 10^{-9})					
Skeleton	4.6	12	4.7	24	21	34
Liver	1.8	3.5	2.8	0.7	1.1	5.0
Kidneys	0.1	0.3	0.8	0.03	0.2	0.07
Spleen	0.03	0.1	0.2	0.04	0.02	0.07
Lungs	5.2(a)	93(a)	92(a)	0.06	0.04	0.06
Other Soft Tissue	3.5	6.3	39	0.7	0.7	0.8
Total Systemic Burden	15(b)	115(b)	144(b)	26	24	42
Excreted in Urine	20	59	>17(c)	1.1	1.2	16
Total Absorbed	35	174	>161	27	25	58

(a) High lung content probably due to inhalation of plutonium resuspended from feces.

(b) If allowance is made for high lung levels, these figures are in reasonable agreement with results from intact microspheres.

(c) Excretion through day 7, when urinary tract infection required removal of catheter.

following feeding of the crushed microspheres would seem to indicate that actual solubilization and absorption from the gastrointestinal tract were less than in the case of intact microspheres. This is somewhat surprising, but may possibly be explained by a more rapid passage of the ground material through the tract. No plutonium was detected in the tract at autopsy, in contrast to the previous experiments where intact microspheres were still present in two-thirds of the pigs at autopsy on the 14th day.

The lower skeleton values in the ground microsphere animals contrast with somewhat higher values in most soft tissues and in urine. It is possible that some very finely ground material was directly absorbed; such material would not be expected to reach the bone. However, there was no evidence from autoradiographs of tissues, or from plutonium deposition in lymph nodes associated with the gut, to suggest that particles could penetrate and move directly across the intestinal membranes.

In any case, absorption of plutonium from ingested ground microspheres seems to constitute no unusual hazard, and is perhaps less hazardous than ingestion of intact microspheres. This of course disregards the obviously greater respiratory hazard of the finely ground material.

INHALATION OF $^{238}PuO_2$ - SOLID SOLUTION MATERIALS BY BEAGLE DOGS

Investigators:

D. H. Willard and J. F. Park

Technical Assistance:

W. Skinner and M. D. Snyder

Solid solution mixtures of $^{238}PuO_2$ with other refractory oxides have displaced $^{238}PuO_2$ microspheres as the fuel form of choice for future SNAP devices. Samples of finely ground $^{238}PuO_2$-ZrO_2 and $^{238}PuO_2$-ThO_2 were obtained from the Los Alamos Scientific Laboratory, and aerosols were inhaled by Beagle dogs. These animals, three in each group, were euthanized at 3 months postexposure and tissues analyzed with results as shown in Table 2. Also shown in Table 2 are plutonium distribution and retention data from dogs exposed to several other forms of $^{238}PuO_2$.

The two solid solution materials behaved quite differently. The shorter retention half-times and higher skeletal deposition of the zirconium oxide material may reflect different physical-chemical characteristics of the two materials, or the difference may be due to the widely different deposition levels. Further exposures will be required to distinguish between these possibilities. Compared to animals that inhaled other forms of $^{238}PuO_2$, the solid solution animals showed no strikingly unusual distribution or retention effects. Lung retention and skeletal deposition, among the limited data available are generally quite variable.

Differences in observed pathology were undoubtedly attributable to the differing deposition levels. The $^{238}PuO_2$-ZrO_2 dogs showed very little damage to the lungs and thoracic lymph nodes. Thoracic lymph nodes of the $^{238}PuO_2$-ThO_2 dogs showed lymphoid depletion and necrosis; the lungs showed some congestion and hemorrhage with subpleural fibrosis and thickening of interalveolar septa.

TABLE 2. Distribution and Retention of Plutonium in Dogs After Inhalation of $^{238}PuO_2$ in Various Forms

Aerosol	Time Post-exposure, months	Retention Half-Time, days		Terminal Body Burden, μCi	Plutonium Distribution (Percent of Terminal Body Burden)			
		Whole-Body	Lungs		Lungs	Thoracic Lymph Nodes	Skeleton	Liver
$^{238}PuO_2$-ZrO_2 (a)	3	400	150	0.1-0.2	72	11	9	3
$^{238}PuO_2$-ThO_2 (a)	3	3600	310	3.3-6.5	83	13	3	1
$^{238}PuO_2$-C.M. (b)	2-3	2200	600	33-162	93	5	1	1
$^{238}PuO_2$-C.M.	22	5600	1100	3.1	72	7	12	7
$^{238}PuO_2$-M (c)	22	--	--	0.8	28	46	15	9
$^{238}PuO_2$-350 °C (d)	1-6	900	300	25-261	90	4	4	2
$^{238}PuO_2$-300 °C (e)	23	--	--	3.0	4	4	64	23

(a) Mean of 3 Dogs

(b) Mean of 6 Dogs exposed to crushed microspheres

(c) Inhaled intact 50 μm microspheres, only fragments retained, pulmonary neoplasia

(d) Mean of 12 Dogs exposed to $^{238}PuO_2$ calcined at 350 °C, 4 Dogs with Pulmonary Neoplasia

(e) Calcined at 300 °C

LONG-TERM EFFECTS OF INHALED $^{238}PuO_2$ IN BEAGLES

Investigators:

D. H. Willard and J. F. Park

Technical Assistance:

W. Skinner and L. R. Richardson

In the course of several years experimentation with various forms of $^{238}PuO_2$, a number of dogs have accumulated that are being held for observation of long-term effects. The status of these animals is summarized in Table 3.

One dog was euthanized due to respiratory insufficiency at 22 months postexposure and was found to contain an alveolar cell carcinoma. Such an early appearance of a tumor at such a low plutonium deposition (0.8 μCi at sacrifice) had not previously been observed. It is interesting that the plutonium deposited in this animal resulted from the inhalation of 50 μm diameter microspheres which were lost from the lung within a few days following exposure. The plutonium

TABLE 3. Summary of Dogs Surviving Exposure to $^{238}PuO_2$ by Inhalation

Form of Oxide	Particle Size CMD, μm	No. of Dogs	Time Post-exposure, months	Lung Burden, μCi[a]	Biological Effects
Calcined at 300 °C	<0.1	10	26	0.1-6.0	Lymphopenia Pulmonary fibrosis Fractured humerus[c]
Crushed microspheres	<0.1	12	28	0.1-4.0	Lymphopenia Pulmonary fibrosis Bronchiolar metaplasia[d]
Intact microspheres[b]	50 μm	6	34	0.1-0.8	Lymphopenia Alveolar cell carcinoma[e]
Intact microspheres[b]	50 μm	5	34	<0.1	Intermittent lymphopenia
Single microspheres (intubated)	50-300 μm	5	34-42	10-2000	None

(a) Estimated from external counting and size of microspheres.

(b) Aerosol contained fragments of microspheres (0.03-1.0 μm, CMD); only these fragments were retained beyond a few days postexposure.

(c) One dog (3.0 μCi body burden) euthanized at 23 months postexposure due to a fractured humerus; histology incomplete.

(d) One dog (3.1 μCi body burden) euthanized at 22 months postexposure with CNS lesions not considered to be caused by plutonium.

(e) One dog (0.8 μCi body burden) euthanized at 22 months postexposure with alveolar cell carcinoma.

remaining was that produced by fragmentation of the microspheres in the lung or during generation of the aerosol.

The dogs with the highest ^{238}Pu lung burdens are those that received single microspheres by intubation. Five of these animals, with depositions of 10 to 2000 μCi, show no ill-effects after harboring these particles for 3 years.

Plutonium analyses were completed on a dog euthanized due to a fractured humerus 23 months after inhalation of $^{238}PuO_2$ calcined at 350 °C (CMD, <0.1 μm). The body burden was 3.0 μCi with 64% in the skeleton, 23% in the liver, 4% in the lungs, 3% in the tracheobronchial and mediastinal lymph nodes, and 3% in the muscle. Another dog, euthanized 22 months after exposure to $^{238}PuO_2$ crushed microspheres, (CMD, <0.1 μm) showed a body burden of 3.1 μCi with 73% in the lungs, 12% in the skeleton, 8% in the tracheobronchial and mediastinal lymph nodes, and 7% in the liver. The contrasting translocation rates in these dogs, both exposed to $^{238}PuO_2$, demonstrate the effect of chemical and/or physical form. Eleven additional dogs now 30 months postexposure to crushed $^{238}PuO_2$ microspheres, and ten dogs now 28 months postexposure to the $^{238}PuO_2$ aerosol described above, are available to supplement these initial results on the two dogs. Samples of the aerosolized material collected during exposure are also available.

REMOVAL OF INTERNAL EMITTERS

The general objective of this project is to develop methods which will hasten the removal, prevent the deposition, or otherwise decrease the damage potential from ingested or absorbed radionuclides. Necessarily associated with this effort is the development of information on the absorption and tissue distribution of radionuclides to help in understanding and predicting their behavior and to serve as a basis for recommendations for the treatment of exposed persons. Studies relating to the removal of inhaled radionuclides from the lung are not included in this project but are covered in the Inhalation Studies project.

EFFECT OF AGE ON PLUTONIUM REMOVAL

Investigator:
V. H. Smith
Technical Assistance:
Alice M. Russell and M. L. Smith

PUBLICATION: REMOVAL OF INTERNAL EMITTERS

SMITH, V. H., R. L. AMSTER, and J. M. THOMAS. "The Biological Disposition of Promethium After Intravenous, Intramuscular, and Subcutaneous Administration to Swine," Health Physics, vol. 17, p. 385. 1969. (Abstract).

The distribution in the rat of plutonium, and its toxic effect, has been shown to be a function of the age of the animal at time of injection. To determine the effect of age on plutonium removal, rats were injected with plutonium citrate at age 1 day (0.09 μCi ^{239}Pu, intracardially), or at age 21 days (1.8 μCi ^{238}Pu, intravenously); and subsequently treated with a single intraperitoneal injection of calcium diethylenetriaminepentaacetate (DTPA), 1.5 mmol/kg.

In the weanling rats DTPA was found to be more effective in preventing

TABLE 1. Effect of Age on Therapeutic Effectiveness of DTPA (1.5 mmol/kg, administered as a single intraperitoneal injection)

Time Between Pu and DTPA Treatments	Deposition of Plutonium in Organs as Percent of Untreated Controls			
	Femur	Liver	Kidney	Spleen
Adult Rats				
1 hr	9	5	17	13
Weanling Rats				
1 hr	6	2	31	9
One-Day-Old Rats				
1 hr	6	18	42	11
1 day	15	18	48	21
4 days	18	43	61	32
10 days	38	44	151	46

deposition, when given 1 hr after plutonium citrate, than similar treatment in adult rats. (Table 1) Thus the treated animals deposited 16 times less plutonium in bone and 47 times less plutonium in liver than did the untreated controls which compares to a corresponding effect in adult rats of 11 and 20 times, respectively. Removal from the kidneys was less effective than in adults, which may have been caused by toxic effects of DTPA on the weanling kidney, although this was not investigated histologically.

The rats injected with plutonium at age 1 day were treated with DTPA 1 hr, 1, 4, or 10 days after the plutonium injection. All rats were sacrificed at age 15 days. The effectiveness of DTPA administered 1 hr after plutonium was not greatly different from that observed in the weanling and adult rats, except that much more plutonium was retained in the livers of the neonatal animals (Table 1). With delay in treatment, effectiveness decreases, as is the case with adult animals.

Severe toxic symptoms were observed in the neonatal animals treated with the calcium salt of DTPA at 1 hr and 1 day after plutonium injection. In further toxicity studies, as much as 140 mmol/kg of Zn-DTPA given subcutaneously at the rate of 2 or 4 mmol/kg/day had little effect on growth of rats when injections were started on day 4 or 10 after birth. The manganese salt of DTPA was also tested and was not well tolerated. Histological estimates of relative toxicity are not yet completed.

METABOLISM OF PROMETHIUM IN MINIATURE SWINE

Investigators:

V. H. Smith and R. L. Amster

Technical Assistance:

Alice M. Russell

In support of the human studies with promethium, conducted in the Radiological Sciences Department, and to better define the biological hazard, the deposition of promethium in miniature swine was studied after intravenous, intramuscular, and subcutaneous introduction of the radionuclide. Five or six animals were injected by each route and sacrificed after 100 days. About 35% of the injected promethium remained at the intramuscular injection site in the ham after 100 days and was retained in a volume about the size of a golf ball. An average of 40% remained at the site of subcutaneous injection, on the neck, but was more variable in its spread (Table 2). In neither case were high lymph node contents noted, suggesting that promethium is likely translocated in an ionic form. This is further supported by the predominant translocation to bone rather than to liver or other reticuloendothelial tissues. Material balances indicate that very little promethium is excreted from intramuscular and subcutaneous depositions, even less than following intravenous injection. Translocation

TABLE 2. Retention of Promethium in Tissues of Miniature Swine 100 Days After Intravenous, Intramuscular, or Subcutaneous Injection of $PmCl_3$ in a pH 3.5 Solution (Means from five or six animals.)

Tissue	Percent of Injected Dose in Tissue		
	Intravenous	Intramuscular	Subcutaneous
Skeleton	55	42	41
Liver	19	22	19
Kidneys	0.77	0.15	0.20
Spleen	0.52	0.20	0.16
Lungs	0.12	0.28	0.32
Injection Site		35	40
Circulating Blood[a]	0.0044	0.010	0.0087

(a) Percent of injected dose/gram blood.

is sufficiently slow so that excision of the deposition site is a practical therapeutic procedure. Promethium hydrolysis products are more subject to chelation attack than similar plutonium compounds and could possibly be mobilized by chelons locally injected.

In the human, 40 to 50% of a dose of about 5×10^{-4} ng/kg of ^{143}Pm (three times less mass than the permissible occupational body burden for ^{147}Pm) was immediately deposited in the liver region and was not translocated from this organ during the ensuing 360 days. In miniature swine, however, an initial deposition of 40% in the liver dwindled to about 20% in 100 days. About 10^6 more mass was involved in the swine experiments, so that it is not clear whether this is a mass effect or a species difference.

Careful measurements were made of promethium concentrations in the blood and excreta of the intravenously injected animals. These results are portrayed in Figures 1, 2, and 3. All of these data demonstrate a long half-time for promethium in the swine, similar to that found in humans. The data suggest that the miniature swine should be a useful model for biological disposition of promethium in man.

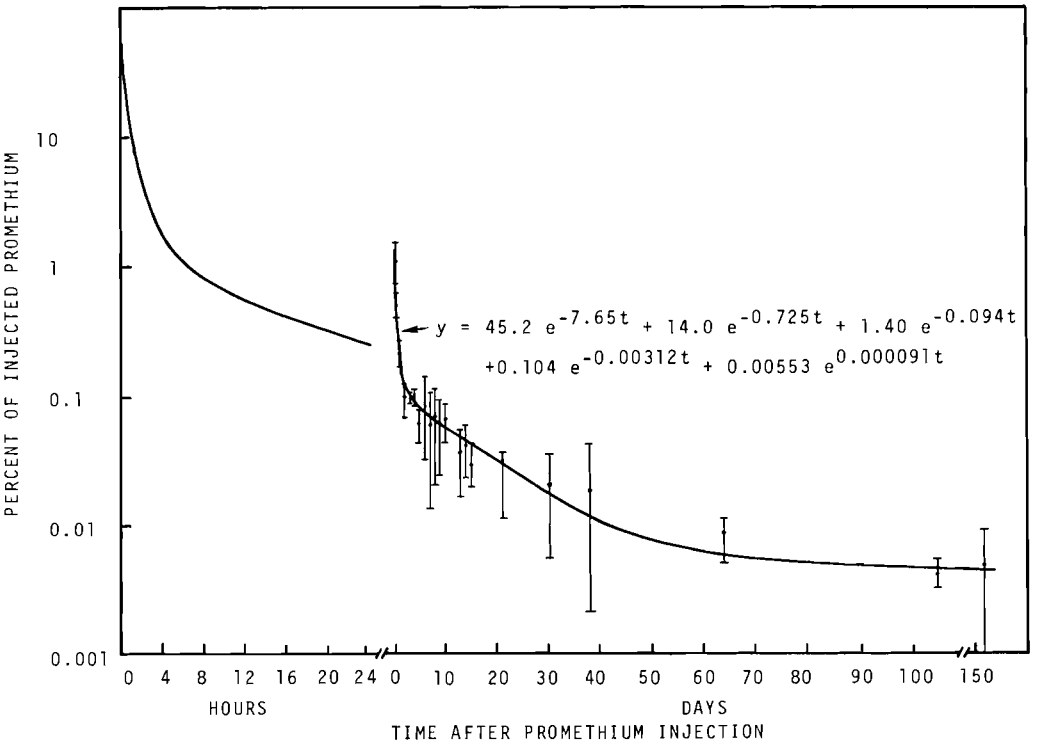

FIGURE 1. Blood Content of Promethium After Intravenous Injection of $PmCl_3$ in Miniature Swine; Data from Six Animals, with 95% Confidence Interval about the Mean

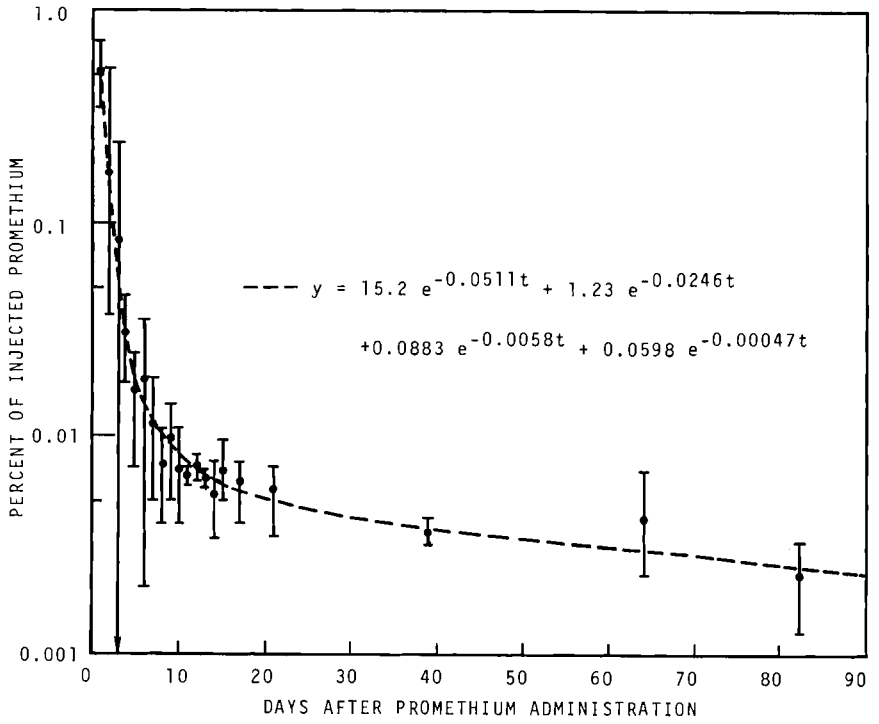

FIGURE 2. Daily Urinary Excretion of Promethium After Intravenous Injection of $PmCl_3$ in Miniature Swine; Data from Six Animals, with 95% Confidence Interval about the Mean

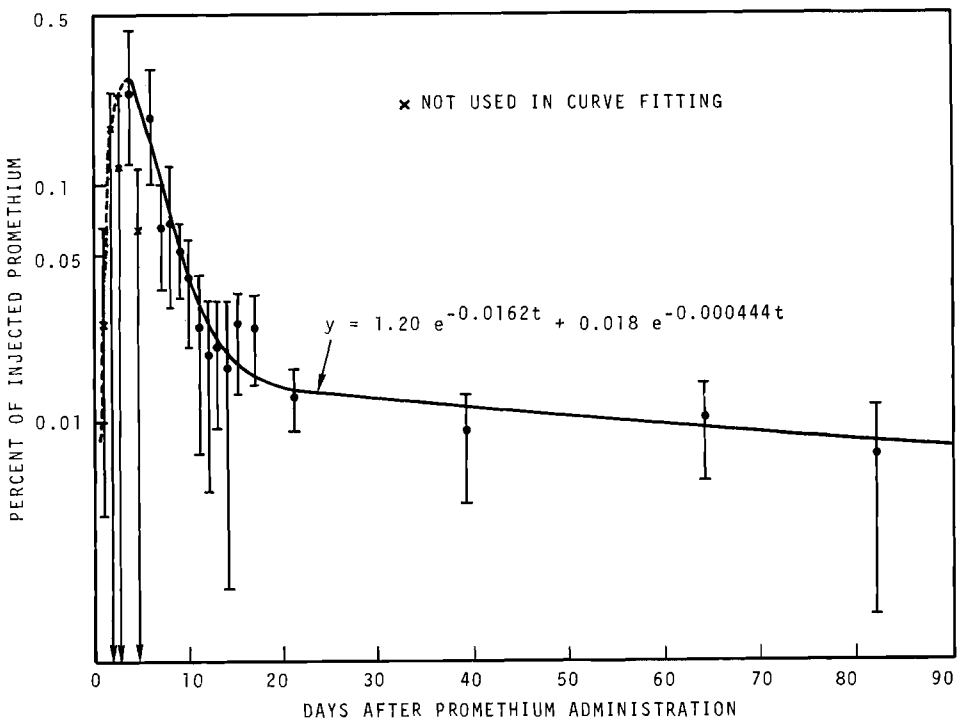

FIGURE 3. Daily Fecal Excretion of Promethium After Intravenous Injection of $PmCl_3$ in Miniature Swine; Data from Six Animals, with 95% Confidence Interval about the Mean

DEVELOPMENT AND EVALUATION OF BLOOD IRRADIATORS

Investigators:

F. P. Hungate, F. T. Cross,
*M. K. Gillis and B. D. Bingham**

Irradiation of blood has been effective in treating some forms of leukemia and complements the action of drugs in suppressing the immune response, thus lengthening organ or tissue transplant time. Such effects are directly related to suppression of lymphocyte concentration, which is usually achieved clinically by extracorporeal irradiation. Osgood has successfully treated leukemic patients with injected ^{32}P. In our studies with inhaled alpha-emitting radioactive particulates, we have observed a consistent severe lymphophenia due apparently to irradiation of blood circulating through capillaries in close apposition to deposited radionuclides.

Parametric calculations indicated that beta emitters with energy sufficient to irradiate a significant volume of blood would produce unacceptably high bremstrahlung dosage in surrounding tissues. Weak photons in the energy range of less than 30 keV seem to provide the most favorable radiation fields (Figure 1). At such low energies, total shielding to protect surrounding tissue is easily attained with <1 mm of tantalum or other heavy metal. Iron-55 was selected as a suitable radionuclide for initial testing.

Two models of irradiators were constructed for in vivo testing. One consisted of a simple thin wall cannula, cast from epoxy, with the ^{55}Fe as a 1-cm wide annulus around the middle. The ^{55}Fe was covered by a tantalum shield which in turn was cast in the epoxy. Short sections of

* *Mechanical Engineering Department, Physics and Engineering Division*

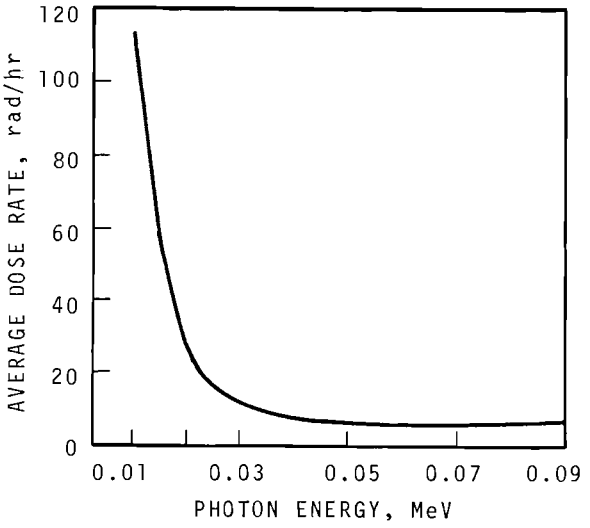

FIGURE 1. Average Photon Dose Rates Within a Cannula 1 cm Long by 0.5 cm Diameter, Containing 1 mCi/cm^2 Activity Density on the Inside Surface

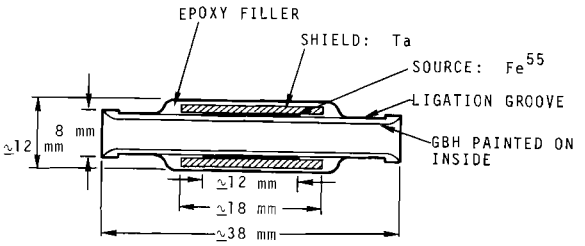

FIGURE 2. Epoxy-Clad Blood Irradiator (Modified at implantation by affixing Dacron velour in the ligation groove with silicone rubber cement. The ends of the cut artery are slipped over the velour, which provides a site for cell ingrowth and more permanent attachment of the vessel to the irradiator.)

dacron prosthetic serve to connect the epoxy device to the cut blood vessel ends. The inner surface of the cannula was GBH (graphite-benzalkonium-heparin) coated and the whole unit was radiation sterilized prior to implantation (Figure 2).

A second model employed a short length of 8 mm dacron prosthetic, coated in its mid-portion with medical grade silastic, a film of Saran Wrap, and with the ^{55}Fe, again placed as a 1-cm annulus around the Saran. A tantalum shield was placed around the ^{55}Fe and the whole encased in silastic, which provided a smooth tissue compatible cover. Both models were constructed in pairs, one with the radiation source and the other without to serve as a surgical control.

These devices were implanted in the thoracic aortas of dogs and hematological changes will be closely followed. From these pilot studies we will hope to obtain information concerning interactions at the blood-device interface, as well as indications of lymphocyte changes to be expected under conditions of chronic blood irradiation.

BNWL-1306
Part 1

MALONALDEHYDE AS A RADIOLYSIS PRODUCT OF BIOLOGICAL MATERIALS

Investigators:
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Technical Assistance:
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Controversy exists over the safety of irradiated foods. Cytotoxic effects have been ascribed to irradiated solutions of pure carbohydrates, yet the nature of the toxic radiolysis products remains obscure. We have shown that irradiation of a number of carbohydrates and of DNA and RNA produces malonaldehyde. Malonaldehyde appears to arise from a deoxy sugar intermediate via a solvated electron reaction; hence, the radiolytic production of malonaldehyde is pH-dependent, higher yields occurring at physiological than at more acidic pH ranges. The identity of malonaldehyde was established by u.v. spectra, by column chromatography and by formation of the 2-thiobarbituric acid derivative. Irradiation of DNA or RNA produced both malonaldehyde and a malonaldehyde-nucleic acid reaction product. This latter product was fluorescent and could be dissociated only by hot acid hydrolysis. Fluorescent reaction products of nucleosides with malonaldehyde show the u.v. absorption spectra and fluorescence excitation and emission spectra characteristic of the 1-amino-3-iminopropene crosslink.

The identification of malonaldehyde as a major radiolysis product of carbohydrates and polynucleotides places the evaluation of the safety of irradiated foods on a more scientific footing. The production of malonaldehyde and its reaction with DNA suggests that the biochemistry of

malonaldehyde may be important to an understanding of the long-term effects of radiation damage as well as the safety of irradiated foods. Since malonaldehyde is also a product of lipid oxidation, it may be of general importance to such problems as aging and carcinogenesis.

1969

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Millwright - F. W. Reynolds

Pipefitter - A. L. Moses

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BAIR, W. J. "Lectures 1 through 8 on Plutonium Inhalation Studies." Technical Aid Program sponsored by Japan Atomic Energy Commission, Japan, February 18 to March 3, 1969.

BAIR, W. J. "Inhalation of Radionuclides and Carcinogenesis." Conference on Inhalation Carcinogenesis, Gatlinburg, Tennessee, October 9-10, 1969.

BAXTER, D. W. and L. K. KNOEBEL. "A Relationship Between Blood Flow and Galactose Absorption in the Dog Jejunum." Federation of American Societies for Experimental Biology, Atlantic City, N.J., April 13-18, 1969.

CRAIG, D. K. "The Effect of Iodine Concentration on the Efficiency of Activated Charcoal Adsorbers." Annual Meeting of Health Physics Society, Pittsburgh, Pennsylvania, June 8-12, 1969.

DIONNE, P. J. and C. L. SANDERS. "A Simulated Model for Determining the Distribution of Alpha-Energy in Alveoli from Inhaled Plutonium Particles." Northwest Simulation Council Meeting, Portland, Oregon, January 24, 1969.

DRUCKER, H., S. BORCHERS, and J. T. YANG. "The Role of Calcium in the Protease Thermolysin." Annual meeting of the Northwest Branch of the American Society of Microbiology, Portland, Oregon, October 3-4, 1969.

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FRAZIER, M. E. and R. N. USHIJIMA. "Isolation of Adenovirus from Sera and Cell Cultures of Leukemic Miniature Swine." Northwest Regional Meeting of the Society of Experimental Biology and Medicine, Portland, Oregon, November 15, 1969.

FRAZIER, M. E., R. N. USHIJIMA, and E. B. HOWARD. "Virus Association with ^{90}Sr-Induced Leukemia of Miniature Swine." Fourth International Symposium on Comparative Leukemia Research, Cherry Hill, New Jersey, September 21-25, 1969.

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HACKETT, PATRICIA L. and GLENDA S. VOGT. "Cellular Alkaline Phosphatase Studies in Miniature Swine Fed ^{90}Sr." Northwest Regional Meeting of the Society of Experimental Biology and Medicine, Portland, Oregon, November 15, 1969.

HOWARD, E. B. "Comparative Pathology of Induced Lung Tumors of Dogs." Pulmonary Cytology Task Force Meeting, Holy Cross Hospital, Salt Lake City, Utah, March 27-28, 1969.

HOWARD, E. B. "Experimental Induction of Porcine Leukemia." Fourth International Symposium on Comparative Leukemia Research, Cherry Hill, New Jersey, September 21-25, 1969.

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HOWARD, E. B. "Histopathology of Induced Pulmonary Neoplasms of Beagle Dogs." Conference of Research Workers in Animal Diseases, Chicago, Illinois, December 1-2, 1969.

HUNGATE, F. P. "Genetic Futures in Plant Breeding." Meeting of the Plant Propagator's Society, Olympia, Washington, September 3, 1969.

MAHONY, T.D. and M. F. SULLIVAN. "The Prevention of Atherosclerosis in Rabbits by Cholecystoileostomy." Second International Symposium on Atherosclerosis, Chicago, Illinois, November 2-4, 1969.

MAHLUM, D. D. and J. D. BERLIN. "Ultrastructural Alterations Associated with Neptunium Induced Fatty Livers." Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 13-18, 1969.

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SANDERS, C. L. "Factors Influencing the Removal of Inhaled $^{239}PuO_2$ Particles by Pulmonary Washing." Society of Toxicology Meeting, Williamsburg, Virginia, March 9-12, 1969.

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