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PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1968
TO THE USAEC
DIVISION OF BIOLOGY AND MEDICINE
VOLUME I LIFE SCIENCES
PART 1 BIOLOGICAL SCIENCES

January 1970

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Part 1

UC-48, Biology and Medicine

PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1968
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
Marguerite S. Stack
Evelyn G. Swezea

January 1970

BATTELLE MEMORIAL INSTITUTE
PACIFIC NORTHWEST LABORATORIES
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This is the 18th 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), and BNWL-714 and 715 (1967).

Part 2 of this volume, BNWL-1050-2, covers work in ecology and earth sciences. Volume II is issued in three parts: (1) Instrumentation, BNWL-1051-1; (2) Radiological Sciences, BNWL-1051-2; and (3) Atmospheric Sciences, BNWL-1051-3.

THIS REPORT IS RESPECTFULLY DEDICATED TO
HARRY A. KORNBERG, MANAGER
OF THE BIOLOGY DEPARTMENT FROM 1948 TO 1968

FOREWORD

This eighteenth in a continuing series of Biology Department Annual Reports follows the general format of the past several years. Each major section of the report is introduced by a few paragraphs reviewing the contents of that section and highlighting what might be considered major accomplishments. Following each of these introductions is a list of 1968 publications in that area of research. A complete list of Biology Department 1968 publications and technical presentations appears at the end of the report.

The year 1968 saw extensive reorganization of the Biology Department. In February, major portions of the Ecology Section and of the Plant Physiology and Agriculture Section were separated from the Biology Department and eventually constituted as a separate Ecosystems Department. In March the Comparative Toxicology Section was combined with the Pathology Section.

As part of a laboratory-wide reorganization in July, the Biology Department became part of the Environmental and Life Sciences Division of Battelle-Northwest. In this move Biology's Technical Services Section became a part of the Radiological Sciences Department and the Biology Administration Section was abolished. A new section was established in the Biology Department, titled Nutrition and Food Technology, managed by D. B. Menzel.

The most significant event of the July reorganization was the transfer of Dr. Harry Kornberg from his position as Manager of the Biology Department to a new post as Associate Manager of the Environmental and Life Sciences Division. Dr. Kornberg had managed the Biology Department since its inception in 1948, and his departure could only be looked upon as the end of an era. Whatever prominence the Biology Department has achieved in scientific circles must be credited, in large measure, to the guidance of its "first manager." The dedication of this Annual Report to him is intended to express the appreciation of all of us who benefitted from this guidance.

W. J. Bair became manager of the Biology Department in July, and his former position as manager of the Inhalation Toxicology Section was assumed by J. F. Park. These numerous changes of assignments are reflected in the listing of Biology Department staff at the end of this report. Reports in the general area of Ecology, formerly included in the Biology Annual Report, appear this year as a separate Part 2 of this volume.

A highlight of the year was the Symposium on Myeloproliferative Disorders of Animals and Man, held in Richland, May 20-23; jointly sponsored by the AEC Division of Biology and Medicine and Battelle-Northwest. Scheduled for May 5-8, 1969 is the Ninth Annual Hanford Biology Symposium on Radiation Biology of the Fetal and Juvenile Mammal.

Many years of planning and frustration culminated in November of 1968 with the start of construction of the new Biology laboratory. This five-million dollar project, located on the Columbia River just south of the 300 Area, and within a few miles of Richland, should be ready for occupancy in mid-1970. In this period of sharply curtailed construction activity, we particularly appreciate the confidence shown in us by this commitment of support by the AEC Division of Biology and Medicine.

W. J. Bair, Manager
Biology Department

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RADIATION EFFECTS STUDIES

Two reports in this section are concerned with water and electrolyte balance in the gastrointestinal radiation syndrome in rats. Two additional reports are concerned with the gastrointestinal syndrome in species other than the rat. Of particular interest are the observations on the miniature swine which differed significantly from those on rats and which may better simulate the human case.

Experience with bile-diversion techniques in the study of gastrointestinal radiation effects prompted an exploratory study of the effects of bile diversion on the development of atheromatous plaques in the aorta of rabbits. The effect of a high cholesterol diet was dramatically reversed by application of these techniques.

A single report describes the initiation of a long-term experiment in miniature swine, designed to study the effect of dose and size of area irradiated on the response of the skin to beta radiation. Early observations of acute effects emphasize the importance of size of irradiated area.

Two reports describe preliminary studies of effects on mice of electrostatic charging and electrostatic fields. Although there was indication of some effect of a negative charge on the hemogram, no evidence of a protective effect was seen in whole-body irradiated mice.

PUBLICATIONS

Berlin, J. D. "The Ultrastructural Localization of Acid Mucopolysaccharides in the Intestine After Irradiation," Radiation Res., vol. 34, pp. 347-356. 1968.

Erdman, H. E. "Ontogeny and Comparative Radiation Sensitivity in the Confused Flour Beetle and Strains of the Red Flour Beetle," J. Econ. Entomol., vol. 61, pp. 123-125. 1968.

Mahony, T. D., J. D. Berlin, J. C. Pekas, and M. F. Sullivan. "The Effect of Bile on the Mucosa of the Small Intestine," In: M. F. Sullivan (ed.) Gastrointestinal Radiation Injury, pp. 42-56. Excerpta Medica Foundation, 1968.

McKenney, J. R. "Electrolyte Transport and Voltage Measurements in a Preparation of the Irradiated Rat

Ileum," In: M. F. Sullivan (ed.) Gastrointestinal Radiation Injury, pp. 206-215. Excerpta Medica Foundation, 1968.

Sullivan, M. F. "Sodium, Water and B₁₂ Absorption in Irradiated Rats: Influence of Bile," In: M. F. Sullivan (ed.) Gastrointestinal Radiation Injury, pp. 216-227. Excerpta Medica Foundation, 1968.

Sullivan, M. F. "Intestinal Radiation Death in Rats Fed a Sodium Deficient Diet," Radiation Res., vol. 35, p. 561. 1968. (Abstr.)

Wachholz, B. W. and T. D. Mahony. "The Influence of Pancreatic Fluid on the Morphological Response of the Intestine to X-Irradiation," Radiation Res., vol. 35, p. 561. 1968. (Abstr.)

SODIUM LOSS IN THE IRRADIATED RAT

A substantial sodium loss was demonstrated in irradiated rats by measurements of whole-body retention and excretion of ^{22}Na . The quantity of ^{22}Na retained is more affected by sodium intake than by radiation.

Investigator:

M. F. Sullivan

Technical Assistance:

Alma L. Crosby

Although it is generally accepted that sodium loss is a major, if not critical, event leading to death following exposure to radiation doses between 1000 and 10,000 R, experiments monitoring whole-body retention of radioactive sodium have led to conflicting interpretations. If sodium loss is, indeed, the primary cause of death, then a substantial alteration of sodium intake might be expected to alter the gastrointestinal radiation syndrome.

Ninety were maintained for 30 days on either a high or low sodium intake by adding sodium (2%) to the drinking water; or by feeding a sodium-deficient diet* containing Katonium,** a cation exchange resin which binds sodium during passage through the alimentary tract. Fifty animals, including controls maintained on a stock diet, were then injected intravenously with ^{22}Na and bled 24 hr later to determine their extracellular sodium space.

Observations

One hundred and sixty Charles River rats were used in these

*Nutritional Biochemical Corporation, Cleveland, Ohio

**Bayer Chemical Company, Surbiton on Thames, England

Some of the rats injected with ^{22}Na and other noninjected rats, with and without dietary restriction, were then exposed to sublethal (1000 R) or lethal doses ranging between 1200 and 1600 R of X-radiation. Only their lower body, distal to the xiphoid process, was irradiated because it has been shown that the effect on sodium loss is similar to that seen after whole-body exposure, and survival is prolonged (5 to 7 days) to permit extended measurements of sodium loss. The mean survival times were: 5.6 days/51 deaths on the low sodium diet, 5.3 days/45

deaths on the stock diet and 5.1 days/17 deaths on the high sodium intake. Sodium elimination was measured both by determining ^{22}Na in the excreta and by measuring that which was retained in the body at intervals after exposure.

Although there was no significant difference in the extracellular sodium space of rats maintained for 30 days on a low sodium diet, a stock diet, or a high sodium diet, and no difference in mortality, the influence of dietary sodium on excretion of injected ^{22}Na was clearly apparent, as shown in Figure 1. The major

FIGURE 1. Cumulative Excretion of ^{22}Na by Irradiated Rats Maintained on Various Levels of Dietary Sodium

fraction of labeled sodium was excreted in the urine of rats on a stock diet, or on a high sodium intake. Substantial excretion occurred even before irradiation. By contrast, animals on a restricted sodium intake lost most of their ^{22}Na in the feces, and appreciable excretion did not occur until the third day after 1300 R, when diarrhea began.

A further indication of the effect of sodium intake on excretion of ^{22}Na from lethally irradiated rats is shown in Figure 2. In this experiment rats were fasted 1 day before ^{22}Na injection and for 7 days thereafter.

Although it is apparent that the excretion of labeled sodium is

markedly influenced by sodium intake, it is also clear that even on a severely restricted sodium intake there is a markedly increased loss of sodium following lethal irradiation doses. This is well shown by the whole-body monitoring data presented in Figure 3. The unirradiated, ^{22}Na -injected controls on a sodium-deficient diet retained essentially all of their ^{22}Na , while there was a dose-dependent loss of ^{22}Na from the irradiated animals. About 8% was lost following a sublethal exposure, 1000 R; over 20% was either excreted or present in the gut lumen at death in animals exposed to 1300 or 1400 R. Others have shown that a loss of 23%

FIGURE 2. Cumulative Excretion of ^{22}Na by Irradiated Rats, Fasted or Maintained on a Stock Diet

FIGURE 3. Whole-Body Retention of ^{22}Na by Sodium-Deficient Irradiated Rats

Conclusions

Although there was no appreciable influence of dietary sodium on mortality or survival time, the present results demonstrate that sodium loss, which is said to be sufficient to cause death, occurs in lethally irradiated rats. This loss can be demonstrated by whole-body counting procedures if nonessential sodium is depleted prior to and during the test period.

of the total-body sodium content is by itself sufficient to cause death in dogs.

WATER AND ELECTROLYTE ABSORPTION AND EXCRETION FROM THE INTESTINE OF SODIUM-DEFICIENT AND/OR IRRADIATED RATS

Sodium and water movement into and out of the small intestine was measured in rats maintained on a low- or high-sodium intake. Radiation reduced insorption (lumen to blood) and caused a net accumulation of sodium in the lumen regardless of the sodium balance of the animal prior to exposure.

Investigator:
M. F. Sullivan

Technical Assistance:
Alma L. Crosby

We have shown that the functions of both absorption and excretion are decreased in the alimentary tract after irradiation and that the net result can be a loss of as much as 20% of extracellular sodium (Annual Reports, 1967, 1968). One might

expect that survival time following lethal intestinal irradiation would be altered in rats depleted of sodium but such was not the case. This suggests that some sodium-conserving mechanism, induced by the deficiency,

may have been operating at the mucosal level.

Observations

Twelve 225-g rats were placed on a low-sodium diet* which contained 2% Katonium,** a cation exchange resin, included to remove sodium during passage through the alimentary tract. Twelve rats on high-sodium intake received a stock diet plus 2% sodium, as NaCl, added to the drinking water. Twelve normal controls were maintained on the stock diet. After 30 days, the animals were exposed to 1250 R lower-body X-radiation (250 KVP). Intestinal function was measured 3 days postirradiation to determine the effect of sodium deprivation or sodium excess on both absorption and excretion as influenced by irradiation.

Measurements of insorption (lumen to blood movement) were made by placing 10 ml of ^{22}Na - and ^3H -labeled phosphate buffer in the small intestine, ligated at the pyloric and ileocecal junctions. The amount not insorbed was removed from the intestine when the rats were killed 30 min later. Exsorption (blood to lumen movement) was measured by injecting 1.0 ml of labeled buffer into the tail vein and measuring the label which appeared in the lumen 30 min later.

The insorption data are shown in Figure 1. The amount of sodium in

the diet did not markedly affect insorption in control animals.

Although sodium insorption was very substantially reduced at 3 days after 1250 R, neither sodium deprivation nor a dietary excess caused a difference in the capacity of the irradiated bowel to transport that cation.

The exsorption data in Figure 2 show that about 3.5 times as much water and sodium, both ^{22}Na and ^{23}Na , were present after radiation as were found in the lumen of control rats maintained on a stock diet. Only in the intestine of irradiated animals on a low-sodium intake was there a substantially lower accumulation of sodium and water, and even in these animals the accumulation exceeded that in low-sodium unirradiated controls.

Conclusions

These results indicate that sodium and water insorption are reduced in irradiated rats (1250 R) to less than one-half their normal level, and that this effect is sufficient to cause accumulation of water and electrolytes in the lumen, despite changes in sodium balance incurred by dietary alterations. The decreased accumulation of electrolytes in the intestine of irradiated rats on a low-sodium diet may help to explain why the restricted sodium intake did not decrease the survival time of rats dying from intestinal radiation injury.

*Nutritional Biochemicals Corporation, Cleveland, Ohio

**Permutit Co. Ltd., London

FIGURE 1. Effect of Irradiation and Sodium Intake on Water and Sodium Insorption from the Small Intestine of Rats (Number of animals in parentheses. Range indicates standard error of the mean.)

FIGURE 2. Effect of Irradiation and Sodium Intake on Water and Sodium Exsorption into the Small Intestine of Rats (Number of animals in parentheses. Range indicates standard error of the mean.)

INTESTINAL RADIATION INJURY IN MINIATURE SWINE

Acute deaths were seen in miniature swine that received more than 400 R whole-body exposure or more than 2700 R lower-body exposure to ^{60}Co gamma radiation. Preliminary histopathologic observations suggest that the ileum was usually the most radiosensitive area of the intestinal tract followed in varying degrees by the cecum, colon, and jejunum, with the duodenum being least radiosensitive. This order is in contrast to that usually reported for other laboratory species.

Investigators:

B. W. Wachholz
T. D. Mahony*
M. F. Sullivan

Technical Assistance:

S. A. Hughes, Jr.

Although gastrointestinal radiation injury has been comprehensively studied in the rodent, the variety of responses seen in other small animals suggested to us that information from an animal species more representative of man would be desirable. Since the Hanford Miniature Swine is an omnivore, exhibits a prodromal response to radiation, attains a size and weight comparable to man, and has a GI tract more similar to the human than that of the rat, we investigated their response to partial- and whole-body ^{60}Co gamma irradiation.

Observations

Mature miniature swine weighing about 75-80 kg were exposed bilaterally to gamma radiation from a ^{60}Co source. Midline doses varied from 250-3500 R for whole-body exposures and from 1150-3500 R for lower-body exposures. Lower-body exposure included everything distal to a point

about 4 cm proximal to the xiphoid process. Animals given total-body exposure doses of 400-3500 R died between 5 and 17 days, the survival time being inversely related to the dose. Animals given lower-body exposure doses of 1150-2750 R were sacrificed at daily intervals from 2-10 days postexposure, or were permitted to survive the immediate post-exposure period; these surviving animals died, or were killed in a moribund condition, between 30 and 135 days postexposure.

In addition to mortality data, results are being accumulated on hematology, blood chemistry, and histopathology. Clinical observations indicated little variation between whole- and lower-body irradiated pigs either in the time of appearance and the frequency of vomiting or in the appearance time and severity of a diarrheal response.

Histopathologic observations on these animals indicate that damage to the small intestine becomes progressively more severe as one proceeds from the duodenum to the

*Consultant Pathologist, Medical
Arts Building, Richland, Washington

ileum, which contrasts with previous interpretations drawn mainly from X-irradiated rodents. This effect appears to be relatively consistent regardless of the time of sacrifice after exposure. That this is not an effect of shielding or of a non-uniform exposure is evidenced by the similarity of response observed in a limited number of pigs which were exposed to radiation with only the head and the neck shielded. A representative picture of the gross pathology in the intestines of these animals is shown in Figure 1.

Histological examination of tissues taken from all areas of the intestinal tract confirms the gross observations, with radiation injury extending from a mild to moderate involvement in the duodenum to severe injury, usually in the ileum, which at times progressed to ulceration (Figures 2-5). In two animals which received 2500 R lower-body exposure, radiation ulcers per-

sisted in the mucosa of the ileum or the rectum for as long as 85 and 101 days postexposure. The degree of contraction due to fibrosis and other changes in the supportive tissue of the submucosa had not reached the point of causing an obstructive stricture in these animals.

Conclusions

Exposure of the gastrointestinal tract of swine to high doses of gamma radiation from ^{60}Co suggested that the ileum is the most severely affected portion of the intestine, closely followed by the cecum, colon and jejunum, with the duodenum being somewhat less involved. These observations indicate that the pig may provide a good model for the late effects of radiation in the human small intestine, where obstruction and ulceration occur most frequently in the ileum.

FIGURE 1. Gross Intestinal Segments of a Miniature Pig Sacrificed 6 Days After 2500 R ^{60}Co Exposure (The head and the neck of the animal were shielded.)

FIGURE 2. Section from the Duodenum of a Miniature Pig 6 Days After 2500 R ^{60}Co Partial-body Exposure

FIGURE 4. Section from the Ileum of a Miniature Pig 6 Days After 2500 R ^{60}Co Partial-body Exposure

FIGURE 3. Section from the Jejunum of a Miniature Pig 6 Days After 2500 R ^{60}Co Partial-body Exposure

FIGURE 5. Section from the Proximal Colon of a Miniature Pig 6 Days After 2500 R ^{60}Co Partial-body Exposure

INTERSPECIES COMPARISON OF GASTROINTESTINAL RADIATION EFFECTS

The gastrointestinal radiation syndrome followed a similar time course and dose response in the rat, guinea pig, and miniature swine. The onset of injury in the bowel, and of regeneration, was delayed in the rabbit.

Investigators:

M. F. Sullivan
T. D. Mahony*
B. W. Wachholz

Technical Assistance:

Alma L. Crosby
S. A. Hughes, Jr.

Although intestinal radiation injury has been followed in several species, few comparative studies have been made which might explain the differing symptomatology and survival time. In fact, it has been questioned whether a typical intestinal radiation syndrome even occurs in species such as the rabbit.

Observations

Rats, rabbits, and guinea pigs were anesthetized with pentobarbital sodium before irradiation; miniature swine were exposed unanesthetized in a restraining cage. That portion of the body proximal to the xiphoid process was shielded by 42 cm of lead while the lower body received 2500 R from a ^{60}Co source. Rats, guinea pigs, and rabbits were killed at 3, 6, 9, or 15 days while pigs were killed at 2, 3, 4, 6, 8, and 10 days for histopathologic study of the gastrointestinal tract. Specimens were taken from the stomach, duodenum, jejunum, ileum, cecum, appendix, and

colon. Comparisons in this report will be limited to the ileum and the colon, which are representative of the small and large intestine and free of any complications due to inadvertent shielding which might have occurred in more proximal segments.

Preliminary X-ray studies indicated that a lower body dose of about 2500 R was necessary to cause a typical intestinal syndrome in rabbits and guinea pigs. Lower body doses less than 2500 R were sublethal for swine. An overall evaluation of the severity of the injury response in the small and large intestine, as a function of time following 2500 R, is shown in Figures 1 and 2. Although diarrhea was a consistent symptom of intestinal radiation injury in rats between days 3 and 6, it did not occur in rabbits or in guinea pigs until 6 days. It usually continued, however, until the animals died or were killed for sectioning at 9 days. Diarrhea persisted in rabbits through the last observation point, 15 days after exposure. It was not, however, a consistent symptom in either of these species at any time. Swine

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FIGURE 1. Radiation Injury to the Ileum of Rats, Guinea Pigs, Rabbits, and Miniature Swine Exposed to 2500 R of Lower-Body Gamma Radiation

FIGURE 2. Radiation Injury to the Colon of Rats, Guinea Pigs, Rabbits, and Miniature Swine Exposed to 2500 R of Lower-Body Gamma Radiation

occasionally developed diarrhea by 3 days, but it was more often seen at 4 or 5 days and continued as long as 9 days postexposure.

Histopathologic observations were made on the intestines from three rats at 3 and 6 days postexposure and were found to be similar to those previously made after 2500 R of X rays. The relationship between small and large bowel damage was consistent with numerous observations previously made at lower X-ray doses. Only one rat survived until 15 days and recovery was almost complete at that time. Eighteen guinea pigs were exposed to 2500 R lower-body irradiation but only one survived until 9 days postexposure. Although regeneration was progressing in the small bowel at that time, the colon was still severely damaged. Observations were made on 29 miniature swine killed between 2 and 10 days postexposure. Damage was more extensive in the ileum at 6 days than it was in either the rat or guinea pig but the colon was less severely injured. Recovery was much slower in the colon than in the small bowel. At least three rabbits exposed to 2500 R were killed at 3, 6, 9, or 15 days postexposure. Although damage to the rabbit intestine was not appreciably more severe than for other species, the recovery process appeared to be substantially slower.

At 15 days, histologic damage was almost as severe as at the 6- to 9-day period of peak response.

Conclusions

The appearance of diarrhea in lower-body irradiated rats, guinea pigs, rabbits, and miniature swine followed the course of mucosal

pathology. Damage to both the small and large bowel reached a maximum severity at about 6 days post-exposure, except in the rabbit large bowel where it was delayed until 9 days. Recovery occurred most rapidly in the rat and most slowly in the rabbit. Differences in the response may be related to mucosal cell kinetics rather than to anatomical differences.

THE PREVENTION OF ATHEROSCLEROSIS IN RABBITS BY CHOLECYSTOILEOSTOMY

Diverting bile from the small intestine reduced the absorption of cholesterol and the subsequent development of atherosclerosis in rabbits maintained on a cholesterol-enriched (1%) diet.

Investigators:

T. D. Mahony*
M. F. Sullivan

Technical Assistance:

Alma L. Crosby

In many earlier studies we have demonstrated the importance of bile as a factor influencing the intestinal radiation syndrome. The methods developed in these earlier studies were applied in the present research to quantitate a well-known action of bile, the facilitation of cholesterol absorption. In addition to its influence on absorption, bile is also involved in cholesterol metabolism, because that steroid is the main pre-

cursor for de novo bile salt production. Results presented in this report demonstrate the influence of bile on cholesterol absorption and on the subsequent development of atherosclerosis.

Observations

Cholecystoileostomy was accomplished in male, albino rabbits weighing about 3 kg by anastomosing

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the gall bladder to the terminal ileum at a point 5 cm proximal to the ileocecal junction. About 10 days following surgery, both operated and control animals were placed on a 1% cholesterol diet. At various times after the initiation of the high cholesterol diet, plasma cholesterol levels were determined by removing blood from the ear vein. Results of these determinations are shown in Figure 1. It is apparent that bile diversion markedly influences plasma cholesterol levels, whether this diversion occurs prior to initiation of the high cholesterol diet or at some time thereafter.

FIGURE 1. Total Plasma Cholesterol Values of Rabbits Maintained on a 1% Cholesterol-Enriched Diet (Arrows indicate time of cholecystoileostomy. The number of animals contributing to the data points are shown in parentheses. Ranges indicate the standard error of the mean.)

The influence of a high cholesterol diet, or biliary fistula, on cholesterol absorption was further studied by intragastric administration of 5 μ Ci 14 C-cholesterol in 2 cm³ of corn oil. Data shown in Figure 2 indicate a 15-fold enhancement of labeled cholesterol absorption in rabbits on the cholesterol-enriched diet. Rabbits on the same cholesterol-enriched diet, but with bile diverted from the small intestine, absorbed about 150-fold less than the nonoperated rabbits, and 10-fold less than normal rabbits on a stock diet. It is not clear from these data whether bile diversion is effective only in reducing the absorption

FIGURE 2. Plasma Cholesterol- 14 C Radioactivity of Rabbits at Intervals After Intragastric Administration (The number of animals contributing to the data points are shown in parentheses. Ranges indicate the standard error of the mean.)

of cholesterol, or whether it may also indirectly affect plasma levels by increasing the use of cholesterol in bile salt synthesis.

The diversion of bile had a dramatic effect on the subsequent deposition of atheromatous plaques in the

aorta of rabbits on a high cholesterol diet, as shown in Figure 3. This effect was further demonstrated by total cholesterol determinations on the aortas from these animals as shown in Figure 4.

FIGURE 3. Aortas from Rabbits Subjected to the Following Treatments: [A. cholecystoileostomy, followed by high cholesterol diet for 180 days (minimal plaque formation); B. high cholesterol diet for 154 days, cholecystoileostomy 14 days before sacrifice (reduced plaque formation compared to C and D); C and D. high cholesterol diet controls (extensive plaque formation); E. stock diet control (minimal plaque formation).]

FIGURE 4. Cholesterol Content of the Aorta of Rabbits Maintained on a High Cholesterol Diet (Each point represents the concentration of cholesterol in one rabbit aorta.)

Conclusions

The absorption of cholesterol from the intestine of rabbits maintained on a high cholesterol diet was markedly decreased by cholecystoileostomy. A further observation of possible clinical importance was the absence of atherosclerosis in operated rabbits maintained on a cholesterol-enriched diet for 3 months.

BETA IRRADIATION OF SWINE SKIN: AN EXPERIMENTAL DESIGN

An experiment was initiated to systematically investigate the effect of dose and size of area irradiated on the response of swine skin to β -radiation. Preliminary results indicate that both factors are important in the acute response.

Investigators:

D. D. Mahlum

R. L. Amster

Technical Assistance:

D. L. Catt

This progress report describes an experiment designed to investigate the relationship between radiation dose and size of area irradiated in producing skin lesions in the Hanford Miniature Swine. Only very preliminary observations of acute effects are as yet available. These studies will be expanded to include rodents, as well as other swine, and will extend for the lifetime of the animals.

Observations

Circular sources were constructed by plating ^{204}Tl on plastic discs having an area of 1.0 mm², 0.5, 1.0, or 5.0 cm². Sufficient ^{204}Tl was deposited to provide dose rates from each source size of 500, 1250, 3125, 7750, or 20,000 rad/hr. Different sized sources were attached to a rectangular

piece of flexible plastic as illustrated in Figure 1. Five such plaques were constructed--one for sources of each dose rate.

FIGURE 1. Arrangement of ^{204}Tl Sources on Exposure Plaque

Hanford miniature barrows were anesthetized with Surital and Nembutal and the hair clipped along each side. The plaques were applied tightly to the skin in dorsal-ventral direction. The order and orientation of the plaques were randomly determined for each side of each pig. Exposures were for 2 hr. Exposed areas were photographed, in color, under carefully controlled conditions, at intervals following irra-

diation. Each photograph included a gray and a color scale to permit comparisons of irradiated areas over an extended period.

Gross changes have not been observed at the 1 mm^2 area of swine skin with exposures up to the maximum of 40,000 rad. Erythema, necrosis, and sloughing are consistently found in 0.5 , 1.0 , and 5.0 cm^2 areas receiving 15,500 or 40,000 rad. Most damaged areas had healed by 60 days post-exposure although they continue to exhibit a slight whitish appearance. Erythema, but not necrosis, is occasionally found in the 5.0 cm^2 area after 6250 rad; the smaller areas showed no response at this dose.

Conclusions

The early response of swine skin to β -radiation from ^{204}Tl sources is dependent on both the dose and the size of the exposed area. Animals will be retained for lifetime observation of tumor response, which will be correlated with the observations of acute response.

EFFECT OF ELECTROSTATIC CHARGING AND ELECTROSTATIC
FIELDS ON SURVIVAL TIMES OF X-IRRADIATED MICE

Young, male, albino mice received a single, whole-body X-ray exposure (700-825 R, 250 kV) while being kept for the duration of the experiment on a metal plate maintained at either +2 kV or -2 kV. No significant effect of electrostatic charges and electrostatic fields on the survival times of the animals was observed.

Investigator:
A. P. Wehner
Technical Assistance:
W. W. Roberts

Animals exposed to a certain dose range of ionizing radiation succumb to hematopoietic death. Since several investigators have reported effects of electroaerosols, electrostatic charging (ESC), and electrostatic fields (ESF) on the hematopoietic system, an investigation was conducted to determine whether ESC and ESF may offer protection against radiation damage in experimental animals.

Observations

An "electrostatic cage" was built, consisting of a 40 × 40 cm perforated aluminum floor and a lucite superstructure, which provided four 20 × 20 × 10 cm cubicles. Each cubicle could house up to six mice. The plate was maintained at +2 kV or -2 kV. A grounded 40 × 40 cm aluminum plate was positioned 23 cm above the floor plate, resulting in an ESF of 87 V/cm. An identical, but uncharged cage, housed the controls. Three experiments were conducted, one with a potential of +2 kV and two with -2 kV at the floor plate. Each experiment involved forty-four 6- to 9-week-old

male albino mice (Charles River CD₁). Fourteen mice were irradiated and "charged," fourteen were irradiated but not charged, eight nonirradiated controls were charged, and there were eight nonirradiated, noncharged controls. Irradiated animals received a whole-body exposure of 725-800 R X-ray at a dose rate of 40 R/min. The experimental animals were kept on the charged plate from time of exposure to the conclusion of the 30-day observation period.

Most of the irradiated animals died between 6 and 28 days post-exposure with symptoms typical of hematopoietic radiation death. Statistical evaluation showed no significant effect of ESC and ESF, of either polarity, on the survival times of the X-irradiated mice. Results are shown in Table I.

Conclusions

Under the described experimental conditions, ESC and ESF had no statistically significant effect on the survival times of irradiated mice. It is conceivable that variation of

radiation dose and ESC might lead to demonstrable effects. It seems more likely, however, that the insult from

a lethal radiation dose would overwhelm any subtle effect which ESC and ESF might exert.

TABLE I. Survival Times of Irradiated Mice Maintained on a Charged Plate

	Survival Time, days	
	Mean	95% Confidence Limits
Expt. 1 825 R		
Charged (-2 kV)	17	13-23
Uncharged controls	18	14-22
Expt. 2 700 R		
Charged (-2 kV)	10	8-13
Uncharged controls	13	11-15
Expt. 3 780 R		
Charged (+2 kV)	13	10-16
Uncharged controls	14	12-17

EFFECT OF ELECTROSTATIC CHARGING AND ELECTROSTATIC
FIELDS ON THE HEMOGRAM OF THE MOUSE

Mice were kept on a metal plate maintained at either +2 kV or -2 kV. The negatively charged mice developed significantly higher lymphocyte counts and significantly lower hematocrit values than their controls.

Investigator:

A. P. Wehner

Technical Assistance:

W. W. Roberts

Several reports in the literature and spot checks during previous experiments suggested that electrostatic charging (ESC) and/or electrostatic fields (ESF) affect the hematopoietic system. Controlled stimulation or suppression of specific parts of the hematopoietic system--particularly by means other than medication--may offer interesting prophylactic and/or therapeutic possibilities.

Observations

An "electrostatic cage," described elsewhere in this Annual Report, housed the experimental animals. In the first experiment, eight 9-week-old, male, albino mice (Charles River CD₁) were kept for 28 days in the cage maintained at -2 kV; in the second experiment, six 6-week-old mice were kept for 38 days in the cage maintained at +2 kV. Control animals were simultaneously maintained in an identical uncharged cage. At the end of each experiment, blood samples were obtained from the ophthalmic venous plexus for hematologic evaluation.

Statistical evaluation of the hematological data from experiment 1

is summarized in Table I. The mean lymphocyte count for the charged mice was significantly higher than controls at the 0.05 level of significance. The increased lymphocyte count was reflected in an elevated mean white blood count. The mean hematocrit value for the charged mice was significantly lower than controls at the 0.05 level. The data also suggest decreased hemoglobin levels in the experimental animals. Serum protein determination by zone electrophoresis on cellulose-acetate medium and albumin-globulin quotients did not show significant differences between experimental and control groups.

Positive ESC and ESF in experiment 2 had no apparent effect on the hemogram.

Conclusions

Results of this exploratory investigation suggest that ESC and ESF may interact with the function of the hematopoietic system. Further and more detailed studies with a larger number of animals are necessary to confirm these preliminary findings

and to define type, magnitude, and mode of action of these effects as well as their dependence on experi-

mental variables such as duration of ESF exposure, ESF strength, and species of animals.

TABLE I. Hematology of Mice Kept for 28 Days on a Charged Plate, -2 kV (Mean values $\pm$ standard error)

	<u>Eight Charged Mice</u>	<u>Seven Controls</u>
Hematocrit (%)	39 $\pm$ 1.9 ^(a)	44.1 $\pm$ 1.2
Hb (g/100 ml)	13.8 $\pm$ 0.5 ^(b)	14.9 $\pm$ 0.3
RBC ($10^6/\text{mm}^3$)	8.20 $\pm$ 0.28	8.63 $\pm$ 0.18
WBC ($10^3/\text{mm}^3$)	7.46 $\pm$ 0.57 ^(b)	5.90 $\pm$ 0.49
Segm. neutr. ($10^3/\text{mm}^3$)	1.10 $\pm$ 0.11	1.34 $\pm$ 0.10
Lymphocytes ($10^3/\text{mm}^3$)	6.07 $\pm$ 0.55 ^(a)	4.21 $\pm$ 0.51
Monocytes ($10^3/\text{mm}^3$)	0.16 $\pm$ 0.04	0.16 $\pm$ 0.02
Eosinophils ($10^3/\text{mm}^3$)	0.13 $\pm$ 0.03	0.18 $\pm$ 0.03

(a) Difference from controls significant at 0.05 level.

(b) Difference from controls significant at 0.10 level.

STRONTIUM-90 STUDIES IN MINIATURE SWINE

Twelve reports in this section are concerned with various aspects of the continuing study of the biological effects of daily ^{90}Sr ingestion by miniature swine in progress in this laboratory since 1958. Approximately 770 Pitman-Moore miniature swine, spanning three generations, have been fed daily doses of this radioisotope ranging from 1-3100 $\mu\text{Ci/day}$, while 200 of their untreated littermates have served as controls. Currently, 150 experimental and 97 control swine are being maintained for lifetime observation and study.

Thus far, 69 experimental and two control swine have developed myelolymphoproliferative disorders. The two cases observed in controls were found in 8- to 9-year-old animals diagnosed as having myelogenous leukemia. In addition, seven of the animals with hematopoietic disorders also exhibited bone tumors. The cumulative skeletal radiation doses in these swine ranged from 20-20,000 rad for those with myelolymphoproliferative disorders, and from 9,000-15,000 rad for those showing bone tumors. The results of this chronic feeding experiment contrast with findings reported by other laboratories of a preponderance of bone neoplasms in experimental animals following single or multiple injections of ^{90}Sr at more mature ages.

With the appearance of hematopoietic neoplasia in control swine it no longer seems necessary, as suggested in last year's Annual Report, to establish feeding levels lower than 1 $\mu\text{Ci/day}$. The major future effort will be directed toward defining the more subtle effects and mechanisms leading to the production of neoplasia in these animals. This change in emphasis is reflected by the number of succeeding reports dealing with the more fundamental aspects of these disease states.

The 1968 Hanford Biology Symposium on Myeloproliferative Disorders of Animals and Man featured results from this program. The symposium was held May 20-23, in Richland, and was cosponsored by the U.S. Atomic Energy Commission and Battelle Memorial Institute. Two hundred scientists representing nine countries participated, with 42 papers presented. Proceedings of the symposium should soon appear as a publication of the AEC Division of Technical Information Extension.

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DOSIMETRY ASPECTS OF CHRONICALLY FED ^{90}Sr SWINE

Measurements of ^{90}Sr content and bone ash weights were utilized in calculating cumulative skeletal dose to animals of the chronic oral ^{90}Sr toxicity study. Differences in ^{90}Sr metabolism among offspring on different levels of daily feeding are noted.

Investigators:
R. F. Palmer
J. M. Thomas*
C. R. Watson**

Previous progress reports have described the experimental design of the chronic oral ^{90}Sr toxicity study in miniature swine and several aspects of uptake and retention of ^{90}Sr in these animals on various radioisotope feeding levels (Annual Report, 1959, et seq.). Knowledge of the ionizing radiation dose delivered to the animals on these studies is essential for proper interpretation of the

observed effects. This report describes differences in ^{90}Sr metabolism observed among the parent and offspring animals of the 1, 5, 25, and 125 $\mu\text{Ci/day}$ levels and the methods used to estimate their cumulative skeletal dose.

Observations

Values of ^{90}Sr skeletal burden and total skeletal ash weight obtained at death from offspring animals were utilized to construct Gompertz growth curves describing the accumulation of ^{90}Sr skeletal growth as a function of age (Figure 1). Dividing the ^{90}Sr skeletal burdens by daily ^{90}Sr intake normalized the curves and revealed

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FIGURE 1. Gompertz Growth Curves for ^{90}Sr Body Burden and Skeletal Growth

that the 1, 5, and 25 $\mu\text{Ci}/\text{day}$ animals accumulated ^{90}Sr similarly, but that the 125 $\mu\text{Ci}/\text{day}$ animals absorbed and/or retained a larger fraction of that ingested. Although there was no significant difference between the skeletal growth curve for the 125 μCi animals and that for the animals receiving less ^{90}Sr , separate equations were derived so that subsequent calculations would involve data from the same animals. Insufficient data were available to treat the 625 μCi per day offspring in a similar manner due to early death of these animals.

Division of the equations for total ^{90}Sr by those for skeletal growth resulted in equations giving the concentration of ^{90}Sr per gram of skeletal ash (Figure 2). For the parent generation, after normalization for daily ^{90}Sr intake which started at approximately 9 months of age, no effect of feeding level was shown and concentration data were fitted by the exponential curve shown in Figure 2.

The following equation for cumulative radiation dose to bone was derived by assuming that live bone was

FIGURE 2. Concentration of ^{90}Sr in Bone Ash of Parent and Offspring Animals

40% ash, that ^{90}Sr and ^{90}Y were in equilibrium, and that 87% of the beta particles from the ^{90}Sr and ^{90}Y were absorbed in bone:

$$\text{Dose} = 20 F \int_0^T C \, dT \text{ rad}$$

where: F = $\mu\text{Ci } ^{90}\text{Sr}$ ingested per day

$$C = \frac{\mu\text{Ci } ^{90}\text{Sr/g ash}}{\mu\text{Ci } ^{90}\text{Sr fed/day}}$$

T = age for offspring or time on ^{90}Sr for parents, in days.

Substitution of the equations shown

in Figure 2 for C in the above equation and integration of the resulting expressions gave the values of cumulative skeletal dose shown in Figure 3. Along the lines adjacent

to the curves in Figure 3 are the numbers of live animals per 500-day interval of age, or days on ^{90}Sr , that remain on the various ^{90}Sr regimens.

FIGURE 3. Cumulative Radiation Dose to the Skeletons of Parent and Offspring Animals and Numbers of Live Animals Remaining on Experiment in Various Dose and Age Categories

Conclusions

This data treatment was felt to be the best method for obtaining estimates of the cumulative skeletal radiation dose to the average animal on each of the ^{90}Sr regimens. Statistical analyses indicate about 50% uncertainty at the 95% confidence level in estimation of cumulative dose to an individual 125 μCi per day offspring and about 35% for the other animals studied.

The slight but statistically significant difference in the ^{90}Sr body-burden curves in Figure 1 is attributed to radiation damage. One possible mechanism leading to this

effect could be an impairment of bone remodeling processes in the 125 μCi animals that would diminish resorption of bone laid down early in life when discrimination against ^{90}Sr was at a minimum. The peaks seen in the concentration curves in Figure 2 for offspring animals are a result of ^{90}Sr attaining its equilibrium body burden at a faster rate and earlier in life than did calcium.

These cumulative skeletal radiation dose data, in conjunction with the results of the bone dosimetry study described in a following paper, will allow the establishment of more effective dose-effect relationships for this study.

DOSIMETRY OF STRONTIUM-90 IN SWINE BONES

Thermoluminescence dosimeters in the form of small LiF rods were placed in and on bones obtained at necropsy of swine fed ^{90}Sr throughout life. Dose measurements agreed with radiochemically derived values and indicated a dose to the endosteum greater than that to the periosteum.

Investigators:
C. R. Watson*
R. F. Palmer

Technical Assistance:
Dorothy A. Ricketts*

Previous reports have described radiochemical and autoradiographic studies of radiation dose distribution in bones from miniature swine

that had ingested ^{90}Sr (Annual Reports, 1962, 1963). Recognizing limitations in these methods, we are developing techniques utilizing small thermoluminescence dosimeters to directly measure radiation dose at selected bone surfaces.

*Radiological Sciences Department

Observations

Recently, LiF became available as small, 1×6 mm, extruded rods,* ideally suited for measurement of beta particle dose in confined locations such as marrow cavities. These dosimeters are reusable and interchangeable; in our read-out device, 200 rods had a coefficient of variation of 3.5% following low-level irradiation.

All bones studied to date have been femurs from animals exposed from conception to various ^{90}Sr regimens. Previous work has shown that ^{90}Sr is deposited uniformly in these bones. Femurs were split lengthwise and, after removal of marrow, dried to simplify dosimeter placement. The halves of the bone were held in apposition for several days, while bare rods were exposed on surfaces and within holes drilled in compact bone (Figure 1). Endosteal surface measurements were repeated with the marrow cavity filled with agar. Measured dose rates are compared in Table I with the average dose rate as derived from radiochemical analysis of the contralateral femur.

To more accurately estimate radiation dose rate to bone in live animals, measurements under more physiologic conditions are needed. As this requires use of wet bones the dosimeters must be encapsulated. The rods are heat-sealed in polyethylene tubing which protects them while attenuating the response by only about 15%. Preliminary measurements on a bone preserved in formalin show

good agreement with the relative dose rates in Table I. In all cases the endosteum receives a greater dose than the periosteum; by a factor of 3 in dry bone without marrow, by a factor of 1.8 in dry bone with simulated marrow, and by a factor of 1.7 in formalin-preserved bone with marrow intact.

Conclusions

These measurements are in good agreement with radiochemically derived values. Further refinement and extension of these measurements are planned, utilizing frozen femurs, jaws, and skulls where osteogenic tumors have been observed, and other pathologic bones from swine on various regimens.

FIGURE 1. Upper Portion - Extruded LiF Rod Dosimeters, Bare and Encapsulated; Lower Portion - Dried Femur, Cut Away to Show Bare Rods on Surfaces (Dark "lines" on the bone are sections of wire placed beside dosimeters for photographic visualization.)

*Harshaw Chemical Co

TABLE I. Mean Radiation Dose Rates to Various Portions of a Typical ^{90}Sr -Containing Dried Swine Femur (Pig 1258, fed 125 μCi of ^{90}Sr /day for 1407 days)

<u>Location</u>	<u>Dose Rate, rad/day</u>	<u>Coefficient of Variation, %</u>	<u>Relative Dose Rate, % of average</u>
Average (a)	15.2	10	100
Compact bone, mid diaphysis	13.3	15	88
Periosteal surface			
Proximal end	3.6	17	24
Proximal 1/3 diaphysis	4.6	17	31
Mid diaphysis	4.8	11	32
Distal 1/3 diaphysis	4.7	12	31
Endosteal surface			
Proximal 1/3 diaphysis	14.7	8	97
Mid diaphysis	15.6	2	103
Distal 1/3 diaphysis	14.4	4	95
Endosteal surface with simulated marrow	7.9	10	52

(a) Derived from radiochemical analysis of the contralateral femur.

FETAL DOSIMETRY IN ^{90}Sr -FED SWINE

An experiment was initiated using thermoluminescent dosimeters to measure the dose rates to fetuses of ^{90}Sr -fed swine.

Investigators:
R. F. Palmer
C. R. Watson*
J. L. Beamer

Results from our chronic, oral, ^{90}Sr toxicity study in miniature swine have suggested the possible importance of fetal exposure to the subsequent development of hemato-

poietic neoplasms (Annual Report, 1967). The fetus is irradiated, not only by the ^{90}Sr - ^{90}Y deposited in its tissues, but also by the ^{90}Sr - ^{90}Y in the tissues and in the intestinal contents of the dam. The total dose

*Radiological Sciences Department

rate to the fetus can be measured by implanting thermoluminescent dosimeters at different stages of gestation. This report presents the experimental design of an experiment still in its early phases.

Observations

Five Pitman-Moore gilts were placed on a regimen of 125 μCi ^{90}Sr /day and bred at the first estrus occurring after 120 days of ^{90}Sr feeding. One animal will be sacrificed at approximately 55, 70, 85, 100, and 110 days postbreeding. The uterus will be exposed surgically and one or more dosimeters implanted deep in the cervical region of each fetus. The incision will be closed, the sow frozen, and held for 2-3 weeks' exposure of the dosimeters. The fetuses will then be removed, the dosimeters recovered, and new dosimeters inserted to measure the dose

rate from fetally deposited ^{90}Sr - ^{90}Y . Following recovery of this second batch of dosimeters, the fetuses and sow will be analyzed for ^{90}Sr content.

Conclusions

Data from this experiment will provide a direct measurement of the radiation dose rate in the thymic area of fetuses at various stages of gestation and will distinguish between the two principal sources of exposure. Correlation of these dose measurements, the values for the sows' ^{90}Sr content, and known values for the ^{90}Sr content of the parent animals in our chronic toxicity study will make it possible to estimate, quite accurately, the radiation doses delivered to a potentially radio-sensitive area of the fetuses of sows on the various ^{90}Sr feeding levels.

HISTOPATHOLOGY OF ^{90}Sr -INDUCED HEMATOPOIETIC
DISORDERS IN SWINE

Three ^{90}Sr -fed and two control Pitman-Moore pigs died during the past year with hematopoietic disorders, bringing the current total of cases to 69 in irradiated and two in the control animals. A proposed sequence of events and development of lesions is presented which may help to provide a unified concept for both experimentally induced and spontaneous myeloproliferative disorders.

Investigators:
E. B. Howard
W. J. Clarke

The criteria for the histopathologic categorization of the leukoproliferative diseases induced by chronic feeding of ^{90}Sr to miniature swine were described in detail in the previous Annual Report. At that time, there were 66 pigs tabulated with a spectrum of hematopoietic disorders, ranging from myeloid metaplasia to granulocytic leukemia to lymphocytic leukemia, with hematologic and histopathologic variables in each group. During the past year, three additional experimental animals have died with lesions of either myeloid metaplasia, myelogenous leukemia, or lymphoma. Two control pigs died with lesions of myelogenous leukemia.

This accumulated histopathologic information was presented at a symposium dealing with bone marrow disorders, convened at this Laboratory in May, 1968, where it received critical evaluation and discussion. Following this symposium, the major point remaining unsolved concerned the frustration encountered in attempting to describe and discuss

radiation-induced hematopoietic disorders while being restricted by conventional terminology applicable primarily to spontaneous disease processes. In an attempt to solve this problem, the following unifying concept for experimentally induced and spontaneous myeloproliferative disorders is presented.

Observations

From histopathologic, cytogenetic, hematologic, and clinical data accumulated from this experiment, we propose that there is a sequential and progressive series of events and lesions in miniature swine chronically ingesting ^{90}Sr , which is as follows:

- 1) Damage to the marrow with induced cell death, vascular damage, fibrosis, and accompanying alterations in biochemical and immunologic features.
- 2) Myeloid metaplasia involving liver, spleen, and nodes, with occasional foci elsewhere.

- 3) Uncontrolled tissue proliferation of myeloid elements widespread in the lymphoreticular system as well as other tissues, and occasional overgrowth of the marrow by blast cells. There may be evidence of a trilineage of cell maturation at this time.
- 4) Selectivity of a primary neoplastic cell line, usually myeloblastic, which predominates in the areas of myeloid proliferation, with invasion, infiltration, and tissue destruction. There appears to be loss of control mechanisms at this time.
- 5) Escape of proliferating neoplastic cells into the peripheral blood, with resultant leukemic hemogram. This is merely a late stage of the soft tissue disease process.

Animals may die of superimposed infection or hemorrhagic syndrome at any stage of this disease process, and the resultant pathologic evaluation may be clouded because the hematopoietic disease was terminated before the late stages described above were reached.

Conclusions

The confusion or lack of knowledge surrounding the nature of certain bone marrow disorders has been apparent for some time. It appears that an animal model system, utilizing chronic ^{90}Sr toxicity to the marrow elements, could provide a means of contributing to the understanding of the pathophysiology of the disease processes classified as the myeloproliferative disorders.

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

Changes in peripheral blood values for cellular elements, urea nitrogen, protein fractions, and serum enzymes are reported for two control swine developing myeloproliferative disorders and five ^{90}Sr -fed swine with hematopoietic dyscrasias and bone lesions.

Investigators:

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Technical Assistance:

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Continuing studies in hematology and serum chemistry of ^{90}Sr -fed swine were performed to determine the clinical status of the animal in regard to its age and health as well as to detect early signs of illness leading to terminal events, and to aid the pathologist in making a final diagnosis.

Observations

As the age of the swine on experiment increases, the early detection of hematopoietic disorders becomes more difficult since the clinical picture is frequently complicated by symptoms resulting from a deteriorating physiological status. This is very evident in the summary of diagnoses made during 1968 (Table I).

A total of five hematopoietic disorders were observed: three myeloproliferative, one myeloid metaplasia, and one lymphoproliferative. The myeloproliferative disease detected in the 125 $\mu\text{Ci/day}$ group ran a relatively predictable course, but the two cases of myeloproliferation in the swine receiving

no ^{90}Sr were somewhat unusual. Both were classified as granulocytic, predominantly eosinophilic type; animal 374 maintained eosinophil levels of 10-15% for several years, while an abnormal appearance of immature basophils characterized the peripheral blood picture of animal 489. These cells, however, were not present in the terminal sample. Overt signs of leukemia were manifested by a high percentage of blast forms in animal 1850, but the increased leukocyte value in animal 489 was due mainly to high segmented and band neutrophil values.

The hemogram of animal 73, showing myeloid metaplasia, is similar to the pancytopenia encountered in swine fed 625 $\mu\text{Ci } ^{90}\text{Sr/day}$ except for the appearance of appreciable numbers of immature forms. Immature cells were also observed in animal 694, which had pancreatitis and hepatitis, and may be attributed to an acute inflammatory reaction.

Decreased erythrocyte values in the one case of aleukemic lymphoproliferation (animal 898) were due to blood loss from a uterine leiomyoma.

TABLE I. Summary of Diagnoses and Terminal Hemograms of Pitman-Moore Swine During 1968

⁹⁰ Sr Level, μCi/day	Animal Number and Generation	Age at Death, months	Histopathologic Diagnosis	Peripheral Blood Values			
				Erythrocytes, millions per mm ³	Leukocytes, thousands per mm ³	Platelets, thousands per mm ³	Immature Cells, percent
0	158	114	Uterine tumor	4.5	10	185	3.4
	374	105	Myeloproliferation, cir- rhosis, hepatomas, car- cinoid tumor, endometritis	6.0	11	391	18
	489	103	Myeloproliferation, pitu- itary adenoma, adrenal lymphoma	8.0	86	111	21
	1248	62	Posterior paralysis	6.7	14	-	0.5
1	1037(F ₂)	74	Lymphoid hyperplasia, mandibular granuloma	6.1	9.4	302	1.5
5	898(F ₂)	81	Lymphoproliferation, uterine tumor	4.0	19	100	4.4
25	694(F ₂)	87	Pancreatitis, hepatitis	6.0	8.5	555	22
	1173(F ₂)	73	Myeloid metaplasia, pneumonia, osteonecrosis	1.3	2.7	16	33
125	1850(F ₁)	35	Myeloproliferation	4.4	85	172	47
625 ^(a)	1666(F ₁)	39	Mandibular giant cell tumor	4.6	8.8	187	3.0
Control				6.5 ± 0.2 ^(b)	12 ± 0.5	250 ± 30	0 ± 0.01

(a) Uterine, milk, and 2 months of ⁹⁰Sr feeding only

(b) Mean and 95% confidence limits for 40 adult swine.

The red cell values of this animal were similar to those of animal 158 that had a uterine leiomyofibroma and no hematologic disorder.

Table II summarizes terminal serum chemistry values of the same swine shown in Table I but categorized by diagnosis rather than by ^{90}Sr feeding levels.

Urea nitrogen levels in hematopoietic disorders apparently correlate with the extent of myeloid cellular invasion of the kidney. Of the three myeloproliferative cases, only animal 1850 did not show a high serum urea nitrogen or extensive cellular damage to the renal filtration apparatus. Values in this animal were very low due to prolonged anorexia. An extremely high serum creatinine value occurred with the increased urea level in animal 489.

Albumen-to-globulin ratios continue to be good early indicators of many abnormalities. Albumen values are almost invariably lower and elevations in globulin fractions are most evident in the alpha fraction in hematopoietic disorders and in the gamma fraction in inflammation.

Increased serum lactic dehydrogenase (LDH) has been correlated with the elevation of peripheral blood leukocytes in myeloproliferative leukemia of swine, but LDH activity is increased in all of the hematopoietic diseases observed this year, even if changes in cellular blood values did not occur. This increase may be due to release of LDH from the various organs invaded by the hematopoietic cells, or from the pro-

liferating cells themselves. Resolution of this question will involve a study of specific tissue LDH isoenzymes.

Serum glutamic-pyruvic transaminase (SGPT) assay is regarded as being somewhat specific for liver damage, and the marked increase in animal 694 is directly related to the extensive loss of functional hepatic cells observed in the histologic section. In comparison, changes in SGPT levels were not as evident in animals with hematopoietic disorders because myeloid or lymphoid cellular infiltration did not produce the same degree of functional impairment. Serum glutamic-oxalacetic transaminase (SGOT) changes are either more sensitive or not as organ-specific as SGPT alterations, since elevated values were obtained in hepatitis as well as in the hematopoietic disorders.

Only modest elevations of serum alkaline phosphatase activity occurred in two cases of myeloproliferation and in one case of hepatitis (Table II), but in the animal developing a mandibular giant cell tumor, the phosphatase activity was unusually high throughout its lifetime as well as just prior to sacrifice (Figure 1). At necropsy the liver appeared normal, so the terminal increase in alkaline phosphatase activity may be attributed to the bone lesion. Acid phosphatase activities, as well as isoenzyme studies, will be performed to define the type and development of these tumors.

TABLE II. Terminal Serum Values for Pitman-Moore Swine During 1968

Classification	Animal Number	Urea N, mg/100 ml	Albumen-to-Globulin	Serum Values			
				A. Pase	Enzyme Units (a)		
					LDH	SGOT	SGPT
Hematopoietic disorder							
Myeloproliferative	374	65	0.25	4.5	-	69	39
	489	255	0.30	4.9	1740	280	44
Myeloid metaplasia	1850	10	0.47	1.5	1020	43	18
	1173	36	0.40	2.3	830	100	47
	898	25	0.49	1.5	1520	120	25
Other disorders							
Uterine tumor	158	-	0.82	1.0	370	27	23
	1248	20	1.0	1.1	510	31	44
	1037	18	0.53	1.3	372	44	29
	694	18	0.48	4.7	-	3150	275
	1666	12	0.35	13.4	530	29	31
Control	18	0.9(b)	0.77 ± 0.15	1.9 ± 0.4	520 ± 34	24 ± 2	30 ± 4

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

(b) Mean and 95% confidence limits for 40 adult swine.

FIGURE 1. Serum Alkaline Phosphatase Values in an Animal that Developed a Mandibular Giant Cell Tumor (1666-GCT) Compared with Control Values and Levels of an Animal with Comparable ^{90}Sr Exposure (1231)

Conclusions

Many of the routine hematologic and serum chemistry tests which have not shown early changes in radiation damage or blood dyscrasias are now more important in detecting complicating or organic diseases in older swine.

CHROMOSOMAL EFFECTS OF ^{90}Sr IN MINIATURE SWINE

Leukocyte cultures and bone marrow preparations from swine ingesting 625 μCi ^{90}Sr daily presented no unusual cell clones. Achromatic lesions were frequently observed. One pair, number 17 which is a small acrocentric chromosome, was often characterized by an isochromatid achromatic lesion occurring in one or both members of the pair.

Investigators:
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The presence of persistent chromosomal damage following exposure to X-rays, neutron sources, and deposited radionuclides in humans and animals has stimulated interest in possible radiation-induced lesions

produced in the chromosomes of swine ingesting ^{90}Sr daily. These studies attempt to correlate the occurrence of hematologic disorders with the observed chromosomal aberrations.

Observations

Karyotypes of both Pitman-Moore and Hanford Miniature Swine were examined to define the chromosomal patterns of these two strains. Arm ratios, centromeric indices, and proportional lengths of their chromosomes were measured to serve as a quantitative documentation of observable qualitative differences, thereby making it possible to identify the chromosomes with reasonable reliability not only on the basis of conformation but on the basis of measurable differences in size and centromere position. The values obtained generally agreed with those reported for domestic pigs. The only exception was a single pair of chromosomes with the centromere in the submedial position which were slightly larger in both the Pitman-Moore and Hanford Miniature pigs studied.

Pair 17, a small acrocentric chromosome, was frequently characterized by an isochromatid, achromatic lesion occurring in one or both members of the pair. These lesions were observed in 19 of 200 metaphases from Pitman-Moore swine and 13 of 326 metaphases from the Hanford Miniature animals. The lesions sometimes appear as a gap across the chromatids, but, more often, merely as a nonstaining region (Figure 1). They have been observed in both leukocyte cultures and direct bone marrow preparations.

FIGURE 1. Acrocentric Chromosomes, Pair 17, Showing Some of the Configurations Assumed by the Achromatic Lesions

Metaphases from 48-hr leukocyte cultures obtained from pigs that had ingested 625 μCi ^{90}Sr daily for 5 months did not show significantly more aberrations than control swine. No dicentrics, tracentrics, or ring forms were seen. Bone marrow samples taken from three Pitman-Moore pigs that died from hematopoietic crises after 7-8 months of ^{90}Sr feeding revealed similar abnormalities; however, analyses of karyotypes from these preparations are incomplete.

Conclusions

Preliminary studies of the effect of chronic daily ingestion of 625 μCi ^{90}Sr on the induction of chromosomal aberrations revealed only chromatid gaps which were also present in controls. No unusual cell clones have been observed at this dose level.

IMMUNOSUPPRESSIVE EFFECT OF CHRONIC STRONTIUM-90
INGESTION BY MINIATURE SWINE

Using a highly antigenic and standardized bacterial antigen, it was demonstrated that prolonged exposure to ^{90}Sr will markedly impair the antibody synthesizing mechanism in miniature swine. It is speculated that this effect of ^{90}Sr may be involved in the leukemogenic process.

Investigators:

E. B. Howard

C. C. Jannke

Some of the spontaneous leukemias appear to be associated with or accompanied by a defective immunologic capability. It is believed that a normal immune system suppresses or eliminates potential leukemogenic agents and altered antigenic tumor cells. Since chronic feeding of ^{90}Sr to miniature swine induces leukemia in these animals, preliminary investigations of possible pathogenic pathways were undertaken, including studies of lymphocyte function and immune response. Previous studies (Annual Report, 1967) indicated that in vitro reactivity of peripheral lymphocytes from swine chronically ingesting ^{90}Sr was considerably decreased when these cells were cultured with phytohemagglutinin.

Observations

This report describes the circulating antibody response of five ^{90}Sr -fed (625 $\mu\text{Ci/day}$) and three normal Pitman-Moore miniature swine. The exposed swine had been on daily ^{90}Sr feeding for approximately 10 months at the time of antigen exposure. The

normal swine were littermate controls.

All eight swine were antigenically stimulated by administering heat-killed, Strain 19, Brucella abortus vaccine (0.2 ml subcutaneously and 0.2 ml intramuscularly). Three preinoculation blood serum samples were obtained for antibody titration. Following antigen inoculation, serum antibody level was determined at twice-weekly intervals. Antibody titers were quantitated by the tube agglutination test using stained B. abortus antigen and with standardized titered antisera as a positive control.

Results are shown in Figure 1. Peak antibody response occurred at 7-10 days postinoculation in both groups of pigs. Compared with data reported previously, the antibody titer of the control pigs reached expected values at the expected time and declined thereafter in a normal manner. The response of the ^{90}Sr -fed swine was markedly impaired, with antibody titers less than one-half the control values at the point of maximal response and for several weeks thereafter.

FIGURE 1. Serum Antibody Titer in Control and ⁹⁰Sr-Fed Pigs Following Inoculation of *Brucella abortus* Vaccine

Conclusions

In other experimental animals, chronic immunosuppression has been shown to be a prelude to the development of leukemia, whether the primary etiologic factor is viral or cytogenetic in character. It is possible that the ⁹⁰Sr-induced immunosuppression demonstrated in the miniature swine of the present study is involved in the development of leukemia in these animals. Irradiation of the marrow stem cells may be partly responsible for this immunologic depression.

SERUM PROTEIN STUDIES IN IRRADIATED ANIMALS

Haptoglobinemia was demonstrated to be a sensitive indicator of radiation damage in miniature swine following ⁹⁰Sr or ⁶⁰Co exposure, and in beagle dogs following inhalation of ²³⁸PuO₂. Haptoglobin was quantitated by measuring the hemoglobin-binding capacity of the serum.

Investigators:

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Previous studies indicated significant alterations in the serum protein distribution of miniature swine chronically exposed to ⁹⁰Sr (Annual Report, 1967). The most marked change on cellulose acetate and acrylamide disc electrophoresis was in the α₂-globulin fraction, which is a heterogeneous group of at least

ten protein species. Further studies in this area are described in the present report.

Observations

Routine serum protein electrophoresis was performed on a Beckman microzone system. Occasionally a

vertical acrylamide slab electrophoresis system* was employed. Haptoglobin was quantitated using the cellulose acetate microzone system with purified hemoglobin added to the serum sample. Haptoglobin levels are expressed as hemoglobin-binding capacity in mg/100 ml of serum.

The range of serum haptoglobin levels in normal Pitman-Moore miniature swine is illustrated in Figure 1. The average value is approximately 50 mg/100 ml and does not appear to be significantly age-dependent.

In nine adult pigs receiving daily feeding of 625 μCi ^{90}Sr , there was an initial increase in serum haptoglobin, occurring within 1 to 2 months, with a return to normal levels after 4 to 5 months. Normal or low normal levels have been maintained in these animals for a period which now extends to 22 months on ^{90}Sr feeding. In swine that developed hematopoietic neoplasms after ^{90}Sr feeding of 125

FIGURE 2. Effect of ^{90}Sr on Swine Haptoglobin

$\mu\text{Ci/day}$ for 2 to 4 years, serum haptoglobin levels remained normal until approximately 6 months before death. There was then a gradual and persistent increase in this protein until death, at which time the level was 2 to 3 times the normal value (Figure 2).

Miniature swine that received whole-body or lower-body ^{60}Co irradiation in single exposure doses of 1500 to 2500 R showed a striking increase in serum haptoglobin, starting 2 to 3 days postexposure and reaching a maximum at the time of death, 5 to 10 days postexposure. At this time the serum haptoglobin levels were found to range from 200 to 300 mg/100 ml (Figure 3).

A preliminary study in beagle dogs receiving inhalation exposure to $^{238}\text{PuO}_2$ has indicated a sharp increase in serum haptoglobin levels within 2 weeks postexposure. Most of these animals reached levels of from 250 to 500 mg/100 ml before death at 3 weeks to 6 months postexposure; the normal range is from 50 to 125 mg/100 ml.

FIGURE 1. Normal Swine Haptoglobin Levels in Pitman-Moore Miniature Swine as a Function of Age

*E-C Apparatus Corp.

FIGURE 3. Effect of ^{60}Co Irradiation on Swine Haptoglobin

Conclusions

Haptoglobin is known to be extremely reactive in a variety of neoplastic and inflammatory diseases. It was shown in the present studies that this protein increases with localized radiation to the marrow and bone tissue by ^{90}Sr , as well as with lung tissue irradiation by ^{238}Pu , and whole-body gamma irradiation from a ^{60}Co source. This must clearly be considered a nonspecific type of response to irradiation.

It is known that the liver is the site of haptoglobin production, and the liver was not affected or irradiated in most of the present studies. It is known also that

chronic hemolysis results in decreased haptoglobin levels; therefore, the mechanism of haptoglobin production is apparently not directly related to hemolysis and hemoglobin release. It would seem that induction of haptoglobin synthesis in the liver must involve a humoral factor released from an irradiated or otherwise damaged cell or tissue type. There is reason to suspect that a factor released from damaged granulocytes may be involved in the induction of certain "acute phase" protein reactants, and also that such a humoral factor may be released from connective tissue cells or ground substance material.

STRONTIUM-90 SWINE TISSUE CULTURE STUDIES

A humoral or leukemic-tissue-associated factor from pigs with ^{90}Sr -induced leukemia showed a stimulating effect on newborn pig spleen cell cultures. Tissue culture and electron microscopic evidence is presented for the presence of a virus in these induced leukemias.

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The presence of unidentified virus particles in the plasma and tumor cells from miniature swine having ^{90}Sr -induced leukemia was previously reported (Annual Report, 1967).

This continuing study is concerned with attempts to isolate virus from pigs with ^{90}Sr -induced leukemia, identify the viruses, and adapt them to tissue culture systems. The study also is concerned with detection of humoral factors in plasma and leukemic tissues which may have an effect on primary or established lines of tissue culture systems. Such factors may be inducers or regulators of hemopoiesis.

Observations

Millipore filter filtrates ($0.45\ \mu\text{m}$) were prepared from plasma and leukemic tissue homogenates obtained from Pitman-Moore miniature swine that had ^{90}Sr -induced hematopoietic tumors. Control tissue and plasma filtrates were prepared from normal animals of similar age without evidence of neoplasia. The filtrates were pooled on the basis of histopathologic and hematologic diagnosis into the following groups:

(1) normal, (2) myelogenous leukemia, and (3) lymphocytic leukemia.

These plasma and tissue filtrates were inoculated into 4-day-old suspension cultures containing from 1.5×10^5 to 1.8×10^5 newborn pig spleen cells/ml. Cultures in 500 ml Erlenmeyer flasks containing 100 ml Fischer's medium enriched with 10% horse serum were inoculated with 0.25 ml of the filtrate. Cell counts were made at various time intervals in a Coulter Model B electronic cell counter, after treatment of the cell-containing aliquots in a 0.25% trypsin-saline solution.

The lymphocytic leukemia filtrate had a marked early stimulating effect on the cultured spleen cells (Figure 1), causing blastic transformation and mitosis of the small lymphoid appearing cells. After about 20 hr of incubation, the rate of increase approached that of the normal tissue filtrate. The myelogenous leukemia filtrate had no effect different from that of the control filtrate.

Electron micrographs were prepared of neoplastic leukocytes from the peripheral blood and of plasma from leukemic pigs. Plasma from leukemic

pigs was also inoculated into stabilized commercial tissue culture cell lines, including porcine kidney, porcine marrow, and porcine cornea, in an attempt to unmask viruses which might be present in leukemic cells and adapt them to an in vitro system for virus production. Cell cultures derived from leukemic pigs on the ^{90}Sr feeding regimen were similarly evaluated.

In the most immature of the neoplastic circulating leukemic cells, there were clusters of particles in the cytoplasm, which had the size and

morphology of viruses (Figure 2). These were not observed in the more mature differentiating leukocytes, but were found in the plasma pellet.

In both the cornea and kidney cultures that were inoculated with plasma, the rate of growth of the cell lines was altered and contact inhibition was partially lost. Electron microscopy revealed both intracellular (Figure 3) and extracellular (Figure 4) particles. Particles were present, but in lesser numbers, which had distinct morphologic similarity to herpesvirus (Figure 5).

FIGURE 1. Spleen Cell Culture, Indicating Stimulation of Cell Proliferation Following Inoculation with Lymphocytic Leukemia Filtrate

FIGURE 2. Blast Cell in the Peripheral Blood of a Pig with ^{90}Sr -Induced Myelogenous Leukemia (Note electron dense cytoplasmic particles)

FIGURE 4. Extracellular Particles a Stabilized Normal Porcine Cornea Culture Following Inoculation with Plasma from a Pig with ^{90}Sr -Induced Myeloproliferative Disease

FIGURE 3. Cytoplasmic Virus-Type Particles in a Fourth Passage Kidney Cell Culture Derived from a Pig with ^{90}Sr -Induced Myelogenous Leukemia

FIGURE 5. Herpes-Type Virus Particle Observed in Fourth Passage Kidney Culture from a Pig with ^{90}Sr -Induced Myelogenous Leukemia

Conclusions

These studies give preliminary indication of the presence of a humoral or tumor cell factor that has a pronounced stimulating effect on cultured newborn pig spleen cells. This factor is present in filtrates from swine with ^{90}Sr -induced lymphocytic leukemia, but not in cases of myelogenous leukemia; nor is it a growth-stimulating factor of normal tissue extract. Further studies on the effects of this factor on lymphocyte and bone marrow cultures are in progress. A similar phenomenon has been observed in human patients with lymphocytic leukemia; myelogenous

leukemia patients lacked this factor. A similar humoral factor has also been observed in patients with autoimmune disorders.

Further evidence was obtained indicating the presence of viral particles in the tumor cells and plasma from miniature swine with ^{90}Sr -induced leukemia. It appears that this virus may be adaptable to stabilized tissue culture systems. Antigenic characterization, in progress, should determine whether this virus is merely a passenger virus that is common to pigs, and whether it has antigenic similarities to known leukemogenic viruses.

BREED COMPARISON OF ^{90}Sr EFFECTS IN MINIATURE SWINE

Three of four Pitman-Moore swine fed 625 μCi ^{90}Sr /day for 8 months died from thrombocytopenia, while all four Hanford Miniatures on a comparable feeding regimen have survived for 13 months.

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Until this year, the only hemato-poietic disorder in our control swine population was observed in a Hanford Miniature male that died at 6 months of age. Histopathologic examination

revealed lymphoproliferation involving several lymph nodes, as well as a hepatic lymphosarcoma. No cases of spontaneous lymphoproliferation have been observed in young control

swine of the Pitman-Moore herd, which is the breed utilized in our chronic ^{90}Sr studies; but recently, two cases of myeloproliferation were observed in 9-year-old swine of this breed.

This experiment was designed to compare the hematologic and biochemical responses of Hanford Miniature and Pitman-Moore swine to daily ^{90}Sr feeding.

Observations

Three separate groups, each with four animals of each breed, were put on a daily regimen of 0, 125, and

625 $\mu\text{Ci } ^{90}\text{Sr}$ feeding at 9 months of age. Routine evaluation of peripheral blood cellular and biochemical levels was performed. No differences between breeds or feeding levels were found in serum chemistry values for calcium, phosphorus, urea, creatinine, protein, cholesterol, alkaline phosphatase, lactic acid dehydrogenase, or the transaminases. Cellular differences were confined to a radiation response in both breeds. Total leukocyte values were decreased in swine receiving 625 $\mu\text{Ci/day}$, this depression being due to a loss of both neutrophils and lymphocytes (Table I). Erythrocyte values were

TABLE I. Neutrophil and Lymphocytic Values for Pitman-Moore (PM) and Hanford Miniature (HM) Swine Fed ^{90}Sr (10^3 cells/mm³)

Breed	⁹⁰ Sr Level, μCi/day	Months of ⁹⁰ Sr Feeding			
		0	3	6	9
Neutrophils					
PM	0	4.7 ± 0.6 ^(a)	3.8 ± 0.3 ^{AB(b)}	2.3 ± 0.4 ^A	3.0 ± 0.5 ^A
HM	0	5.7 ± 0.8	4.4 ± 0.6 ^A	3.3 ± 0.5 ^A	3.6 ± 0.6 ^A
PM	125	4.4 ± 0.7	2.9 ± 0.4 ^{AB}	3.2 ± 0.7 ^A	3.4 ± 0.2 ^A
HM	125	4.8 ± 0.7	2.9 ± 0.5 ^{AB}	3.3 ± 0.5 ^A	2.6 ± 0.3 ^A
PM	625	5.4 ± 1.3	1.7 ± 0.1 ^B	0.8 ± 0.3 ^B	
HM	625	4.0 ± 0.2	1.9 ± 0.2 ^B	1.0 ± 0.1 ^B	1.0 ± 0.2 ^B
Lymphocytes					
PM	0	10.9 ± 0.6	7.2 ± 0.5	7.0 ± 0.6 ^{AB}	5.0 ± 0.4 ^{AB}
HM	0	13.8 ± 1.2	9.0 ± 0.6	7.0 ± 0.7 ^A	6.9 ± 1.2 ^A
PM	125	10.2 ± 1.3	7.7 ± 0.7	5.9 ± 0.1 ^{BC}	4.5 ± 0.1 ^{AB}
HM	125	12.0 ± 0.5	6.9 ± 0.7	5.9 ± 0.6 ^{BC}	6.2 ± 0.6 ^A
PM	625	11.2 ± 1.3	6.2 ± 0.7	4.4 ± 0.4 ^C	
HM	625	12.1 ± 1.5	6.6 ± 0.7	4.1 ± 0.1 ^C	3.1 ± 0.1 ^B

(a) Standard error of the mean.

(b) Values with different capital letters are significantly different ($P < 0.01$, using a Duncan multiple range test).

also decreased in this group, but the greatest decline was observed in platelet levels (Figure 1). After 6 months of 625 μCi ^{90}Sr feeding, values for Pitman-Moore animals were lower ($P < 0.05$) than Hanford Miniature Swine; and at 8 months, three of the four Pitman-Moore swine died from a hemorrhagic syndrome caused by the thrombocytopenia.

Table II summarizes the survival times for all previously exposed Pitman-Moore swine on a 625- μCi ^{90}Sr /day regimen. In the present experiment, after 13 months of ^{90}Sr

feeding, one Pitman-Moore and all four of the Hanford Miniatures are past the median survival time of 10 months of feeding and 19 months of age. With the exception of one animal that showed myeloproliferation, all nonsurviving swine in Table II died with a hematopoietic crisis.

Conclusions

No firm conclusions concerning ^{90}Sr toxicity can be drawn from data on just four animals of each breed, but it is interesting that all of the Hanford Miniatures fed 625 μCi of ^{90}Sr have survived as long as they have. It will be of interest to see if the terminal events, after longer periods of ^{90}Sr exposure, involve cellular proliferation or pancytopenia. Critical periods in the development of the toxicity syndrome in the 125- μCi animals should be evident next year.

FIGURE 1. Platelet Values in Pitman-Moore and Hanford Miniature Swine Fed ^{90}Sr

TABLE II. Summary of Survival Times for All Pitman-Moore Swine Fed 625 μCi $^{90}\text{Sr}/\text{Day}$

Number of Animals	Age at Initiation of ^{90}Sr Feeding, months	Age at Death, months	Duration of ^{90}Sr Feeding, months
5	9	17	8
1	9	18	9
1	9	19	10
1	9	(a)	13
1	9	25	16
4	32	(a)	22
13	(Median) 19		10

(a) Live animals

LEUKOCYTE ALKALINE PHOSPHATASE VALUES IN HEMATOPOIETIC DISORDERS OF SWINE

Preliminary cytochemical studies indicate that decreased leukocyte alkaline phosphatase activities may occur in swine developing myeloproliferative diseases.

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Cytochemical studies for alkaline phosphatase activity in ^{90}Sr -fed swine were performed to differentiate between complications involving neutrophilic leukemoid reactions and the myeloproliferative state. In man, the enzyme activity is observed in the granulocytic series and is usually confined to segmented and band neutrophils although it is occasionally seen in metamyelocytes (1). Positive alkaline phosphatase reactions have also been reported in myelocytes in leukemia (2).

Observations

Initial observations on the leukocyte alkaline phosphatase (LAP) reactivity of control Pitman-Moore swine, graded on a rather subjective basis of intensity of the reaction utilizing a modified azo-dye technique (3), are shown in Figure 1. Since over 80% of the observed cells show some order of reactivity, the resultant average control LAP score of 202, obtained by multiplying reactivity by percentage of cells

scored (Table I), was found to be higher than the range of 30-70 typically observed in human samples.

Leukocyte alkaline phosphatase scores accumulated thus far for a number of hematopoietic disorders are shown in Table I. Decreased LAP scores are found in both the myeloproliferative state and myeloid metaplasia. These lower scores are often observed for long periods of time before the onset of acute hematologic signs in swine fed 125 $\mu\text{Ci/day}$ so

FIGURE 1. *Distribution of Cellular Alkaline Phosphatase Reactivity in Leukocytes of Control Swine*

TABLE I. Leukocyte Alkaline Phosphatase Values for Pitman-Moore Swine

<u>Hematopoietic Disorder</u>	<u>Number of Animals</u>	<u>LAP Score</u>
Control	19	202 $\pm$ 12 ^(a)
Hemorrhagic syndrome	3	176 $\pm$ 19
Lymphoproliferation	2	108 - 202 ^(b)
Myeloproliferation	8	114 $\pm$ 32
Myeloid metaplasia	3	116 $\pm$ 31

(a) Mean $\pm$ 95% confidence interval about the mean (see text)

(b) Range

that it was initially believed that prolonged ^{90}Sr ingestion produced an unreactive cell population; however, recent values obtained from animals fed 625 $\mu\text{Ci/day}$, showing pancytopenia and a terminal hemorrhagic syndrome in a much shorter period of time (Table I), are not lower than control scores. Further investigations of the possible effect of the ^{90}Sr -feeding level on induction periods for cellular enzyme changes are in progress.

Figure 2 shows a comparison between granulocyte maturity and cellular phosphatase activity in an animal with myeloproliferative leukemia. It is notable that the percentage of unreactive cells increased before the appearance of appreciable numbers of immature cells in the peripheral blood and that the LAP score was low despite the presence of a large number of mature neutrophils. If a deficiency of cellular phosphatase activity may be taken as one of the criteria of myeloproliferation or myeloid metaplasia in swine, the

LAP score will provide a better basis for appraising the status of animals showing abnormal hematologic profiles. As an example, Figure 3 compares values from two live, 9-year-old swine, both offspring of 25 $\mu\text{Ci } ^{90}\text{Sr}$ per day animals, both with abnormally high leukocyte and neutrophil values, but with a remarkable difference in their LAP scores. Although the LAP score is normal for animal 396, the unreactive cells are slightly above control levels while reactive cells have shifted to a more positive state, suggesting a complicating leukemoid reaction. Neutrophils from animal 401 are almost devoid of phosphatase activity implying the possible development of myeloproliferation or myeloid metaplasia.

Conclusions

The evaluation of leukocyte alkaline phosphatase activity appears to be a promising method for the early detection of myeloproliferation or myeloid metaplasia. Preliminary

FIGURE 2. A Comparison of Granulocyte Values and Cellular Alkaline Phosphatase Activity in Myeloproliferative Leukemia

FIGURE 3. Cellular Alkaline Phosphatase Activity and Granulocyte Distribution in Two Offspring of Swine Fed 25 μCi $^{90}\text{Sr}/\text{Day}$

data do not indicate that phosphatase scores may be utilized to distinguish between the two disorders. The determination of the percentage of unreactive cells may be more useful than evaluation of the LAP score in the event of complicating leukemoid reactions.

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A METHOD OF QUANTITATIVE THERMOLUMINESCENCE

Simultaneous recording of the thermoluminescence glow curve and its integral eliminates errors caused by variations in dosimeter readout parameters.

Investigator:
C. R. Watson*

Thermoluminescence dosimetry readout devices measure light emitted by rapidly heated radiosensitive phosphors. Two methods of quantitation of this light are commercially available. In the integral method, the total light emitted throughout the heating period is measured, usually by converting the photomultiplier signal to pulses which are counted. The peak height method measures maximum light output, usually obtained from the glow curve which is

a plot of photomultiplier signal versus heating time. We found it desirable to measure both of these variables simultaneously, and have modified our readout device accordingly.

Observations

Our thermoluminescence readout system was of the integral type, the limits of integration being the start and end of the heating cycle. Any change in readout parameters, such as switching dosimeter types, necessitated readjustment of the heating time to insure that the integral

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limits coincided with the significant portion of the glow curve.

When we examined many glow curves of several dosimeter materials, we concluded that integral limits defined by the heating cycle were unsatisfactory because small variations in readout conditions changed the shape of the glow curve. For the same reason we concluded that peak height analysis was unreliable unless all readout parameters were held rigidly constant.

Our primary problem was in the use of powdered dosimeter material. Small changes in the distribution of a 25-mg sample on the heater surface resulted in large variations in glow curve shape. This was due to non-uniform heating of the sample. When some of the powder was in poor thermal contact with the heater, it reached the thermoluminescent temperature later than the rest of the sample, causing smearing of the glow curve.

Attachment of a Disc* integrator to the strip chart recorder has been an inexpensive and reliable solution to this problem. This mechanical device records the integral in the form of traverses of a pen at the bottom of the chart. By selecting that portion of the glow curve which is significant, and projecting from its end points to the integral trace, one obtains a number proportional to the integral under the peak by

FIGURE 1. Glow Curves of Two Samples of LiF Exposed to 10 R and Readout in 22 sec at Different Heating Rates (The Peak heights are different, while the area under the peaks is constant.)

counting traverses of the integrator pen between these limits (Figure 1). The device has been employed for over a year, and is reliable and accurate.

This method is independent of variations in readout conditions. For example, Figure 1 shows that the integral measured this way is constant despite a significant change in heating rate with its subsequent dramatic change in glow curve shape.

Conclusions

Simultaneous recording of the glow curve and its integral has increased the flexibility and reliability of our thermoluminescence readout system.

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INHALATION STUDIES

A large number and a wide variety of reports on inhalation studies are included in this section. Several other reports concerned with inhalation are included in the section on Radioelement Removal Studies.

Three reports are concerned with pathologic effects in beagles of PuO_2 inhalation: the current status of the chronic effects experiment, now 10 years in progress; results of acute toxicity studies with ^{238}Pu ; and indications of a possible association between effects of radiation on immune response and tumor development. Four reports are concerned with comparisons of the metabolic behavior of three isotopes of plutonium: ^{237}Pu , ^{238}Pu , and ^{239}Pu , and of americium with plutonium.

The role of phagocytic cells in the distribution of particulate plutonium has been studied following intraperitoneal injection and inhalation of PuO_2 . Three reports describe these studies, and a fourth applies the results to the development of a model for determining the distribution of alpha energy around deposited $^{239}\text{PuO}_2$ particles.

Several years retention data are reported for inhaled ruthenium oxide in dogs; also data on the retention of uranium oxide and on the metabolic fractionation of different members of the uranium decay chain. Plans are described for a major chronic exposure study of uranium mine atmosphere pollutants employing hamsters and beagles; this is a project which receives partial support from the National Institute for Environmental Health Sciences, Department of Health, Education, and Welfare. Two reports deal with the biochemistry of lung subcellular fractions.

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

Of 26 dogs that have died or were sacrificed when death was imminent, 29-103 months after exposure to $^{239}\text{PuO}_2$ aerosols, 18 showed pulmonary neoplasia. Data thus far available suggest that alveolar deposition of more than 5 nCi/g of lung might be expected to result in premature death due to pulmonary neoplasia.

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A long-term study of the biological effects of inhaled $^{239}\text{PuO}_2$ in dogs is in its tenth year. Of 40 beagle dogs given single 10 to 30 min exposures to $^{239}\text{PuO}_2$ aerosols, only six remain alive. This report summarizes the present status of the experiment.

Observations

Plutonium analyses and histopathologic examinations have been completed on 31 animals; 18 of these showed evidence of pulmonary neoplasia. An additional three animals, recently sacrificed, show gross evidence of pulmonary neoplasia, but analyses and tissue examinations are not complete. Six dogs are still alive; three of these show radiographic evidence of pulmonary neoplasia.

Ten dogs died or were sacrificed when death was imminent due to respiratory insufficiency. Pulmonary neoplasia, when observed in these dogs, was an incidental finding at necropsy or was diagnosed a few months prior to death in thoracic radiographs. In 14 other dogs, pulmonary tumors were diagnosed in thoracic radiographs prior to clinical signs of severe respiratory insufficiency. One dog died with meningitis and another with cardiovascular disease, conditions not presently considered to be associated with plutonium exposure. Five dogs were sacrificed to determine the tissue distribution of plutonium in asymptomatic dogs.

Figure 1 shows the relationship between the quantity of alveolar deposited plutonium and the survival time of dogs, with an indication of those

FIGURE 1. Relationship Between Deposition of Inhaled $^{239}\text{PuO}_2$ and Survival Time of Dogs

which died with lung tumors. Also included in Figure 1 are data from other experiments concerned with acute effects of larger quantities of inhaled $^{239}\text{PuO}_2$. Assuming the life span of the beagle dog is 15 years, these data suggest that alveolar deposition of as little as 5 nCi/g of lung might be expected to result in a premature death due to pulmonary neoplasia.

Figure 2 summarizes retention and translocation data from several experiments with dogs that inhaled $^{239}\text{PuO}_2$. Alveolar deposited plutonium was retained in the lung with a biological half-life of about 1,000 days. Approximately 30% of the plutonium was eventually translocated to the tracheobronchial and mediastinal lymph

nodes, 15% to the liver, and 5% to the skeleton. The highest concentration of plutonium occurred in the tracheobronchial lymph nodes followed in descending order by mediastinal lymph nodes, lungs, liver, and skeleton.

The lung tumors were classified as bronchiolo-alveolar carcinomas, bronchiolar adenocarcinomas, bronchial carcinomas, squamous cell carcinomas, and epidermoid carcinomas. They were usually multicentric in origin and metastases were observed in the tracheobronchial and mediastinal lymphatics, liver, kidneys, diaphragm, and skeleton. In addition to bronchiolar adenocarcinomas one dog had a capillary hemangioma and another dog a lymphangiosarcoma in the lungs. Two dogs developed secondary hypertrophic pulmonary osteoarthropathy with extensive skeletal lesions. The lungs of all the dogs showed extensive fibrosis.

The tracheobronchial and mediastinal lymph nodes showed severe lymphatic necrosis with replacement by dense sclerotic tissue. One dog had a lymphangiosarcoma and another a capillary hemangioma in the mediastinal lymph nodes. Although the lymph nodes were severely damaged and contained the highest concentration of plutonium, the lungs were the principal site of neoplasia, and pathology in the lungs was the primary cause of death.

The livers showed minimal cellular degeneration and necrosis. The bone showed no outstanding lesions except the periosteal proliferation in the two dogs with secondary pulmonary osteoarthropathy.

FIGURE 2. Retention and Translocation of $^{239}\text{PuO}_2$ in Dogs

Conclusions

If the deposition of $^{239}\text{PuO}_2$ in the lung of the beagle dog is not so high as to cause early death from pulmonary fibrosis, one may expect lung tumors after a latent period of about 3 years. The latent period lengthens

with decreasing plutonium deposition and may approach the life span of the dog at alveolar depositions of less than about 5 nCi/g of lung. Pathology in the lungs has been the primary cause of death in all cases involving toxicity of inhaled $^{239}\text{PuO}_2$.

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

Beagle dogs died of pulmonary insufficiency related to plutonium-induced changes in the lungs 27 to 185 days after inhalation of $^{238}\text{PuO}_2$. The body burdens at death ranged from 17 to 261 μCi with 90% in the lungs. The biological effects were similar to those previously observed in beagles after inhalation of similar radioactivity levels of $^{239}\text{PuO}_2$.

Investigators:

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Technical Assistance:

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M. D. Snyder

The acute biological effects of inhaled $^{239}\text{PuO}_2$ in beagles were earlier shown to be primarily related to plutonium-induced changes in the lungs. Since ^{238}Pu has a specific activity of 17.4 Ci/g, as compared to 0.062 Ci/g for ^{239}Pu , injury might be expected to be 282 times more severe for ^{238}Pu when the two isotopes are compared on an equal weight basis. For identical radioactivity levels, the mass of ^{238}Pu is 282 times less than that of ^{239}Pu , which could result in different toxic responses or affect the relative distribution of the two isotopes within the body. This report describes the acute toxicity of inhaled $^{238}\text{PuO}_2$ in beagle dogs and compares the results with those obtained in previous inhalation studies with $^{239}\text{PuO}_2$.

Observations

Twelve beagle dogs were exposed for 15 min to $^{238}\text{PuO}_2$ aerosols prepared by calcining the oxalate at

700 °C for 7 hr. The aerosol concentration ranged from 4 to 35 $\mu\text{Ci/liter}$. The count median diameter of the particles was 0.05 μm with a standard geometric deviation of 1.9. Six dogs were maintained as controls.

The dogs died or were sacrificed when death was imminent 27 to 185 days postexposure. The body burden at death ranged from 17 to 261 μCi with 77 to 94% in the lungs, 2 to 13% in the skeleton, 1 to 9% in the tracheobronchial and mediastinal lymph nodes, 1 to 5% in the liver and approximately 1% in all other tissues (Table I). The highest concentration occurred in the tracheobronchial lymph nodes followed in descending order by lungs, liver, and skeleton.

Whole-body retention curves were determined for each dog by adding to the body burden at death the cumulative daily excretion values. Extrapolation of the clearance curve to the ordinate at zero time gave an estimate of the fraction of alveolar-deposited plutonium. Figure 1 shows the whole-body and lung retention curves for a representative dog.

From 30 to 85% of the total deposited

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TABLE 1. Dog Mortality and Tissue Distribution
After Inhalation of $^{238}\text{PuO}_2$

Dog Number	Survival, days after exposure	Initial Alveolar Burden, μCi	Terminal Body Burden, μCi	Plutonium Distribution, percent of terminal body burden			
				Lungs	Lymph Nodes(a)	Skeleton	Liver
390	27	268	261	92	4	2	1
403	30	176	167	94	2	2	2
408	35	177	168	93	1	3	2
395	56	120	112	92	3	4	1
450	56	79	74	91	4	4	1
393	61	146	140	94	2	3	1
460(b)	70	91	84	90	3	4	2
442(b)	76	92	88	90	4	3	2
399	77	61	58	91	5	2	1
449(b)	94	47	44	91	3	4	1
413(b)	125	19	17	80	9	7	3
400	180	27	25	77	4	13	5

(a) Tracheobronchial and mediastinal lymph nodes

(b) Pulmonary neoplasia

FIGURE 1. Whole-Body and Lung Retention of Inhaled $^{238}\text{PuO}_2$ in a Representative Dog

plutonium was retained in the body with biological half-times ranging from 400 to 1600 days (Table II). The lung retention half-time ranged from 160 to 410 days. Assuming uniform dispersal of plutonium and radiation in the lungs, the radiation dose ranged from 8,000 to 25,000 rad (Table II).

The dogs showed intermittent anorexia associated with a 20 to 50% loss of body weight prior to death. Respiratory rate gradually increased from a normal range of 20 to 40 respirations per minute to over 100 respirations per minute the week prior to death. Minute volume increased and tidal volume decreased. The partial pressure of O_2 in arterial blood de-

creased and pCO_2 increased. Hemoglobin and pH were within the normal range (Table III).

The mean lymphocyte count of the exposed dogs was significantly less than the control dogs when the first blood count was completed 1 week after exposure, and decreased to less than 10% of control values at 28 days, where it remained throughout the subsequent period (Figure 2). The mean total leucocyte count of the exposed dogs was significantly less than that of control dogs, primarily due to the decrease in lymphocytes. There was no significant change compared to the controls in the neutrophil, monocyte, or eosinophil counts. Hemoglobin,

TABLE II. Deposition and Retention of Inhaled $^{238}PuO_2$

Dog Number	Time of Death, days after exposure	^{238}Pu Deposited		Retention Half-Time, days		Estimated Average Dose to Lungs, krad
		Total, μCi	Alveolar, percent	Whole Body	Lung	
390	27	318	84	710	160	25
403	30	232	76	400	180	21
408	35	245	72	440	190	24
395	56	343	35	530	250	24
459	56	89	79	590	240	15
393	61	193	76	1060	400	26
460	70	181	50	600	260	18
442	76	113	81	1320	370	24
399	77	81	75	1460	410	19
449	94	58	82	980	410	15
413	125	19	77	1040	270	8
400 (a)	185	27	29	1650	380	14
Mean			68 ± 12	898 ± 265	293 ± 60	

(a) Mean $\pm$ 95% confidence interval

TABLE III. Pre-Exposure and Terminal Respiratory Volumes and Arterial Blood Gas Analysis in Dogs After Inhalation of $^{238}\text{PuO}_2$ (Means with 95% confidence intervals)

	Pre-Exposure	Terminal (a)
Respirations/min	22.0 $\pm$ 4.0	124.0 $\pm$ 29.0
Minute volume (liter)	3.9 $\pm$ 0.7	7.0 $\pm$ 2.0
Tidal volume (ml)	184.0 $\pm$ 38.0	57.0 $\pm$ 11.0
pO ₂ (mm Hg)	99.0 $\pm$ 5.0	58.0 $\pm$ 16.0
pCO ₂ (mm Hg)	37.0 $\pm$ 2.0	51.0 $\pm$ 11.0
pH	7.39 $\pm$ 0.01	7.37 $\pm$ 0.03
Hemoglobin (g/100 ml)	17.6 $\pm$ 0.9	16.3 $\pm$ 1.5

(a) Values during the week prior to death

FIGURE 2. Effects of Inhaled $^{238}\text{PuO}_2$ on the Leucocyte Counts of Beagles

erythrocytes, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations were within normal range.

There were no significant changes in serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, potassium, calcium, sodium, phosphorus, or bicarbonate. Serum chloride levels were significantly lower in the exposed dogs only during the week before death. This coincided with the time of highest pCO_2 levels in arterial blood. There were no significant changes in total serum proteins, albumin, and globulin.

The dogs that died within the first 3 months after exposure showed free blood in the feces during the week prior to death and a tendency toward hematoma formation after arterial blood sampling. The mean thrombocyte count of the controls was 295 ± 80 compared to terminal value of 121 ± 47 (thousand/mm³) for the exposed dogs.

Thoracic radiographs showed uniform mottling of the lung with increased densities which progressed to patchy increased density during the week prior to death. Auscultation of the thorax and necropsy findings confirmed the pulmonary edema. The lungs were two to three times normal weight due to the accumulation of fluid in the parenchyma and airways.

Histologic alterations in the lungs of animals that died approximately 1 month after exposure were of an acute and primarily vascular nature; there was edema and some hemorrhage.

Fibrinous transudate filled many of the alveoli and bronchioles. The alveolar septal walls were thickened, edematous, and hyalinized. There were small focal areas of necrosis and a sparse proliferation of alveolar macrophages. The epithelium of the bronchi and bronchioles was not significantly altered. There was very little indication of fibrosis or collagenization in these lungs. Autoradiographic examination indicated a diffuse scattering of ²³⁸Pu throughout, with only slight indication of localization toward the apical or pleural surfaces of the lung. At this time, most of the ²³⁸Pu was found to be associated with the walls of the bronchioles and alveoli, with relatively few particles localized in the interstitium. Some ²³⁸Pu particles resided in free alveolar macrophages.

The dogs that survived 2 to 6 months after exposure showed more chronic changes superimposed on the lesions previously noted. There were edema, hemorrhage, focal necrosis, and extensive fibrinous deposits in the bronchiolo-alveolar spaces. Septa were markedly damaged, being thickened, hyalinized, and in some instances ruptured, resulting in extensive emphysema. There was a somewhat greater proliferation of alveolar macrophages although this was still not extensive. Interstitial, peribronchial, and subpleural fibrosis and scarring were more prominent, with the pleura being quite thickened in some areas. In animals surviving the longer time period there was a

varying amount of epithelial hyperplasia in the bronchi and bronchioles. In four of these dogs, there were multiple foci of neoplastic proliferation of bronchiolar epithelium, resulting in small carcinomas in situ, or developing bronchiolo-alveolar carcinomas.

Histologically, there was almost total lymphoid necrosis of the tracheobronchial, mediastinal, and sternal lymph nodes, with few viable lymphocytes remaining, a scattering of hemosiderin-laden macrophages, and the tissue replaced by proliferating fibroblasts and collagen deposition. In those nodes that did not undergo necrosis, such as the mandibular, cervical, axillary, prescapular, popliteal, and mesenteric nodes, there were varying amounts of reactivity with macrophage production and some hemosiderin deposits. Autoradiographically, the most severely damaged nodes contained a very heavy deposition of plutonium particles that ranged from large particles with innumerable dense tracks to very small particles or ionic ^{238}Pu , as evidenced by the presence of single tracks in the NTA emulsion after a 14-day exposure period. In those nodes that showed a reactive process, there was only ionic ^{238}Pu . In most areas of the lymph nodes, ^{238}Pu was seen to be within macrophages, associated with the hemosiderin pigment, regardless of whether it was particulate or ionic in form.

A fairly consistent microscopic finding was focal necrosis of marrow elements, especially in the cellular red marrow of the vertebrae and ribs.

There were pinpoint hemorrhages and congestion in the marrow, focal areas of necrotic cells, fibrin, and early fibrous strands. Autoradiographic examination showed heavy deposits of ionic ^{238}Pu in the bone, with greatest localization associated with the endosteum of the trabecular bone and cortex. There was also a considerable deposition in the marrow associated with blood pigments in mononuclear cells, or along the reticular network. There was a lesser amount in the ossified trabecular bone and still less in the cortex. In two of the animals, dying less than 50 days after exposure, there was some myeloid proliferation in the spleen; probably a compensatory reaction to the marrow damage.

The livers of exposed animals showed minimal changes in the parenchymal cells which were often swollen and contained a finely vacuolated or ground glass appearance to the cytoplasm. A few early indications of degeneration were seen in the nuclei, such as marginated chromatin. These lesions could have been due to a direct effect of ^{238}Pu on the cells, or to early anoxic degeneration associated with passive congestion, since the sinusoids were dilated and congested. Autoradiographically, the ^{238}Pu in the liver was seen as single tracks in the reticuloendothelial and parenchymal cells.

Conclusions

The mean whole-body retention half-time of alveolar-deposited $^{238}\text{PuO}_2$ of approximately 900 days compares with

a value of approximately 750 days noted in previous acute toxicity studies with $^{239}\text{PuO}_2$. The mean lung retention half-time of alveolar-deposited $^{238}\text{PuO}_2$ of 300 days compares with a value of approximately 400 days noted in previous studies with $^{239}\text{PuO}_2$. The apparent shorter retention time in the lung of $^{238}\text{PuO}_2$ correlates with a larger translocation of the ^{238}Pu to other tissues than occurred for ^{239}Pu . Approximately 3% of the ^{238}Pu at death was located in the tracheobronchial and mediastinal lymph nodes, 3% in the skeleton and 1% in the liver. Approximately 4% of the ^{239}Pu at death was located in the tracheobronchial and mediastinal lymph nodes and less than 1% was present in other tissues. The particle size of the $^{239}\text{PuO}_2$ was $0.6\text{ }\mu\text{m}$ compared to $0.05\text{ }\mu\text{m}$ for the $^{238}\text{PuO}_2$. Since previous studies have shown that translocation of $^{239}\text{PuO}_2$ tends to increase with decreasing particle sizes, it is possible the small particle size of the $^{238}\text{PuO}_2$ could account for the increased translocation. However, studies in rats comparing the acute toxicity of inhaled $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ of the same particle size also showed greater translocation of $^{238}\text{PuO}_2$ than $^{239}\text{PuO}_2$.

The clinical effects of acutely lethal quantities of inhaled $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ were similar. The dogs exposed to $^{238}\text{PuO}_2$ showed more extensive vascular damage in the lung, while the $^{239}\text{PuO}_2$ -exposed dogs had a more generalized fibrosis. The neoplastic response was similar to that previously described after inhalation of $^{239}\text{PuO}_2$ except that it did

not arise in areas of scarring but appeared to arise near the terminal bronchi or beginning bronchioles.

The $^{238}\text{PuO}_2$ -exposed animals showed lesions in the skeleton and liver related to translocation of plutonium to these tissues. Lesions in these tissues were not observed in dogs after inhalation of $^{239}\text{PuO}_2$ because very little ^{239}Pu was translocated to these tissues.

Figure 3 compares the plutonium concentration in the lungs at death of 26 dogs dying 55 to 168 days after inhalation exposure to $^{239}\text{PuO}_2$ particles with a CMD of approximately $0.6\text{ }\mu\text{m}$ prepared by calcination of the oxalate at 300 to $430\text{ }^\circ\text{C}$, with the 12 dogs in the present study that died 27 to 185 days after aerosol exposure to $^{238}\text{PuO}_2$ particles with a CMD of $0.05\text{ }\mu\text{m}$ prepared by calcination at $700\text{ }^\circ\text{C}$. The microcurie concentration of plutonium in the lungs at death was significantly higher for ^{238}Pu - than for ^{239}Pu -exposed dogs dying at a similar time postexposure. Previous studies with rats showed no significant difference in the lung dose-survival relationship for inhaled $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ aerosols; however, in these experiments the CMD of the $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ aerosols were both $0.1\text{ }\mu\text{m}$ and both oxides were prepared by calcination at $350\text{ }^\circ\text{C}$. The indication from the present study that inhaled $^{239}\text{PuO}_2$ in dogs was more toxic than inhaled $^{238}\text{PuO}_2$ may be due to the difference in particle size of the two plutonium aerosols employed. In any case, the difference may be considered negligible for purposes of hazard evaluation and establishment

FIGURE 3. Relationship Between Quantity of Plutonium in the Lung at Death and Survival Time

of exposure limits. Because of the relatively higher rate of translocation of ^{238}Pu , the long-term toxicity of these two plutonium isotopes might

be expected to show greater differences, both quantitatively and qualitatively.

IMMUNOCOMPETENCE OF BEAGLES EXPOSED TO $^{238}\text{PuO}_2$ AND $^{239}\text{PuO}_2$

There was a dose-response relationship between the quantity of $^{238}\text{PuO}_2$ deposited in dogs' lungs and their heterophile antibody titer. There was also a correlation between reduced antibody response and the occurrence of tumors.

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Technical Assistance:
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It was previously shown that rats had a reduced antibody-forming capacity after inhaling $^{239}\text{PuO}_2$ at higher doses and an apparent stimulation of this mechanism at low doses (Annual Report, 1967). This study has now been extended to include further observations of the long- and short-term effects of ^{238}Pu and ^{239}Pu in beagle dogs.

Observations

Eleven 3-1/2 to 4-year-old beagles that had inhaled aerosols of $^{238}\text{PuO}_2$ 4 weeks earlier were injected, together with six control dogs of the same age, with 50 million washed sheep red blood cells. The animals were assayed for heterophile antibodies at periods of from 1 to 13 weeks following the single antigen stimulation. The effects of the plutonium burden on antibody-forming ability and on the half-life of the antibodies formed are shown in Figure 1. Controls had a maximum antibody titer of about 1:180 and an antibody half-life of about 6 weeks. The response of the experimental ani-

mals varied with the amount of plutonium deposited. Only two of the animals survived the entire experimental period. None exhibited an antibody half-life as long as that of the controls. Two animals with plutonium deposits of 58 and 44 μCi showed a marked early stimulation of antibody titer, but a much shortened half-life.

The duration of the antibody depression following plutonium inhalation is not known. However, studies were made on several dogs (82-117 months old) that had carried low-level $^{239}\text{PuO}_2$ burdens from 68-100 months. Three dogs (65-69 months old) that had inhaled $^{239}\text{PuO}_2$ 8 months previously and three unexposed controls (76-105 months old) were also included in these tests. The results are shown in Table I, with animals divided into groups according to the pathology exhibited. The controls and the old exposed animals free of tumors had essentially normal antibody titers. Animals with tumors, or suspected of having tumors, showed a marked reduction of antibody-forming capacity. The recently exposed animals were most completely inhibited.

FIGURE 1. Antibody Titers of Beagle Dogs Exposed to Inhaled $^{238}\text{PuO}_2$ (The numbers by each line indicate the initial quantity of ^{238}Pu deposited in each dog.)

TABLE I. *Heterophile Antibody Titer of Dogs Exposed to $^{239}\text{PuO}_2$*

Number of Dogs	X-Ray Diagnosis for Tumors	Average Antibody Titer
<u>Unexposed controls</u>		
3	--	1:128
<u>Animals exposed 8 months previous</u>		
3	--	1:14
<u>Animals exposed 68-100 months previous</u>		
4	Negative	1:96
7	Suspected tumors	1:36
3	Positive	1:50

Conclusions

The inhalation of ^{238}Pu and ^{239}Pu oxides by beagle dogs produces a variable response to antigenic stimulation by washed sheep red blood cells. The half-life of the antibodies formed in all of the experimental animals was shortened, regardless of whether the animal demonstrated an initial stimulation or inhibition. This may indicate either an increased rate

of destruction of the antibody, or of the antibody-carrying or -synthesizing cells. More work is needed to elucidate this mechanism. The results of studies on dogs with old plutonium burdens indicate that the depression of antibody synthesis may last for years. However, this can only be confirmed and quantitated by a series of challenges with sheep red cells to the same animal over an extended period of time.

The correlation between the depressed antibody titer and the occurrence of lung tumors is most interesting. Recently exposed animals may exhibit a depressed immunocompetence which will either remain depressed or recover with time. If recovery occurs, as appeared to be the case in the four old dogs without tumors, then the postexposure period may be carcinogenically uneventful. If recovery of immunocompetence is delayed too long, or does not occur at all, then a condition may exist which is most favorable for the propagation of mutant cells, leading to neoplastic growth in the area of the radiation exposure. Further studies are in progress to investigate this possibility.

COMPARATIVE METABOLISM OF ^{237}Pu AND ^{239}Pu IN BEAGLES

$^{237}\text{Pu}(\text{NO}_3)_4$ given intravenously to dogs showed a different tissue distribution and a higher rate of excretion than $^{239}\text{Pu}(\text{NO}_3)_4$. These results are ascribed to the large difference in specific activity between the isotopes and the consequent difference in mass of material injected.

Investigators:

W. J. Bair
I. C. Nelson*
D. H. Willard

Technical Assistance:

W. Skinner

It has been proposed that ^{237}Pu may be a suitable plutonium isotope for in vivo studies of the metabolic behavior of plutonium in humans. Plutonium-237 decays principally by electron capture with a half-life of 45.6 days. The electron capture gives rise to 17, 60, and 100 keV photons which are detectable by current in vivo counting techniques. For the safe use of ^{237}Pu in human studies, it must be administered free of more hazardous isotopes such as ^{238}Pu and ^{239}Pu .

The purpose of this experiment was to compare the metabolic behavior of intravenously injected $^{237}\text{Pu}(\text{NO}_3)_4$ with $^{239}\text{Pu}(\text{NO}_3)_4$. Since the specific activity of ^{237}Pu is 12 $\mu\text{Ci}/\text{ng}$, its metabolic behavior might be expected to differ significantly from that of other lower specific activity plutonium isotopes, which are generally deposited in the body in μg quantities as a result of industrial accidents.

Observations

The plutonium was formed by a $^{235}\text{U}(\alpha, n)^{237}\text{Pu}$ reaction produced by

irradiating a 93% enriched ^{235}U metal foil 1/2 in. $\times$ 1 in. $\times$ 1 mil thick with 27.5 MeV helium ions in the 88-in. cyclotron at the Lawrence Radiation Laboratory, Berkeley, California. The principal contaminants in the plutonium are shown in Table I.

Two dogs were given 0.8 μCi of ^{237}Pu in 0.4 ml of 0.14 N HNO_3 by injection into the right cephalic vein. The dogs were sacrificed after 30 days. Fecal and urine samples were collected daily and the dogs were monitored periodically with a 4 $\times$ 8 in. NaI crystal scintillation counter. The results are compared in

TABLE I. Contaminants in ^{237}Pu

Radio-nuclide	pCi/ μCi ^{237}Pu	pg/ μCi ^{237}Pu
^{236}Pu	91	0.2
^{238}Pu	53	3
^{239}Pu	32	520
^{228}Th	10	0.01
^{233}U	6	630

1 μCi ^{237}Pu weighs 83 pg

* Radiological Sciences Department

Table II with those from three dogs similarly treated with $^{239}\text{Pu}(\text{NO}_3)_4$ in a previous experiment.

About 80% of the 30-day retained burden of ^{239}Pu was present in the liver, 6% in the skeleton, 4 to 10% in the spleen, and about 3% in muscle. The distribution of ^{237}Pu was much different; 30 to 40% in liver, 40 to 50% in skeleton, less than 1% in spleen, and 6 to 10% in muscle. Also, the amount of ^{237}Pu excreted was significantly greater than ^{239}Pu ; 20 to 30% for ^{237}Pu and about 1% for ^{239}Pu .

The differences in distribution and excretion of these two plutonium isotopes can be attributed to the dif-

ference in mass of plutonium administered, about 350 μg in the case of ^{239}Pu and about 1 ng in the case of ^{237}Pu (including the mass of contaminants, 420 pg ^{239}Pu and 500 pg ^{233}U). The small mass of ^{237}Pu would have provided less opportunity for colloid formation. It is known that colloidal plutonium injected into the blood deposits primarily in the reticulo-endothelial tissues such as liver and spleen. On the other hand, plutonium complexed with citrate to prevent colloid formation at neutral pH deposits principally in the skeleton. The ^{237}Pu in this experiment behaved much like citrate-complexed plutonium.

TABLE II. Comparative Tissue Distribution and Excretion of ^{237}Pu and ^{239}Pu 30 Days After Intravenous Injection of $\text{Pu}(\text{NO}_3)_4$ (Percent of retained body burden)

Dog No.	^{237}Pu		^{239}Pu		
	322	300	22	27	243
<u>Tissue</u>					
Liver	33	44	85	83	78
Skeleton	50	43	5.8	6.4	6.9
Kidney	0.88	0.93	0.12	0.13	0.088
Spleen	0.75	0.82	4.4	5.8	9.6
Lung	0.46	0.60	0.23	0.30	0.31
Muscle	10	5.7	3.2	2.7	3.2
Pu excreted in urine(a)	19	7.2	0.48	0.43	0.47
Pu excreted in feces(a)	9	13	0.64	0.95	0.62
μCi injected	0.85	1.1	25	21	21
μg injected	0.0011 ^(b)	0.0014 ^(b)	400	340	340

(a) Percent of injected Pu

(b) Includes ^{239}Pu and ^{233}U contaminants

COMPARATIVE DISTRIBUTION OF ^{238}Pu AND ^{239}Pu IN RATS FOLLOWING
INHALATION OF THE OXIDE

Rats which inhaled $^{238}\text{PuO}_2$ showed a much greater translocation of the radionuclide to systemic organs than was found for inhaled $^{239}\text{PuO}_2$ of the same particle size and crystalline form. At times greater than 1 year after single inhalation exposures, 1/4 to 1/2 of the total body burden of ^{238}Pu was found in the skeleton.

Investigator:
B. O. Stuart

Technical Assistance:
J. C. Gaven

The mortality patterns of more than 200 rats, exposed to several concentration levels of $^{238}\text{PuO}_2$ or $^{239}\text{PuO}_2$ aerosols (CMD 0.1 μm), were described previously (Annual Report, 1967). Representative animals from the groups at each dose level were sacrificed periodically for histopathology and tissue radioactivity analyses when deaths began to occur. Calculated total lung doses received by rats that died more than 100 days after exposure to $^{238}\text{PuO}_2$ were somewhat smaller than doses received by rats dying after similar times following inhalation of $^{239}\text{PuO}_2$, suggesting

that damage due to the translocation of the material to other tissues may have contributed to the death of the $^{238}\text{PuO}_2$ -exposed animals.

Observations

Table I shows the tissue levels of ^{239}Pu in rats at increasing times after single inhalation exposures to the oxide as percentages of total body burdens. The relatively large percentage of ^{239}Pu contained in the gut at 1 week after exposure is typical for an inhaled insoluble material

TABLE I. Tissue Distribution of ^{239}Pu in Rats After Inhalation of $^{239}\text{PuO}_2$ (Percent of body burden at death)

Rat No.	Days after Exposure	Tracheo-bronchial Lymph Nodes	Lungs	Spleen	Kidneys	Liver	Skeleton	GI Tract Plus Contents
95	7	0.1	67	0	0	0	0.01	32.9
63	13	0.3	97	0	0	0.02	0.01	2.4
73	28	1.4	96	0.02	0	0.4	0.1	2.3
30	68	1.8	97	0.04	0	0.3	0.1	0.1
4	113	-	99	0	0	0.3	0.2	0

in the rat. Virtually all of the tissue-bound ^{239}Pu remained in the lungs; after a few months, 1 or 2% was found in the tracheobronchial lymph nodes, only 0.3 to 0.4% in the liver, and about 0.1% in the skeleton.

Table II shows the corresponding tissue distribution data for $^{238}\text{PuO}_2$. In marked contrast to the negligible translocation of ^{239}Pu , 1/10 of the ^{238}Pu body burden was found in the skeleton at 3 weeks after exposure, increasing to 1/4 to 1/2 after a year. The liver and kidneys also contained up to 3 to 4% of the ^{238}Pu body burden. External whole-body monitoring of both $^{238}\text{PuO}_2$ - and $^{239}\text{PuO}_2$ -exposed rats showed very similar long-term retentions, indicating that the higher percentage values of ^{238}Pu for the systemic organs also reflected much higher absolute translocation rates.

Conclusions

The much greater translocation to systemic organs of inhaled ^{238}Pu , as compared with ^{239}Pu , reaching as high as 50% of the total body burden in the skeleton at 1-1/3 years after exposure, may have contributed to the earlier-than-expected deaths in the lowest exposure level groups of rats that inhaled $^{238}\text{PuO}_2$. Since other studies have shown that plutonium which accumulates in the skeleton is predominantly in an ionic or complexed form, inhaled $^{238}\text{PuO}_2$ seems to be more soluble in vivo than $^{239}\text{PuO}_2$ having the same particle size, crystalline structure, and method of preparation.

TABLE II. Tissue Distribution of ^{238}Pu in Rats After Inhalation of $^{238}\text{PuO}_2$ (Percent of body burden at death)

Rat No.	Days after Exposure	Tracheo-bronchial Lymph Nodes	Lungs	Spleen	Kidneys	Liver	Skeleton	GI Tract Plus Contents
84	20	0.4	82	0.1	0.5	2.4	11	2.1
67	48	0.6	84	0.2	0.4	1.3	12	0.5
39	78	0.8	77	0.2	0.4	1.9	23	0.4
50	127	3.4	78	0.2	1.1	1.7	15	-
59	320	3.3	64	0.7	0.5	1.6	30	0.4
5E	465	0.1	40	0.6	3.3	3.6	48	0.7
4E	481	3.3	68	0.5	0.5	3.6	23	0.5

PLUTONIUM-AMERICIUM RATIOS IN BEAGLES AFTER INHALATIONOF $^{239}\text{PuO}_2$ AND $^{239}\text{PuF}_4$

Preliminary data show that americium and plutonium did not separate in lungs of dogs that inhaled $^{239}\text{PuF}_4$ or in those that inhaled $^{239}\text{PuO}_2$ heavily enriched with higher isotopes of plutonium and ^{241}Am . However, liver samples from the latter dogs showed increased levels of ^{241}Am , relative to plutonium.

Investigators:
W. J. Bair
P. O. Jackson*

The purpose of these studies is to determine whether ^{241}Am , a gamma emitter, is a valid tracer for ^{239}Pu in whole-body monitoring of humans. Plutonium-amerium ratios in tissues from a number of ^{239}Pu experiments are being compared with the ratios of the two radioelements in the aerosol sample collected at the time of exposure to determine whether separation of plutonium and americium occurred in the body. Results were previously presented for several forms of plutonium dioxide (Annual Reports, 1966, 1967).

Observations

Preliminary results are available

for dogs which inhaled $^{239}\text{PuF}_4$ and for dogs which inhaled $^{239}\text{PuO}_2$ enriched with ^{240}Pu , ^{241}Pu , ^{242}Pu , and ^{241}Am (Table I). None of the tissues thus far analyzed from these dogs indicate significant separation of the americium and plutonium except for liver of dogs which inhaled the enriched plutonium. These values suggest several-fold increased concentrations of americium relative to plutonium.

Conclusions

Until these studies are completed, the possible separation of ^{241}Am from ^{239}Pu after inhalation cannot be evaluated.

* Radiological Sciences Department

TABLE I. Plutonium-Amercium Ratios in Dog Tissues after Inhalation of Plutonium

Dog Number	Compound Inhaled	Ratio: $^{239}\text{Pu} + ^{240}\text{Pu}/^{241}\text{Am}$			
		Aerosol	Lung - Left Diaphragmatic Lobe	Bronchial Lymph Node	Liver
429	PuF_4 (a)	6.5	8.3	6.6	
434			6.5	6.5	
436			8.1	-	
441			5.5	5.5	
448			7.2	-	
451			7.1	-	
330	$^{239}\text{PuO}_2$ (b)	1.9	1.9	1.8	0.44
338			1.8	1.8	0.33
350			1.9	1.8	-

(a) Tissues sampled 90 days after exposure.

(b) ^{239}Pu enriched with ^{240}Pu , ^{241}Pu , ^{242}Pu , and ^{241}Am . Tissues sampled 150 days after exposure.

DETERMINATION OF THE RATIO OF ^{241}Am TO $^{239,240}\text{Pu}$ IN SOFT TISSUES

An electrodeposition procedure for the simultaneous deposition of plutonium and americium allowing precise determination of the ratio $^{241}\text{Am}/^{239,240}\text{Pu}$ in ashed tissue samples by means of alpha energy analysis is evaluated.

Investigator:
P. O. Jackson*

Technical Assistance:
T. A. Jokela*

For the precise determination of plutonium-to-americium ratios in biological samples, an analysis technique was needed which minimized the risk of preferential loss of either radionuclide. An electrodeposition procedure which simultaneously depos-

its both actinides on a platinum disc suitable for alpha energy analysis has the advantage of a single-step process with minimal manipulation. Such a plating procedure was developed⁽¹⁾ and its application is described in this report.

* Radiological Sciences Department

Observations

The method may be briefly described as follows:

- Ash sample with HNO_3 , 30% H_2O_2 , HClO_4 . Store in 4M HNO_3 .
- Convert suitable aliquot to chlorides with HCl . Evaporate.
- Add ~0.1 g hydroxylamine hydrochloride and dissolve salts in 4 ml saturated NH_4Cl solution. Transfer to electroplating cell.
- Add 10 μl 2.7M HF , 5 μg U, and three drops methyl violet.
- Electroplate at 2A onto a platinum disc.
- Add 5 μg U again after 5 min and 15 min.
- After 45 min, add 3 ml of concentrated NH_4OH and remove current.
- Rinse electrolyte to beaker with water, acidify with concentrated HNO_3 , evaporate, and mount for alpha assay.
- Dismantle cell and rinse disc with water and ethanol. Dry and flame disc.
- Use silicon surface barrier detector for alpha energy analysis with multichannel analyzer.

The ratio of 5.48 MeV alphas (^{241}Am and ^{238}Pu) to 5.15 MeV alphas ($^{239,240}\text{Pu}$) is determined on an aliquot of the total sample. The ratio of 5.48 MeV alphas (^{238}Pu) to 5.15 MeV alphas ($^{239,240}\text{Pu}$) is determined by the same procedure after extracting Pu into 0.45M TTA in xylene from 1M HNO_3 and back extracting into 8M HNO_3 . From these two measurements, one can calculate the ratio of ^{241}Am to $^{239,240}\text{Pu}$.

A 270 keV energy band was summed for each alpha peak in alpha energy analysis calculations. Peak resolution was approximately 60 keV (F.W.H.M.) for thin sources. Counting periods were sufficient to achieve a minimum of 50,000 counts in each peak. Correction for alpha tailing was made by assuming that the 5.48 MeV peak overlapped the 5.15 MeV peak in proportion to a similar peak overlap between the 5.15 MeV and the region at 4.82 MeV.

Careful techniques were necessary to achieve consistent quantitative recoveries. Excessive salts accompanying the samples had an inhibiting effect on radionuclide deposition. A reduced plating efficiency was always accompanied by a white or gray deposit on the cathode. Bone samples always had a heavy white deposit and serious plating loss. Liver samples formed a visible deposit but quantitative recoveries were achieved when 0.1 to 1% of the organ was used. Quantitative thin deposits were normally attained for the lung and lymph node aliquots. Recoveries below 90% were accompanied by a preferential loss of plutonium.

The reproducibility of $^{241}\text{Am}/^{239,240}\text{Pu}$ ratios in replicate electrodepositions with recoveries exceeding 95% is shown in Table I. An average and maximum precision of $\pm 1.4\%$ and $\pm 3.6\%$, respectively, is indicated. Based on these results, a difference of 7% between two independent ratio determinations would be significant.

TABLE I. Precision of Replicate Analyses

Sample	Recovery, %	²⁴¹ Am 239, 240Pu	Range	σ (a)	$\sigma/\bar{x}$
1	99	0.515	0.0018	0.0016	0.0031
	98	0.517			
2	95	0.547	0.022	0.020	0.036
	95	0.525			
3	96	0.526	0.0011	0.00098	0.0019
	97	0.525			
4	100	0.531	0.0187	0.0110	0.020
	100	0.546			
	100	0.550			
5	97	0.569	0.0065	0.0058	0.010
	96	0.576			

Average Coefficient of Variation ($\sigma/\bar{x}$) = 0.014

(a) σ based on range estimate

Conclusions

This method has been satisfactory for the high specific activity soft tissue samples resulting from laboratory exposures to plutonium and americium compounds. For bone samples or low specific activity tissues, a method of removing interfering con-

stituents such as calcium is under investigation.

Reference

1. Jackson, P. O. Annual Report for 1967 to the USAEC Division of Biology and Medicine, Volume II: Physical Sciences, Part 2, Radiological Sciences, pp. 1-4, BNWL-715 2. Battelle-Northwest, Richland, Washington. 1968.

CONCENTRATION OF $^{239}\text{PuO}_2$ PARTICLES WITHIN PHAGOCYtic CELLS

A marked concentration of intraperitoneally injected plutonium particles within mononuclear phagocytes was demonstrated by kinetic studies and by electron microscopy.

Investigator:
C. L. Sanders

Technical Assistance:
R. R. Adee

Recent studies in this laboratory have described the interaction of plutonium particles with phagocytic cells. Intraperitoneally injected plutonium particles are rapidly phagocytized and transported to the lymphatics of the omentum and mesentery (Annual Report, 1967). The radiation dose distribution from radionuclide particles can be greatly affected by this process, resulting in intense "hot spots" of radioactivity around phagocytes, as demonstrated by the experiments described in this report.

Observations

Female rats were intraperitoneally injected with 1.4 μCi or 19 μCi $^{239}\text{PuO}_2$ particles (CMD, 0.12 μm), suspended in 2 ml saline. Peritoneal fluid cells were isolated from subsequent washings of the peritoneal cavity and examined under light and electron microscopes. The quantity of plutonium in the wash and the cellular composition were determined at intervals following injection. The number of cells in the wash was determined by a total DNA-deoxyribose analysis, dividing this value by the aver-

age amount of DNA-deoxyribose in a diploid rat cell. The intracellular accumulation of particles in phagocytes was studied autoradiographically.

Limited aggregation of $^{239}\text{PuO}_2$ particles was observed in extracellular spaces prior to their engulfment by the peritoneal mononuclear phagocytes (PMP). However, the degree of extracellular aggregation was small compared to that which occurred within phagosomes of the PMP.

Only a few large particles of 1 to 2 μm diameter were phagocytized during the first 15 min following administration. Most particles of less than 1 μm diameter remained in extracellular spaces at this time. Very few particles were extracellular by 60 min following administration. They were engulfed by PMPs, either concentrated in one large phagocytic vacuole of several micrometers diameter (Figure 1), or in several closely spaced phagosomes (Figure 2).

Many phagocytes sampled during the first hour following plutonium administration exhibited marked ultrastructural alterations which would no doubt lead to the death of the cells

18,400X

FIGURE 1. Peritoneal Mononuclear Phagocyte at 1 hr after Administration of 19 μ Ci $^{239}\text{PuO}_2$ Particles (Numerous plutonium particles are scattered within a large phagocytic vacuole.)

(Figure 2). No such changes were observed following only saline administration. Following plutonium, many whole dead cells were found within membrane-limited phagosomes of PMPs. Other PMPs contained numerous inclusion bodies resulting from the engulfment of cell debris. There was a moderate cytoplasmic vacuolization and an increased number of lysosomes in some PMPs. In the most affected PMPs, the mitochondria were swollen and vacuolated, the Golgi complex and the endoplasmic reticulum were dilated or

disrupted, and the nucleus was swollen and of decreased electron opacity, indicative of lysis of nuclear material. The overall picture was one of cellular necrosis and phagocytosis of dead cells or cell debris.

Some PMPs exhibited a remarkable ability to engulf large quantities of plutonium and yet maintain a reasonably normal structural configuration. Unfortunately, the residence time of particles in the PMPs was unknown;

18,000X

FIGURE 2. Peritoneal Mononuclear Phagocyte at 1 hr After Administration of 19 μ Ci $^{239}\text{PuO}_2$ Particles (Well defined individual plutonium particles are present within several phagocytic vacuoles. This phagocyte displays degenerative changes leading to the probable death of this cell and release of engulfed plutonium particles.)

particles could have been phagocytized only minutes before sampling. In general, the severity of cellular alteration was greatest in PMPs containing the greatest amount of plutonium. Alpha irradiation was undoubtedly the damaging agent since phagocytized thorium dioxide colloid or latex beads did not produce such changes.

The qualitative observation of plutonium particle accumulation within the PMP was corroborated by a quantitative calculation of phagocytized plutonium. Data required for this computation are shown in Table I. The average amount of plutonium (μ Ci) phagocytized by each active phagocyte (i.e., phagocyte which has engulfed plutonium particles as distinguished

TABLE I. Parameters Used for Determining the Amount of Plutonium in Peritoneal Mononuclear Phagocytes at Various Times After Intraperitoneal Injection of 1.4 μCi $^{239}\text{PuO}_2$ Particles [All values are means ($N = 3-5$) from data accumulated during this study.]

Time Post-injection, hr	^{239}Pu in Wash		Peritoneal Fluid Nucleated Cell Count		Percent of Mononuclears with Phagocytized ^{239}Pu
	Total, μCi	Percent Phagocytized	Total, $\times 10^6$	Percent Mononuclear Cells	
0.5	0.98	40	7.5(a)	87	10
1.0	0.92	71	8.9	83	16
2.0	0.82	92	9.2(a)	78	28
3.0	0.73	92	9.5(a)	74	32
4.0	0.67	96	10.0	72	35
5.0	0.45	94	11.0(a)	70	19
6.0	0.26	93	12.5	67	9
7.0	0.06	92	13.0(a)	65	3

(a) Estimated by interpolation

from one which has not) was calculated from the expression: $\mu\text{Ci Pu/phagocyte} = \mu\text{Ci } ^{239}\text{Pu in wash} \times \text{percent } ^{239}\text{PuO}_2 \text{ particles phagocytized/mononuclear count} \times \text{fraction of mononuclears with phagocytized } ^{239}\text{PuO}_2$. The equivalent number of 0.12- μm diameter* particles ingested per active phagocyte is obtained by dividing this expression by $5.8 \times 10^{-10} \mu\text{Ci/0.12-}\mu\text{m diameter } ^{239}\text{PuO}_2 \text{ particle}$. The marked accumulation of plutonium particles per PMP is shown in Figure 3 as a function of time after administration of particles. The accumulation of particles was highest at 30 min and then fell during the ensuing 3 hr to about half of this 30-min

value. During the period from 3 to 7 hr, the average active phagocyte contained the equivalent of approximately 500, 0.12- μm diameter plutonium particles.

Such packing of plutonium particles within phagosomes of several micrometers diameter results in hot spots of intense alpha activity. Approximately 10% of the emitted energy may be absorbed by the phagocyte, depending on the size of the phagocyte and the intracellular location of particles. Self-absorption of alpha energy by a 1- μm diameter, or smaller $^{239}\text{PuO}_2$ particle, amounts to <10%. An average active phagocyte, containing the equivalent of 500, 0.12- μm diameter particles, or the equivalent of one, 1- μm diameter particle, will receive an estimated alpha dose of about 225 rad/hr.

* This value was chosen since the particles administered in this study had a mean diameter of 0.12 μm .

FIGURE 3. Average Equivalent Numbers of 0.12- μm Diameter Particles Phagocytized by Individual Peritoneal Mononuclear Phagocytes at Intervals Following Intraperitoneal Administration of 1.4 μCi $^{239}\text{PuO}_2$ Particles (These average values were computed from data presented in Table I.)

MAINTENANCE OF PHAGOCYTTIC FUNCTION FOLLOWING $^{239}\text{PuO}_2$ PARTICLE ADMINISTRATION

Latex beads were phagocytized at a normal rate by phagocytes in the peritoneal fluid despite the deposition of large quantities of plutonium particles in the peritoneal cavity. The phagocytosis of beads was reduced in only those phagocytes which had previously engulfed large plutonium particles.

The resistance of the reticulo-endothelial system (RES) to external irradiation has been well documented. The functioning of the RES in an iso-

Conclusions

Phagocytes appear to play an important role in determining the early fate of deposited $^{239}\text{PuO}_2$ particles. Plutonium particles found in the peritoneal fluid after the first few hours are nearly all within phagocytes, irrespective of the amount of administered plutonium. The phagocytized particles are concentrated within cytoplasmic vacuoles. The alpha dose distribution is therefore largely influenced, in the early stages, by the distribution of phagocytes and their concentration of plutonium particles.

Investigator:
C. L. Sanders

Technical Assistance:
D. L. Gayman

lated body cavity, such as the peritoneal cavity, following deposition of plutonium particles has not been

tested. Previous studies have demonstrated the ability of peritoneal mononuclear phagocytes to rapidly phagocytize, concentrate, and transport plutonium particles. Marked cell damage and death were observed following deposition of plutonium particles, the severity of which depended on the amount of plutonium deposited in the peritoneal cavity. Latex beads were injected into the peritoneal cavity as a test particulate for the determination of phagocytic function following plutonium particle deposition.

Observations

Female rats were given intraperitoneal injections of either 1.4 μCi or 19 μCi $^{239}\text{PuO}_2$ particles (mean diameter, 0.12 μm) suspended in 2 ml saline. Animals were selected at intervals following plutonium injection and injected with 1 mg latex beads (2.0 μm mean diameter). Peritoneal fluid cells were isolated from washes of the peritoneal cavity and examined by light microscopy.

Intraperitoneally injected latex beads were rapidly engulfed by mononuclear phagocytes. Over 80% of the beads were found within phagocytes by 15 min postinjection. Plutonium particles were engulfed at a slower rate with an 80% intracellular accumulation occurring between 60 and 120 min (Table I). The difference in rate of phagocytosis of latex beads and plutonium particles may be due to particle size or particle concentration differences. After the first few hours, essentially all the plutonium parti-

cles in the peritoneal fluid were found within phagocytes. The number of beads per phagocyte increased with time, paralleling in rate the intracellular accumulation of beads (Table II).

Plutonium particle administration resulted in a marked loss of mast cells, eosinophils, and small lymphocytes from the peritoneal fluid. Beyond the seventh day, nearly all cells found were large mononuclear cells (Table III). The number of mononuclear cells found on cytosmears was greatly increased at 7 to 14 days after 19 μCi $^{239}\text{PuO}_2$ particle administration. Administration of up to 19 μCi plutonium did not depress the rate or extent of latex bead phagocytosis at 7 to 14 days post-plutonium injection (Table IV). The number of beads per phagocyte was decreased at 7 to 14 days following plutonium (Table II) due largely to the increased number of phagocytes in the peritoneal fluid, which, in effect, diluted the number of beads available for engulfment per phagocyte.

Plutonium particles were observed within phagocytes either visually or by autoradiography. Large cytoplasmic vacuoles often enclosed large plutonium particles within phagocytes. Numerous latex beads were seen together with plutonium particles in the same phagocyte. The two kinds of particulates were usually segregated into different cytoplasmic areas. Numerous intact phagocytes containing large plutonium particles were undergoing nuclear degeneration. Many phagocytes, at 7 to 14 days following plutonium, contained cell

TABLE I. Phagocytosis of Latex Beads and Plutonium Particles by Peritoneal Mononuclear Cells

Time Post-Injection, min	No. of Rats	Percent of Particles Phagocytized ($\bar{x} \pm SD$)	Phagocytic Index ^(a) ($\bar{x} \pm SD$)
<u>Latex Beads</u>			
2	5	34 $\pm$ 23	42 $\pm$ 7
5	6	63 $\pm$ 6	46 $\pm$ 23
10	5	71 $\pm$ 11	34 $\pm$ 20
15	5	83 $\pm$ 8	55 $\pm$ 14
30	10	76 $\pm$ 16	60 $\pm$ 8
60	6	87 $\pm$ 7	46 $\pm$ 15
<u>Plutonium Particles</u>			
10	6	14 $\pm$ 5 ^(b)	6 $\pm$ 3
30	5	34 $\pm$ 9 ^(b)	13 $\pm$ 5
60	7	73 $\pm$ 9 ^(b)	18 $\pm$ 4
120	4	92 $\pm$ 5 ^(b)	26 $\pm$ 4

(a) Percentage of mononuclear cells with engulfed particulates.

(b) Values probably low due to difficulty in resolving intracellularly aggregated submicron-size particles; extracellular aggregation was minimal.

TABLE II. Influence of Exposure to Irradiations from ^{239}Pu on the Distribution of Injected Latex Beads within Peritoneal Phagocytes

Treatment	Time After Bead Injection, min	No. of Rats	Percentage of Bead-Containing Cells That Contain a Specified Number of Beads							
			1	2	3	4	5	6	7	>8
Control (No ^{239}Pu)	2	5	32.5	22.9	13.5	10.2	5.7	2.6	4.2	8
	5	6	29.5	19.5	13.3	9.7	5.7	4.9	4.9	12
	10	5	17.9	16.1	10.9	9.6	10.9	7.4	7.3	19
	15	5	12.9	11.3	11.9	8.7	8.2	7.3	5.1	34
	30	10	8.5	8.1	6.6	7.1	5.8	6.0	5.9	52
	60	6	9.2	10.0	8.4	7.9	8.7	6.1	8.4	41
1.4 $\mu\text{Ci } ^{239}\text{Pu}$, 7 days prior to bead injection	2	6	20.6	12.7	14.6	10.9	8.3	8.8	5.4	18
	60	6	12.5	11.5	10.7	6.7	7.4	6.5	5.4	39
19 $\mu\text{Ci } ^{239}\text{Pu}$, 7 days prior to bead injection	60	6	13.1	13.2	10.7	8.4	5.7	6.9	5.7	36
19 $\mu\text{Ci } ^{239}\text{Pu}$, 14 days prior to bead injection	60	6	13.8	11.6	8.2	11.3	11.1	6.0	6.8	31

TABLE III. Influence of Exposure to Radiations from ^{239}Pu on the Cellular Composition of the Peritoneal Fluid

Treatment (a)	Time After Injection	No. of Rats	Cell Differential ($\bar{x} \pm \text{SD}$) (c)		
			Mast Cells	Eosinophils	Mononuclear Cells (d)
None	-	9	5.4 $\pm$ 2.0	13 $\pm$ 2	82 $\pm$ 4
Saline	60 min	6	14 $\pm$ 9	11 $\pm$ 3	75 $\pm$ 9
Latex beads	0-60 min (b)	37 (b)	11 $\pm$ 5	12 $\pm$ 6	77 $\pm$ 7
1.4 $\mu\text{Ci } ^{239}\text{Pu}$	0-60 min (b)	18 (b)	8.4 $\pm$ 4.7	7.3 $\pm$ 4.3	84 $\pm$ 6
1.4 $\mu\text{Ci } ^{239}\text{Pu}$	7 days	12	<0.1	6.0 $\pm$ 5.2	94 $\pm$ 5
19 $\mu\text{Ci } ^{239}\text{Pu}$	7 days	6	0.0	1.0 $\pm$ 1.2	99 $\pm$ 1
19 $\mu\text{Ci } ^{239}\text{Pu}$	14 days	6	0.0	0.5 $\pm$ 0.8	99 $\pm$ 1

(a) Particulates injected with 2 ml saline.

(b) Cumulative totals for all animals sampled during the first hour.

(c) Neutrophils, not included, accounted for less than 5% of all nucleated cells at these times.

(d) Includes lymphocytes, monocytes, and macrophages.

TABLE IV. Influence of Exposure to Radiations from ^{239}Pu on the Phagocytosis of Latex Beads by Peritoneal Mononuclear Cells

^{239}Pu Injected, μCi	Time of Bead Injection, days post ^{239}Pu	Time of Observation, min postbead injection	No. of Rats	Percent of Beads Phagocytized ($\bar{x} \pm \text{SD}$)	Phagocytic Index (a) ($\bar{x} \pm \text{SD}$)
1.4	7	2	6	42 $\pm$ 18	48 $\pm$ 18
1.4	7	60	6	95 $\pm$ 3.2	67 $\pm$ 15
19.0	7	60	6	97 $\pm$ 0.9	39 $\pm$ 5.7
19.0	14	60	6	96 $\pm$ 1.9	34 $\pm$ 5.0

(a) Percentage of mononuclear cells with engulfed beads.

debris and dead cells as well as latex beads and/or plutonium particles (Figure 1).

The phagocytosis of latex beads was depressed in only those phagocytes which contained visible large plutonium particles. When plutonium

and latex beads were administered simultaneously, about 90% of phagocytes that contained visible plutonium particles also contained one or more latex beads. This value decreased to 61% when bead injection was delayed for 7 days and to 37%

1280X

FIGURE 1. Photomicrographs Demonstrating the Phagocytosis of Plutonium Particles, Latex Beads, and Cell Debris by Peritoneal Mononuclear Cells (Giemsa Stain)

- [(a) Engulfed latex beads and a large plutonium particle (arrow) within a phagocyte 1 hr after the simultaneous injection of 1 mg latex beads and 19 μCi $^{239}\text{PuO}_2$ particles.
- (b) Autoradiographic demonstration of phagocytized small plutonium particles in a cell also containing latex beads; 7 days after injection of 1.4 μCi $^{239}\text{PuO}_2$ particles and 60 min after injection of latex beads.
- (c) Large plutonium particle (arrow) found within a large cytoplasmic vacuole of a phagocyte; 7 days after injection of 19 μCi $^{239}\text{PuO}_2$ particles and 60 min after injection of latex beads. Note the whole cell and cell debris and the absence of latex beads.
- (d) Engulfment of latex beads by phagocytes, 14 days after the injection of 19 μCi $^{239}\text{PuO}_2$ particles and 60 min after injection of latex beads. Note the whole cell and cell debris also present within these phagocytes.
- (e) Same as d; note the engulfed large plutonium particle (arrow) and cell debris and the absence of latex beads.
- (f) Same as d; note the large engulfed plutonium particle (arrow) and the destruction of the cell's nucleus.]

when bead injection was delayed for 14 days. The number of beads per phagocyte also decreased with increasing time after plutonium administration (Table V).

Conclusions

This study confirms previous indications that the RES retains its

normal ability to engulf foreign particulates despite the deposition of large amounts of alpha-emitting nuclides, either systemically or within an isolated body cavity. The phagocytosis of latex beads was reduced in only those phagocytes which had previously engulfed large plutonium particles.

TABLE V. Phagocytosis of Latex Beads by Peritoneal Mononuclear Phagocytes Which Had Also Engulfed Large Plutonium Particles, Visible at 1600X under the Light Microscope (The latex beads were injected either simultaneously with 19 μ Ci $^{239}\text{PuO}_2$ particles or were injected 7 or 14 days after injection of 19 μ Ci plutonium particles. Samples of peritoneal cells were obtained at 60 min after latex bead administration. All values are means of six animals.)

Time of Bead Injection, days post ^{239}Pu	Phagocytic Index ^(a) ($\bar{x} \pm \text{SD}$)	Percentage of Bead-Containing Cells That Contained a Specified Number of Beads							
		1	2	3	4	5	6	7	>8
0	89 $\pm$ 4	6.3	7.0	5.8	6.5	5.7	6.8	8.5	53
7	61 $\pm$ 9	14.0	9.2	11.1	6.3	4.1	7.8	5.7	41
14	37 $\pm$ 7	17.1	12.9	16.7	9.6	9.0	4.8	5.3	24

(a) Percentage of mononuclear cells containing visible plutonium particles, that also contained one or more latex beads.

DISTRIBUTION OF INHALED $^{239}\text{PuO}_2$ PARTICLES WITHIN PULMONARY MACROPHAGES

Inhaled plutonium particles were rapidly phagocytized and retained within pulmonary macrophages of the rat for at least 40 days. Phagocytic indexes correlated well with the log ^{239}Pu content of the lung.

Investigator:
C. L. Sanders

Technical Assistance:
V. T. Faubert

Pulmonary macrophages are thought to constitute an important clearance vector due to their ability to phagocytize particulate material and "migrate" to the terminal bronchioles where they are cleared via ciliary mucous flow. The objective of the present study was to demonstrate, in a quantitative manner, the role played by pulmonary macrophages in effecting the early distribution and fate of inhaled plutonium particles.

Observations

Female rats were exposed to an aerosol of $^{239}\text{PuO}_2$ particles. At intervals following inhalation, rats were sacrificed and their lungs removed and washed with up to 19 consecutive 12 ml saline washes. The lungs and selected lung washings were assayed for ^{239}Pu . Particles and cells from lung washings were examined microscopically and by autoradiography.

Washing the lung with 19 separate 12 ml saline washes did not appear to result in a loss of cellular elements comprising the alveolar walls. Indeed, free pulmonary macrophages were often found in alveoli even after

this aggressive washing regimen. The phagocytic index (percent of macrophages containing particles) in the first lung wash was essentially the same as that found in all succeeding washes, irrespective of the time at which washing occurred after inhalation of particles (Table I). This indicates that the first lung wash provides a sample which is representative of the alveolar distribution of plutonium particles phagocytized by macrophages.

The presence of plutonium particles within pulmonary macrophages was autoradiographically demonstrated in lung sections and cytosmears (Figure 1). There was an early phase of rapid but incomplete accumulation of plutonium particles within pulmonary macrophages. From 10 to 55% of all plutonium particles found in the first wash during the first 3 hr following inhalation had been phagocytized by macrophages. Usually over 90% of these particles were found within macrophages from the second to the fortieth day after inhalation (Figure 2). The percentage of phagocytized particles found in lung washes during the first 10 hr after

TABLE I. Phagocytosis of Plutonium Particles by Pulmonary Macrophages Sampled by Serial Lung Washing Following Inhalation of 1 to 4 $\mu\text{Ci } ^{239}\text{PuO}_2$ (Each value is an individual measurement.)

Time After Inhalation	Percent of Macrophages Containing Particles (Phagocytic Index)							
	Wash Number							
	1	4	7	9	12	14	17	19
1 hour	40	47	43	48	49	42	45	45
3 hour	58	74	65	60	58	67	64	67
6 hour	76	75	79	69	--	--	--	72
8 hour	84	76	83	81	79	73	76	81
10 hour	74	79	83	81	75	80	81	74
2 days	84	75	70	71	75	81	76	80
8 days	80	--	86	--	--	81	--	76

	Percent of Particles in Macrophages							
1 hour	9	51	74	79	83	65	62	84
3 hour	17	83	78	69	78	74	75	82
6 hour	22	77	83	90	--	--	--	94
8 hour	38	85	85	88	93	88	93	85
10 hour	47	66	71	90	85	88	89	91
2 days	90	91	90	94	93	91	92	95
8 days	96	--	97	--	--	97	--	96

1040X

FIGURE 1. Autoradiographic Demonstration of the Phagocytosis of $^{239}\text{PuO}_2$ Particles by Pulmonary Macrophages [A. Lung section embedded in Epon, 7 days after inhalation (Azure II and methylene blue stains). B. Pulmonary macrophage from a cytosmear (Giemsa stain).]

FIGURE 2. Intracellular Accumulation of $^{239}\text{PuO}_2$ Particles Within Pulmonary Macrophages, as Measured in the Initial Lung Wash, at Intervals After Inhalation (Each point represents an individual measurement.)

inhalation increased with each succeeding wash of the same lung. This dependence on the sequence of lung washes was no longer apparent by the second day postinhalation (Table I).

The phagocytic index increased during the first few hours following inhalation and thereafter remained relatively steady during the ensuing 16 days although considerable variation among individuals was observed. A trend toward a decreasing phagocytic index was observed from 16 to 40 days after exposure (Figure 3). The phagocytic index was higher in animals with larger amounts of plutonium deposited in the lung. A good correlation was found between the log

^{239}Pu content of the lung and the phagocytic index, as shown in Figure 4, which includes all data from 1 hr to 18 days after inhalation.

Upwards of 50 to 60% of lung plutonium was removed by 19 lung washings, essentially all this plutonium being present within pulmonary macrophages. The therapeutic implications of this finding are considered elsewhere in this report.

Conclusions

The rate of phagocytosis of inhaled plutonium particles by pulmonary macrophages was similar to that observed previously in the peritoneal

FIGURE 3. Phagocytic Indexes of Pulmonary Macrophages as Measured in the Initial Lung Wash, at Intervals Following Inhalation of $^{239}\text{PuO}_2$ Particles (Each point represents an individual measurement. Symbols represent plutonium deposition levels as defined in Figure 2.)

cavity (Annual Report, 1967). Most of these phagocytized particles were probably initially deposited in the alveoli. Particles initially deposited in the trachea, bronchi, and bronchioles are rapidly cleared during the first day following exposure. The first lung wash of rats, during the 1 to 10 hr period following inhalation, probably sampled mostly those particles present in the trachea, bronchi, and bronchioles, thus leading to comparatively low values for the intracellular accumulation of particles within macrophages. Succeeding washings would sample proportionally fewer of these particles since they would have been preferentially

removed in the first few washings. The similar phagocytic indexes found in serial washes of the same lung indicated that the macrophages and associated plutonium particles were sampled, in a predictable manner, from the alveoli.

The number of particles phagocytized or the number of macrophages containing engulfed particles was dependent on the number of particles deposited in the alveoli. Thus, it should be possible to predict, within limits, the amount of plutonium present in the lung during the first few weeks following inhalation exposure by washing macrophages from the lung during this period.

FIGURE 4. *Correlation Between Phagocytic Index and Log of Plutonium Present in the Lung After One Wash. [Symbols have the significance defined in Figure 2 and represent data from individual measurements made from 1 hr to 25 days after inhalation. The center solid line is the regression line (correlation coefficient, 0.91). The outer solid lines represent the 95% confidence intervals around the regression line. The outermost dashed lines represent the 95% confidence intervals for individual samples.]*

The technique of lung washing combined with autoradiography, in living or killed animals, offers an experimental approach to the quantitative

study of the deposition, phagocytosis, distribution, and retention of inhaled radioactive particles.

DISTRIBUTION OF ALPHA ENERGY IN ALVEOLI FOLLOWING DEPOSITION
OF $^{239}\text{PuO}_2$ PARTICLES

A hybrid computer, alveolar model, was developed for determining the distribution of alpha energy around deposited $^{239}\text{PuO}_2$ particles. The energy distribution is modified by the position of the particle in the alveolar wall and by phagocytosis by alveolar macrophages.

Investigators:
C. L. Sanders
P. J. Dionne*

Recent studies have demonstrated the rapid phagocytosis of inhaled $^{239}\text{PuO}_2$ particles deposited in the alveoli of rat lung. Many of these particles are retained within pulmonary macrophages for extended periods of time, resulting in an altered distribution of emitted alpha energy. Using hybrid computer techniques, we have made a preliminary effort to model the dosimetric situation in the lung, particularly as influenced by the exact position of the particle with respect to the alveolar cells.

Observations

A 0.5- to 1.0- μm thick section of rat lung, fixed in gluteraldehyde and embedded in Epon 812, was placed on a glass slide and stained with methylene blue and Azure II. A photomicrographic montage of a representative alveolus was prepared and an outline of the alveolus and surrounding tissue, measuring about 220 μm $\times$ 220 μm ,

was traced onto graph paper. The grid points of the alveolar walls and the surrounding alveolar air spaces were placed into a FORTRAN program and displayed on an oscilloscope screen (Figure 1). A digital portion of a hybrid computer generates this alveolar map while an analog computer is used to plot the distribution of alpha energy over the map.

FIGURE 1. *The Computer Array of the Alveolus as Displayed on an Oscilloscope Screen (The arrow represents a hypothetical placement of a plutonium particle. The dark areas in the alveolar wall represent nuclei.)*

* Control and Instrumentation Department, Systems and Electronics Division

Plutonium particles were arbitrarily located in the alveolar wall or in the alveolar air space. Particles in the air space were either free or were placed within a 12- μ m diameter alveolar macrophage, superimposed on the map. The scanning program traces out 360 vectors (corresponding to 360 alpha particle tracks at 1° intervals). The appropriate Bragg alpha absorption curves for tissue were used for computing alpha energy release along the paths of alpha emissions. The absorption of alpha energy in air saturated with water is negligible (maximum penetration of 38 mm in air) as compared to absorption in tissue (maximum penetration of 48 μ m in tissue). The new array, in the form of a shadow representing alpha energy distribution, is displayed on the oscilloscope and a picture is taken with a Polaroid camera. The fraction of the total grid spaces covered by each dose array is recorded for each particle configuration.

The distribution of alpha energy from a point source, such as a plutonium particle, is influenced both by

the position of the particle in the alveolar array and by the phagocytosis of the particle. An example of an alpha energy distribution is shown in Figure 2. The plutonium particle was placed in the alveolar space and the energy distribution compared when the particle was free or engulfed by a 12- μ m diameter pulmonary macrophage.

Conclusions

Preliminary results indicate that a large fraction of the alpha dose is deposited within the alveolus and the immediately surrounding alveolar walls. Engulfment of particles by alveolar macrophages significantly shields the cells in the adjacent alveolar walls. Further studies are in progress to determine the influence of position of the particle in the alveolar array and the effect of size of phagocytizing alveolar macrophage on the distribution of alpha dose. It is hoped that such studies will provide a better understanding of the factors influencing alpha dose distribution in the lung.

FIGURE 2. Alpha Dose Distribution in the Alveolar Array
[The dose distribution is represented by the light area
comprised of lines emitted from a plutonium particle
(arrows) at same position as in Figure 1.]

- 2a. Full energy (5.0×10^6 eV) absorption display from free plutonium particle.
- 2b. Half energy (2.5×10^6 eV) absorption display from free plutonium particle.
- 2c. Full energy absorption display from plutonium particle in center of 12- μ m diameter macrophage.
- 2d. Half energy absorption display from plutonium particle in center of 12- μ m diameter macrophage.

LONG-TERM RETENTION AND TRANSLOCATION OF INHALED ^{106}Ru - $^{106}\text{RhO}_2$ IN BEAGLES

External counting of six dogs that received single inhalation exposures to ^{106}Ru - $^{106}\text{RhO}_2$ showed whole-body biological retention half-times ranging from 1750-3230 days. Longitudinal scanning and periodic sacrifices of these animals showed avid pulmonary retention and minimal translocation of the isotope pair to systemic organs. Two animals showed 11 and 17% of the total-body burden in the tracheobronchial lymph nodes at 39 months after exposure.

Investigator:
B. O. Stuart

Technical Assistance:
J. C. Gaven

Six beagle dogs received single inhalation exposures to ^{106}Ru - $^{106}\text{RhO}_2$ particles of 0.07-0.12 μm count median diameter and were sacrificed at intervals extending to 39 months after exposure. External gamma-ray monitoring of the total body showed little elimination of the isotope-pair beyond the first few weeks after exposures, with effective retention curves closely paralleling the 1.0 year physical decay of ^{106}Ru (Annual Report, 1966). This report describes the whole-body retention and distribution patterns of ^{106}Ru - $^{106}\text{RhO}_2$ at various times after initial pulmonary deposition of 20-30 μCi of the oxide.

Observations

Single exponential curves fitted to whole-body counting rates over 20 to 200 days after exposure indicated biological retention half-times of 900 to 1540 days (Annual Report, 1966), but continued whole-body count-

ing showed significantly longer half-times ranging from 1750 to 3230 days when curves were fitted from 100 to 540 days after exposure. These whole-body retention curves are shown in Figure 1. Longitudinal scanning of these animals showed radioactivity peaks located over the thorax at all times after exposure.

Table I lists the tissue distribution of ^{106}Ru - ^{106}Rh in beagle dogs as percent of total-body burden. At 7 months after exposure, two dogs showed 98% of their total-body burden in the lungs. By 3-1/4 years after exposure, the lung content was reduced to 82-85% of total-body burden, with the remainder translocated predominantly to lymph nodes. The significant translocation to the skeleton in one animal, reported earlier (Annual Report, 1966), was found to be in error.

None of the animals showed significant biological effects from these exposures; a slight lymphopenia

FIGURE 1. Whole-Body Retention of Inhaled ^{106}Ru - $^{106}\text{RhO}_2$ in Beagle Dogs (Uncorrected for radioactive decay)

TABLE I. Distribution of ^{106}Ru - ^{106}Rh in Beagles After Inhalation of ^{106}Ru - $^{106}\text{RhO}_2$ (Percent of total body burden at sacrifice)

Time after Exposure (months)	7		24	39	
Dog Number	348	353	358	354	356
Tracheobronchial lymph nodes	1.53	0.94	2.82	11.1	16.9
Mediastinal lymph nodes	0.083	0.003	0.32	3.6	---
Lungs	97.6	98.4	95.5	85.0	82.0
Spleen	0.007	0.013	0.003	0.005	0.003
Kidneys	0.006	0.007	0.013	0.005	0.033
Liver	0.056	0.080	0.034	0.116	0.356
Skeleton	0.17	0.24	0.16	0.090	0.250
Muscle	0.29	0.34	1.05	0.034	0.13
Pelt	0.146	0.019	0.008	0.001	0.014

seemed to develop within a month after exposure, but all blood cell levels showed no difference from controls at 1 year after exposure.

Conclusions

Studies of the biological disposition and effects of inhaled ^{106}Ru - $^{106}\text{RhO}_2$ particles in beagle dogs over several years showed extremely long

whole-body retentions with half-times approaching 9 years. Results of periodic sacrifices showed radioactivity remaining almost exclusively in the lungs, with less than 20% translocation to the tracheobronchial lymph nodes at 39 months after exposure. Initial alveolar depositions of 20-30 μCi of this isotope pair showed little effect on circulating blood cells.

EXPOSURE OF BEAGLES TO OAK RIDGE U_3O_8 ASH

In dogs, the thoracic retention half time for ^{235}U inhaled as a constituent of an industrial ash ranged from 131 to 171 days. This compares with values of 380 to 1470 days obtained for humans accidentally exposed to similar dust at Oak Ridge.

Investigators:

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Technical Assistance:

M. D. Snyder
W. Skinner

A number of persons were accidentally exposed to U_3O_8 enriched to about 90% ^{235}U at Oak Ridge, Tennessee.⁽¹⁾ Because the thoracic retention half time of ^{235}U ranged from 380 to 1470 days in these individuals compared to the 120-day half time quoted in the literature for U_3O_8 in animals, it was considered useful to test the actual exposure material in experimental animals.

Observations

Samples of dust suspected of being similar to that inhaled by the personnel at Oak Ridge were introduced into an aerosol chamber for exposure of four beagle dogs. The CMD of the aerosol was $0.23 \mu m$ and the σ_g was 1.9. The dogs were monitored periodically with a chest counter comprised of two $3/8 \times 5$ in. NaI crystals, and excreta were collected. The dogs were sacrificed after about 290 days.

Initial alveolar deposition ranged from 4.5 to 16 mg of ash or 0.1 to $0.36 \mu Ci$ ^{235}U (Table I). The body burdens at sacrifice, after 290 days, ranged from 0.023 to $0.076 \mu Ci$ ^{235}U . These values, determined from assay of all tissues, agreed reasonably well with values obtained from in vivo counting. During the observation period, the dogs excreted nearly 80% of the alveolar-deposited ^{235}U .

Figure 1 shows the thoracic retention of ^{235}U in two of the four dogs. Single exponentials were fitted to the data and the constants for all dogs are given in Table II. The retention half times ranged from 131 to 171 days for the period studied.

At time of sacrifice, 55 to 63% of the body burden of ^{235}U was in the lungs (Table III) and 21 to 40% in the tracheal bronchial lymph nodes. Less than 10% was distributed throughout all the other tissues, the kidney containing about 1%.

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TABLE I. ^{235}U Ash Content of Dogs After Inhalation Exposure

	Dog Number			
	471	475	476	477
Initial alveolar deposition (a)				
$\mu\text{Ci } ^{235}\text{U}$	0.36	0.19	0.10	0.20
mg Ash	16	8.1	4.5	8.7
Final body burden				
$\mu\text{Ci } ^{235}\text{U}$ (tissue assay)	0.076	0.039	0.023	0.062
$\mu\text{Ci } ^{235}\text{U}$ (in vivo counter)	0.078	0.046	0.032	0.056
Excreted - $\mu\text{Ci } ^{235}\text{U}$	0.28	0.15	0.08	0.14

(a) By extrapolation of thoracic retention curve to 0 time.

TABLE II. Constants for Thoracic Retention of ^{235}U in Beagle Dogs

Constants for Equation, $Y = Ae^{-bt}$	Dog Number			
	471	475	476	477
A (cpm)	866	440	245	473
b	-0.0053	-0.0048	-0.0040	-0.0043
Half time, $T_{1/2}$ (days)	131	145	171	160

TABLE III. Tissue Distribution of ^{235}U in Dogs 290 Days After Inhalation of ^{235}U Ash (Tissue content - % of body burden)

Tissue	Dog Number			
	471	475	476	477
Lung	62.8	63.5	55.3	55.3
Tracheobronchial lymph nodes	21	30.9	33.2	41.4
Spleen	6.3	0.32	0.22	0.0009
Skeleton	3.7	2.2	4	0.95
Muscle	2.8	0.9	0.86	0.34
Kidney	1	0.85	1.1	0.7
Mediastinal and sternal lymph nodes	1	0.25	1.7	0.59
Liver	0.6	0.44	1.7	0.32

FIGURE 1. Thoracic Retention of ^{235}U in Beagle Dogs

Conclusions

The results of this experiment with $^{235}\text{U}_3\text{O}_8$ inhaled as a constituent of a production ash agree in general with previously published results from studies of U_3O_8 . The thoracic retention half times were much shorter than those observed for accidentally exposed humans at Oak Ridge. Therefore, it can be tentatively concluded that either the humans inhaled an entirely different material than that given the dogs, or dogs and humans do not clear this par-

ticular ^{235}U dust from their lungs at the same rate. To resolve this, it will be helpful to compare additional data from the dog and human exposures. For example, accumulation of ^{235}U in lymph nodes should be compared in the two species as well as the response of the respective in vivo counting systems to this accumulation of ^{235}U .

Reference

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NON-EQUILIBRIUM TISSUE DISTRIBUTIONS OF ^{238}U & ^{234}U , ^{230}Th , ^{210}Pb , AND ^{210}Po IN BEAGLES AFTER INHALATION OF URANIUM ORE

A dog sacrificed 2 years after a short series of exposures, and another sacrificed 3 months after 6 months of exposures, showed marked non-equilibrium ratios of ^{234}U , ^{230}Th , ^{210}Pb , and ^{210}Po in both pulmonary and systemic organs. Levels of ^{230}Th were 5-20 times higher than ^{234}U in the lungs, tracheobronchial lymph nodes, liver, and kidneys. Levels of ^{230}Th ranged up to two orders of magnitude higher than ^{210}Pb levels in the spleen and kidneys of the dog sacrificed 2 years after exposures. Even in these long-term animals, the lungs remained the major reservoir for all radionuclides studied.

Investigator:
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Technical Assistance:
J. C. Gaven
W. Skinner

Previous studies involving the chronic inhalation exposure of rats to a pitchblende ore showed a rapid separation of ^{238}U & ^{234}U and ^{230}Th constituents of the inhaled material

in the lungs, and very dissimilar levels of these radionuclides in systemic organs, both during and after 8 weeks of exposures (Annual Report, 1964). Subsequently, four beagle

dogs were exposed to the same concentrations of ore dust (1 mg/liter) for 1 hr daily, but exposures were terminated after 8 days because of acute renal toxicity. Two of these were sacrificed when moribund, approximately 2 weeks after exposure, and showed roughly two times more ^{230}Th than ^{238}U in the tracheobronchial lymph nodes and lungs, indicating a rapid separation of these radionuclides (Annual Report, 1967). The other two dogs survived and were retained for long-term uranium-chain radionuclide distribution studies. Four additional dogs were subsequently exposed for 1/2 hr, twice daily, for 6 months, to airborne ore dust concentrations of 0.1 mg/liter.

Observations

Table I shows the tissue concentrations of various uranium-chain ra-

dionuclides determined in a dog (412) sacrificed 2 years after surviving 8 days of exposure to aerosols of 1 mg/liter pitchblende ore dust. The second dog (381) was sacrificed 3 months after cessation of 6 months of daily exposures to the same ore dust at concentrations of 0.1 mg/liter. Radiochemical analyses of the ore dust used in these studies showed secular equilibrium for all members of the chain; thus similar translocation and excretion rates for the various radionuclides would be indicated by equal radioactivity levels in the separate tissues. However, much higher levels of thorium than of uranium were found in all tissues.

Conclusions

Earlier studies involving exposure of rats and dogs to pitchblende

TABLE I. Organ Content of Uranium-Chain Radionuclides Following Inhalation of Pitchblende Ore Dust by Beagles (See text for experimental details)

Organ	U, μg	^{234}U , d/m	^{230}Th , d/m	^{210}Pb , d/m	^{210}Po , d/m
Dog 412					
Lung (a)	96	75 $\pm$ 8 (c)	500 $\pm$ 50	56 $\pm$ 10	70 $\pm$ 7
Lymph node (b)	5.3	4.1 $\pm$ 0.4	33 $\pm$ 7	3.6 $\pm$ 1	4.2 $\pm$ 1
Liver	88	69 $\pm$ 7	400 $\pm$ 100	17 $\pm$ 2	35 $\pm$ 4
Kidneys	15	12 $\pm$ 1	280 $\pm$ 30	2.1 $\pm$ 1	7.4 $\pm$ 1.5
Spleen	1.1	0.9 $\pm$ 0.1	24 $\pm$ 5	0.2 $\pm$ 1	0.8 $\pm$ 0.4
Dog 381					
Lung (a)	0.42	0.33 $\pm$ 0.04	5.5 $\pm$ 1	1.7 $\pm$ 1	4.6 $\pm$ 1
Lymph node (b)	0.09	0.07 $\pm$ 0.04	0.4 $\pm$ 1	0.3 $\pm$ 1	0.07 $\pm$ 0.2
Liver	0.49	0.38 $\pm$ 0.04	4.1 $\pm$ 1	1.2 $\pm$ 1	7.2 $\pm$ 1.5
Kidneys	0.44	0.34 $\pm$ 0.04	1.5 $\pm$ 1	0.0 $\pm$ 1	2.0 $\pm$ 0.7
Spleen	0.09	0.07 $\pm$ 0.04	1.1 $\pm$ 1	0.5 $\pm$ 1	0.0 $\pm$ 0.2

(a) Right diaphragmatic lobe

(b) Tracheobronchial nodes

(c) Estimated maximum error derived from the magnitude of known sources of bias and the intrinsic precisions of the methods

ore dust showed rapid in vivo separation of thorium from the parent uranium in the lungs, and totally different patterns of build-up and removal of these radionuclides in the systemic organs. The presently re-

ported long-term dog studies confirm an avid retention of ^{230}Th in the lungs and support the early findings of essentially independent translocation and retention patterns for different members of the uranium chain.

CHRONIC EXPOSURE OF HAMSTERS AND BEAGLES TO SIMULATED URANIUM MINE ATMOSPHERE POLLUTANTS*

The requisite laboratory facilities and inhalation exposure systems are nearing completion for life-span studies, using 600 hamsters and 60 beagle dogs, related to the problem of increased lung cancer in uranium miners. The hamsters are being exposed daily to combined or separate mine air contaminants of radon and its short-lived daughters, carnotite ore dust, and diesel exhaust fumes. The dogs will receive daily inhalation exposures to cigarette smoke and radon plus daughters.

Investigator:
B. O. Stuart

Technical Assistance:
J. C. Gaven
W. Skinner
K. C. Upton

The high incidence of lung cancer in uranium miners has emphasized the urgent need for studies to clarify the cause-effect relationships between inhalation exposure to a variety of mine air pollutants and the subsequent pulmonary response. Although it is generally assumed that alpha radiation from inhaled radon daughters may be the primary agent in the induction of tumors, a variety

of other toxic materials are found in mine air, including ore dust and diesel exhaust fumes. Most of the miners are also cigarette smokers.

Observations

The hamster experiments are designed to study the effects of inhalation of two levels of radon and daughters and the possible synergistic or contributing effects of carnotite ore dust and/or diesel exhaust aerosols. One hundred animals per group will be exposed daily 6 hr/day, 5 days/week, according to the following schedule:

* These studies are jointly supported by the Atomic Energy Commission and the National Institute for Environmental Health Sciences, Department of Health, Education and Welfare.

- Group 1. (Low level): 7 nCi radon per liter (90% RaA, 60% RaB, 40% RaC and C') → 30 Working Levels of Rn daughter radioactivity on laboratory air dust.
- Group 2. (High level): 0.14 μ Ci radon/liter (daughters as above) → 600 Working Levels on laboratory air dust.
- Group 3. (High level plus carnotite ore dust): Radon and radon daughter concentrations as in Group 2. Ore concentration of 50 million particles per cubic foot.
- Group 4. Controls.
- Group 5. Diesel exhaust.
- Group 6. Diesel exhaust (as in Group 5) plus high level radon and ore dust (as in Group 3).

Figures 1 and 2 show the exposure chambers and individually compartmented cages constructed for these studies. Exposure of Groups 1-4 is underway. Animals sacrificed from Groups 1 and 4 after 6 weeks of exposure show no difference in the lungs between control and low-level animals; however, the tracheas of the latter show slight

cellular and glandular changes in the lamina propria, with concomitant increase in connective tissue.

The dog experiments will involve three groups of 18 dogs each plus a group of controls to test specifically the possible contributory or synergistic effects of radon plus its daughters and cigarette smoking. The dogs will smoke cigarettes in a manner strictly analogous to humans.

The experimental design is as follows:

- Group 1. Radon at 0.14 nCi/liter with radon daughters with carnotite ore. Exposure for 4 hr/day, 5 days/week. Sham exposure to cigarette smoke.
- Group 2. Radon plus radon daughters with ore as in Group 1 plus two working shifts of smoking (total 10-20 cigarettes per day with resting on alternate hours).
- Group 3. No radon; smoking as in Group 2.
- Group 4. Controls. Sham exposure only.

Dog-holding boxes and a multiple-dog exposure chamber fabricated for this experiment are shown in Figure 3.

FIGURE 1. Typical Chamber for Daily Exposure of 100 Hamsters to Constituents of Uranium Mine Atmosphere.

FIGURE 2. *Wedge of 17 Individual Compartments for Hamster Inhalation Exposure*

FIGURE 3. Exposure Chamber Plus Ventilated Dog Holding Boxes for Simultaneous Exposure of Ten Dogs to Radon Daughters with Uranium Ore Dust

DETERMINATION OF TRACE LEVELS OF URANIUM, ^{230}Th , ^{210}Pb ,
AND ^{210}Po IN SOFT TISSUES

A method was developed for the determination of trace concentrations of U, ^{230}Th , ^{210}Pb , and ^{210}Po in soft tissues of animals exposed to airborne uranium ore dust. The techniques were applied to urine and such tissue samples as lung, liver, spleen, kidney, and lymph node.

Investigators:

*P. O. Jackson**
B. O. Stuart

Technical Assistance:

*T. A. Jokela**

The evaluation of radiation exposure to uranium miners from the inhalation of airborne uranium ore dust requires information on the concentration of uranium and its daughters in the various body organs. In this study, several existing techniques were adapted for the determination of trace concentrations of U, ^{230}Th , ^{210}Pb , and ^{210}Po in soft tissues, such as beagle dog lungs, livers, spleens, kidneys, lymph nodes, and bioassay materials.

Observations

The sample is decomposed to a slurry in warm 2M HNO_3 . Uranium-233, ^{234}Th , and ^{208}Po tracers are added and the sample is wet ashed with HNO_3 , 30% H_2O_2 , and HClO_4 . HF is used to volatilize any silicious material. The salts are dissolved in dilute nitric acid, filtered, and the filters are alpha- and beta-counted to determine that dissolution is complete before proceeding. The subsequent procedure is outlined in Figure 1.

Thorium-230 d/m is calculated by applying the usual detector efficiency, volumetric, and decay corrections. The radiochemical recovery is determined from a ^{234}Pa beta count taken through a 54 mg/cm^2 aluminum absorber eliminating ^{234}Th betas. Significant ^{228}Th (plus its decay products) may be present since the ^{233}U tracer contains ^{232}U . The tracers should be purified immediately before analysis, and the thorium should be separated within 1 or 2 weeks to minimize ingrowth. Thorium-228 ingrowth is calculated from the time interval between ^{233}U purification and the thorium separation using the ^{232}U content measured by alpha energy analysis. Thorium-228 decay products (^{224}Ra , ^{220}Rn , ^{216}Po , ^{212}Bi , and ^{212}Po) are estimated assuming their ingrowth from the time they are volatilized from the mounted thorium fraction.

The total uranium mass in the sample is determined fluorimetrically. Results must be corrected for the mass of added ^{233}U tracer present and the radiochemical yield of the separation. The alpha and the fluorimetric results

* Radiological Sciences Department

FIGURE 1. Flow Chart Detailing Overall Separation Scheme

contain contributions from both natural uranium and ^{233}U . These contributions are determined by successive approximations to the following equations before the radiochemical recovery is determined and applied:

1. $[\text{Net } ^{233}\text{U d/m}] = [\text{gross alpha d/m}] - [1.55 (\mu\text{g U})]$
2. $[\text{Net } \mu\text{g U}] = [\text{gross } \mu\text{g U}] - [4.75 \times 10^{-5} (\text{d/m } ^{233}\text{U})]$

The net d/m ^{233}U measures the radiochemical yield. The d/m of ^{234}U (parent of ^{230}Th) is computed from the net uranium mass.

The measured fraction of the alpha spectrum from 4 to 6 MeV, which is due to ^{210}Po , is multiplied by the gross alpha d/m of the source, giving net ^{210}Po d/m. Radiochemical recovery is measured similarly from the product of the fraction of ^{208}Po alphas and the gross alpha d/m. The usual differential equation solution and decay corrections are applied for ingrowth from ^{210}Pb present at sacrifice.

The ^{210}Pb is determined from measurements taken on ^{210}Po separated after a period of ingrowth following lead separation. The calculation technique is identical with the above except for appropriate changes in the buildup and decay parameters. Lead-212 gammas at 0.24 MeV are used to measure the radiochemical recovery during the lead separation. Polonium-208 is used for the polonium separation yield. Recovery during ashing is not determined, but losses should not exceed those measured with ^{208}Po or ^{234}Th .

The ashing procedure was designed to minimize losses of ^{210}Po by vola-

tilization and losses of all the measured radionuclides by coprecipitation with insoluble residues. Losses of organically bound polonium may occur at temperatures as low as 100 °C during dry ashing. High recoveries of ^{208}Po tracer do not preclude losses of unexchanged organically bound ^{210}Po during wet ashing. However, the slow initial decomposition used in the method allows a maximum opportunity for solvating polonium, allowing early exchange with the tracer. In addition, formation of insoluble residues, especially from liver samples, was reduced by fuming with perchloric acid. A fine precipitate tended to form on dilution with 4M HNO_3 when samples were ashed with $\text{HNO}_3 + \text{H}_2\text{O}_2$ alone. An alpha and beta analysis of the filterable residues indicated negligible losses of the added tracers.

Table I presents radiochemical recoveries measured for separations performed on several typical samples. The average recoveries for ^{208}Po , ^{233}U , and ^{234}Th were satisfactory approximations for computation purposes. Those tracers could be omitted once the average is established for laboratory operations.

Contamination levels encountered in reagent blanks are presented in Table II. These blanks demonstrate that the tracers added were often the major source of contamination. The ^{228}Th and ^{233}U from ^{233}U tracer have been discussed previously. The ^{210}Pb contamination apparently came from traces of ^{230}Th in the ^{232}Th used for milking, since its level varied with the time between milkings.

TABLE I. Radiochemical Recoveries Measured for Samples of Ashed Beagle Organs

Sample		Radiochemical Recovery (%)			
		^{233}U	^{234}Th	^{208}Po (a)	^{212}Pb (b)
Reagent blank	1	89	87	--	--
	2	83	65	--	--
Liver	1	76	88	105	67
	2	82	78	107	71
Lung	1	80	85	110	90
	2	84	88	99	83
Kidney	1	93	85	97	97
	2	92	84	102	90
Lymph node	1	92	82	93	87
	2	90	85	88	94
Spleen	1	93	83	99	89
	2	94	84	92	90
Average		87	83	99	86

(a) ^{208}Po recovery measurements were $\pm 10\%$ because of propagation of counting statistic variations in the calculation technique and the nonuniformity of tracer material directly mounted for comparator sources.

(b) ^{212}Pb recoveries are the product of recoveries measured for the extraction and ion exchange column steps.

Extracting and discarding lead 24 hr prior to the milking for tracer use would have reduced the level of ^{210}Pb to <0.1 d/m. The ^{210}Po added with ^{208}Po was only 0.06% of the 5.1 MeV ^{208}Po alphas. Polonium-209 is also present in the tracer to an extent of approximately 1%; however, its 4.87

MeV alphas do not interfere in the procedure.

In this group of samples, the interference would have been reduced had less tracer been used. However, the choice of tracer levels was based on a higher expected level of sample activity.

TABLE II. Reagent Blank Activity Levels

Determination	d/m from Tracers	(Source)	d/m from Reagents ^(a)
Fluorimetric U (as ^{234}U)	0.06	(~ 2000 d/m ^{233}U) ^(b)	0.03
^{230}Th	1.2-3.6	(~ 2000 d/m ^{233}U [^{228}Th])	0.5
^{210}Pb	1.6 ^(c)	($\sim 30,000$ d/m ^{212}Pb)	---
^{210}Po	0.08 ^(d)	(~ 140 d/m ^{208}Po)	<0.4

(a) Fluorimetric U and ^{230}Th results are residual reagent blanks after correction for tracer source contribution; ^{210}Po result was based on runs with no tracer added.

(b) Contribution from ^{233}U was based on its mass, interpreted as natural uranium in calculating the d/m of ^{234}U .

(c) ^{210}Pb added with ^{212}Pb tracer was determined from the ingrowth of ^{210}Po in an aliquot of that tracer. ^{212}Pb d/m is immediately following separation.

(d) No decay correction included.

INCORPORATION OF PALMITATE INTO LIPIDS BY LUNG SUBCELLULAR FRACTIONS

The esterification of palmitate by lung mitochondria and microsomes is dependent on the presence of CoA, ATP, and D, L- α -glycerolphosphate. Concentrations of D, L- α -glycerolphosphate in excess of 5 mM increased the incorporation of palmitate into diglycerides without affecting incorporation of triglycerides or lecithin.

Investigator:
E. G. Tombropoulos

Technical Assistance:
W. G. Morrow

Our past studies of the biosynthesis of lung surfactant by subcellular fractions showed mitochondria to be most active in synthesizing long

chain fatty acids (Annual Report, 1965). Esterification of palmitate occurred in both microsomal and mitochondrial fractions (Annual

Report, 1967). Some of the factors affecting the esterification of palmitate by lung mitochondrial and microsomal fractions are described in this report.

Observations

Lung tissue from 1-1/2 to 2-month old female, Syrian, golden hamsters was homogenized and fractionated by centrifugation into mitochondrial and microsomal fractions. Incorporation of ^{14}C -labeled palmitate was studied in incubation mixtures, buffered with glycyl-glycin, and adjusted to pH 7.5. At the end of 40 min incubation, the mixture was extracted with chloroform methanol (2:1), the lipids fractionated and isolated by column and thin layer chromatography. Requirements for various cofactors were studied by varying their concentration in the incubation mixture (Tables I, II,

and III). Boiled subcellular fractions were used as controls.

The incorporation of palmitate into triglycerides and lecithin was linear over incubation intervals of from 15 to 120 min (Figure 1). Similar linearity was observed for the incorporation into diglycerides. Incorporation of palmitate was also directly proportional to the amount of subcellular protein present in the range from 0.25 to 4.5 mg/ml (Figure 2).

Conclusions

Both mitochondria and microsomes require ATP, CoA, and D, L- α -glycerolphosphate (Tables I, II, and III) for the incorporation of palmitate into triglycerides and lecithin. The optimal concentrations of these factors are similar for both subcellular fractions. Concentrations of D, L- α -glycerolphosphate

TABLE I. Effect of ATP Concentration on the Incorporation of Palmitate into Lipids of Lung Subcellular Fractions (Average results from three experiments.)

ATP Concentration, mM	Nanomoles Palmitate Incorporated per mg Protein			
	Triglycerides		Lecithin	
	Mitochondria	Microsomes	Mitochondria	Microsomes
0	0.22	0.50	2.0	1.3
3	0.43	0.80	3.8	3.2
6	0.65	3.4	4.8	5.5
12	1.6	4.0	6.0	6.2
18	2.2	4.5	8.0	10
25	2.6	5.0	7.8	14
31	2.6	5.5	7.0	14
43	2.5	5.2	6.8	14

TABLE II. Effect of CoA Concentration on the Incorporation of Palmitate into Lipids of Lung Subcellular Fractions

CoA Concentration, mM	Nanomoles Palmitate Incorporated per mg Protein			
	Triglycerides		Lecithin	
	Mitochondria	Microsomes	Mitochondria	Microsomes
<u>I</u>				
0	0.1	0.05	1	1.1
0.02	1.5	1.2	5.0	7.5
0.04	2.3	2.4	5.5	12
0.08	4.0	3.5	8.4	16
0.20	4.9	4.5	10	18
0.40	5.3	5.1	17	25
<u>II</u>				
0	0.3	0.2	1.2	0.80
0.16	5.0	7.0	7.5	11
0.32	5.7	7.0	8.2	13
0.64	5.3	6.2	8.5	13
1.30	5.5	5.5	9.4	12

TABLE III. Effect of D, L- α -Glycerolphosphate Concentration on the Incorporation of Palmitate into Lipids of Lung Subcellular Fractions (Average results from two experiments.)

D, L- α -Glycerol- Phosphate Concentration. mM	Nanomoles Palmitate Incorporated per mg Protein					
	Diglycerides		Triglycerides		Lecithin	
	Mito- chondria	Micro- somes	Mito- chondria	Micro- somes	Mito- chondria	Micro- somes
0	1.4	1.3	1.2	1.2	3.6	3.8
5	2.0	3.8	2.7	3.0	6.8	12.0
10	2.4	4.3	2.6	3.1	6.2	11.0
20	3.0	5.0	2.5	3.3	6.3	10.0

higher than 5 mM increased the incorporation of palmitate into diglycerides but had no further effect on incorporation into triglycerides and lecithin. At these higher concentrations of D, L- α -glycerolphosphate other factors may become limiting for the conver-

sion of diglycerides into triglycerides and lecithin. Microsomes may show slightly higher requirements for ATP than mitochondria, which could be due in part to the regeneration of ATP by oxidative phosphorylation in the mitochondrial fraction.

FIGURE 1. Time Course of Incorporation of Palmitate by Lung Subcellular Fractions

FIGURE 2. Effect of Subcellular Fraction Protein Concentration on the Incorporation of Palmitate

INCORPORATION OF CHOLINE INTO LECITHIN BY LUNG
SUBCELLULAR FRACTIONS

Lung mitochondria and microsomes incorporate choline into lecithin. This incorporation is stimulated by CTP and CaCl_2 . The pathway stimulated by CTP appears to be a bio-synthetic pathway similar to the phosphorylcholine pathway in the liver.

Investigator:
E. G. Tombropoulos

Technical Assistance:
A. Jacqueline Clary

This report presents results from a series of experiments designed to study the biosynthesis by lung tissue of dipalmitoyl lecithin, the most active component of lung surfactant. It was reported in previous Annual Reports that: 1) long chain fatty acids are synthesized mainly by lung mitochondria via elongation, and 2) the incorporation of long chain fatty acids into lecithin is mediated by the lung microsomal and mitochondrial fractions. The presently reported studies are concerned with factors affecting the incorporation of choline into lecithin.

Observations

Lungs from 2- to 3-month-old, female Syrian hamsters were used in this study. The techniques for lung fractionation were described in previous Annual Reports. One to two mg of lung subcellular fraction-protein was incubated in 6.6 mM glycyl-glycin buffer, pH 7.8, containing potassium citrate (5 mM), MgCl_2 (20 mM), dipalmitin (2 mM), D, L- α -glycerolphosphate (5 mM),

L-cysteine (5 mM) and choline (5 mM). When indicated, the flask also contained 0.65 mM CoA and 16 mM glutathione, and/or 26 mM ATP, 1.5 mM CaCl_2 , 1.5 mM CTP. At the end of the incubation period (40 min), the reaction was stopped by addition of trichloroacetic acid to a final concentration of 7%. Lipids were extracted with chloroform-methanol (2:1). Lecithin was isolated from the lipid extract by thin-layer chromatography using two solvent systems in succession on 20 x 40 cm plates.

Typical experimental results are shown in Table I. The mitochondrial and microsomal fractions of lung tissue were more active than the whole homogenate in incorporating choline ($\text{Me-}^{14}\text{C}$) into lecithin. This incorporation of choline into lecithin is increased by CaCl_2 and CTP (cytidine triphosphate) but not by CMP (cytidine monophosphate). ATP and CoA further stimulated the choline incorporation induced by CaCl_2 in the mitochondrial, but not in the microsomal fraction. This may indicate that CaCl_2 stimulates a biosynthetic

TABLE I. Incorporation of Choline (Me-¹⁴C) into Lecithin by Lung Subcellular Fractions

Experiment	Additions	Nanomoles Choline Incorporated/mg Protein		
		Mito-chondrial	Micro-somal	Whole Lung
1	ATP + CoA	0.7	0.91	0.27
	ATP + CoA + CaCl ₂	1.5	2.2	0.51
	ATP + CoA + CTP	1.0	1.3	0.37
2	None	0.13	0.20	
	KF	0.16	0.06	
	CaCl ₂	0.36	0.70	
	CaCl ₂ + ATP	0.57	0.40	
	CaCl ₂ + ATP + CoA	0.60	0.75	
	CaCl ₂ + ATP + CoA + KF	0.34	0.20	
3	None	0.30	0.47	
	ATP + CoA	0.30	0.36	
	ATP + CoA + KF	0.23	0.07	
	CTP	0.37	0.45	
	ATP + CTP	0.44	0.86	
	ATP + CoA + CTP	0.59	0.93	
	ATP + CoA + CTP + KF	0.30	0.16	
	CTP + CaCl ₂	1.0	1.9	

reaction in the mitochondrial fraction. Possibly, in microsomes, CaCl₂ stimulates a phospholipase which liberates choline from phosphatidyl choline; but in the presence of excess choline the reverse reaction could become significant. Similar results have been obtained with rat liver microsomes.

CTP stimulates the incorporation of choline by both mitochondrial and microsomal fractions. This represents a biosynthetic mechanism as both subcellular fractions require ATP in addition to CTP. The effects of CTP and CaCl₂ appear to be

additive. Incorporation of choline into lecithin was inhibited by KF in all cases tested.

Conclusions

Lung mitochondrial and microsomal fractions incorporate choline into lecithin by biosynthetic mechanisms similar to those known for phosphorylcholine in the liver, where CTP is required. The mitochondrial fraction, in addition to the above mechanism, may utilize another pathway requiring CaCl₂. Both mechanisms are inhibited by KF.

RADIOELEMENT TOXICITY STUDIES

Included in this section are reports on a miscellaneous group of radioelement toxicity studies not associated with the major programs covered in the other sections. Three reports are concerned with effects of age on various manifestations of radionuclide toxicity. The first two, on plutonium and radioiodine, detail recent findings in a continuing study of these radionuclides. The third report on radiozinc describes preliminary results of age dependence studies with this element. These reports attest to the very significant effects of age on the metabolism of radionuclides, and on the biological effects resulting. Reflecting our interest in this area, the 1969 Hanford Biology Symposium will be held May 5-8 on the topic, "Radiation Biology of the Fetal and Juvenile Mammal."

The report entitled "Radioiodine in Sheep and Swine" describes recent results in an 18-year-old chronic toxicity experiment which is completed except for observation of a few surviving animals.

The final report in this section describes a cooperative study with Oregon State University, supported by the Richland Graduate Fellowship Program.

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

Decreases in femoral breaking strength and changes in water and ash content of the femur appear to be dependent on radiation dose and on the maturity of the bone at time of plutonium administration. Hepatic parenchymal cell function, as evaluated by rose bengal clearance, was more affected by polymeric plutonium in the adult and by monomeric plutonium in weanling and newborn rats. An increased incidence of mammary tumors was observed in female rats injected as weanlings or adults, with doses of 6 or 12 $\mu\text{Ci/kg}$, but not in rats which survived higher doses.

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

Technical Assistance:
D. L. Catt
Joan O. Hess
C. L. Mason

This report continues the description of an experiment (Annual Report, 1967), that involved the intravenous injection of monomeric and polymeric plutonium preparations into newborn, weanling, and young adult rats of the Charles River CD strain. Dose levels ranged from 6-90 $\mu\text{Ci/kg}$ body weight. Animals were sacrificed and studied at intervals ranging from 1 day through 9 months after exposure.

Observations

Maximal skeletal deposition occurred in animals injected with monomeric plutonium. The average cumulative

radiation doses to the femur, calculated from more extensive data than were available for last year's report, are shown in Figure 1. Since deposition and retention were independent of the size of the injected dose, within the range studied, results are presented as the rad dose accumulating from each $\mu\text{Ci/kg}$ administered. The general shape and magnitude of these curves are reasonably explained by the extent of initial deposition and subsequent dilution due to growth. They do not, of course, reflect localized differences in radiation dose which may be a more important factor in determining damage than the average dose.

FIGURE 1. Cumulative Radiation Dose to Femur after Administration of 1 $\mu\text{Ci/kg}$ of Monomeric ^{239}Pu to Rats of Various Ages

More extensive data on the breaking strength of the femur are now available, and a more rigorous expression for strength, taking into account the cross-sectional area and length, has been employed in deriving the relationships shown in Figure 2. Breaking points were determined at 9 months postinjection and are related to the dose received by the femur during the first 45 days after exposure. The effect is greatest, and most clearly dose-dependent, in the weanling animals. The radiation dose received by the femur of newborn animals was so small as to have negligible effect.

The chemical composition of the femurs of animals injected as weanlings is plotted as a function of radiation dose in Figure 3. Ash content decreased and water content increased with increasing radiation dose. The level of other measured constituents was not significantly affected.

More extensive data on the retention of plutonium in the liver were

45-DAY RADIATION DOSE TO FEMUR, krad

FIGURE 2. Effect of Age on the Relation between Radiation Dose and Femoral Breaking Strength S (grams $\times$ length/2 $\div$ cross sectional area)

FIGURE 3. Effect of Radiation Dose on Several Constituents of Femur at 9 Months after Injection of ^{239}Pu in Weanling Rats

employed to construct cumulative dose curves. Figure 4 shows the curves obtained following injection of the polymeric form of plutonium which deposits preferentially in the liver. As in the case of skeletal deposition, the age-related differences are reasonably explained in terms of

FIGURE 4. Cumulative Radiation Dose to Liver after Administration of 1 $\mu\text{Ci/kg}$ of Polymeric ^{239}Pu to Rats of Various Ages

initial deposition and subsequent dilution due to growth.

The functional capability of the hepatic parenchymal cells was evaluated 9 months after administration of ^{239}Pu by measuring the blood clearance rate of radioiodine-labeled rose bengal. As would have been predicted from the dose curves, the largest effects were seen in animals injected as adults (Table I). An explanation for the greater effectiveness of the monomer, as compared to

the polymer, in the newborn and weanling groups is not readily apparent.

Further data were obtained on the long-term effects of plutonium in rats. Of notable interest was the increased incidence of mammary tumors in animals surviving the 3-month postexposure period of acute effects. Female rats injected with 6 or 12 $\mu\text{Ci/kg}$ of either monomeric or polymeric plutonium, as adults or weanlings, showed a substantially increased mammary tumor incidence (Table II). This effect was not seen at higher dose levels, nor was it seen in rats exposed as newborns. The significance of this observation is not clear and invites further study.

Conclusions

The patterns of biological response seen in rats injected at various ages, with two physicochemical forms of plutonium, are a complex function of these variables. These results indicate the importance of giving consideration to such variables in the establishment and application of permissible exposure limits.

TABLE I. Effect of Administration of ^{239}Pu to Rats of Various Ages on the Clearance of Rose Bengal from the Blood at 9 Months after Exposure (Each value is based on determination in four to eight rats.)

Injected Dose, $\mu\text{Ci/kg}$	Percent Cleared from Blood in 15 Min					
	Adult		Weanling		Newborn	
	Polymer	Monomer	Polymer	Monomer	Polymer	Monomer
0	73		76		75	
6	69	--	78	65	79	74
12	64	78	68	73	78	80
18	48	80	74	58	78	72
30	--	24	75	62	72	60
60	--	--	--	60	74	41

TABLE II. Effect of Age at Time of Exposure on Mammary Tumor Incidence in Surviving Females at 9 Months Postexposure to Monomeric or Polymeric ^{239}Pu

Injected Dose, $\mu\text{Ci/kg}$	Adult		Weanling		Newborn	
	Monomer	Polymer	Monomer	Polymer	Monomer	Polymer
0	0/17		0/6		0/14	
6	3/7	1/7	1/4	0/4	0/1	0/3
12	1/5	3/6	2/4	1/4	0/2	0/1
18	0/3	0/6	0/2	0/3	0/1	0/2
30	0/7	-	0/3	0/2	0/5	0/4
60	0/5	-	0/3	-	-	0/3

AGE DEPENDENCE OF RADIOIODINE METABOLISM AND TOXICITY IN RATS

The effect of radiation from injected ^{131}I on the morphology of the thyroid gland was dependent on the age of the rat at the time of administration. In adult animals, cellular necrosis and secondary repair occurred, while in perinatal animals there was an inhibition of tissue proliferation and maturation. Examination of effect in other organ and tissue systems indicated that some were a result of the whole-body radiation dose while others may be attributed to the resulting hypothyroid state of the animals.

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

Technical Assistance:
D. L. Catt
Joan O. Hess

It was found from a comparison of the physiological damage resulting from administration of graded doses of radioiodine, that radiosensitivity of the thyroid glands decreased with increasing age (Annual Reports, 1966, 1967). Studies were performed to relate these decreases in physiologic function to the associated morphologic damage. Studies were also performed to establish whether physiologic alterations noted in other systems are due to whole-body radiation exposures from the administered radioiodine, or to the induced hypothyroid state.

Observations

Rats of four ages (19-day fetuses, newborns, weanlings and young adults) were injected with 0, 0.5, 1.0, or 3.0 $\mu\text{Ci/g}$ of carrier-free ^{131}I . The fetal rats were exposed to radiation from ^{131}I by intraperitoneal injection of the dam; postnatal animals were injected intraperitoneally. Rats were placed on a low-

iodine diet 1 week prior to exposure and maintained on this diet until sacrifice or for 1 week after exposure. Randomly selected animals from each age group were sacrificed at 1, 3, and 7 days after injection and the thyroid glands excised and processed for histologic examination. Other animals were kept on a stock diet for 4 months, placed on a low-iodine diet for 1 week, given a 1.0 μCi tracer dose of ^{131}I intraperitoneally, and killed 24 hr postinjection. The thyroid glands and other tissues, such as the liver, kidney, spleen, gonad, and thymus were processed for histologic examination.

As early as 1 day after administration of the 3.0 $\mu\text{Ci/g}$ dose, the adult animals showed severe destruction of the thyroid with necrosis and sloughing. By 3 days the effect was more pronounced and by 7 days little could be seen other than hemorrhagic masses of collagen and connective tissue. At the 1 $\mu\text{Ci/g}$ level, by 7 days, the thyroid glands were almost completely necrotic and

replacement by eosinophilic fibrous connective tissue and collagen had begun. At the lowest dose level of 0.5 $\mu\text{Ci/g}$, significant damage, including degeneration of epithelial elements and interstitial fibrosis, was evident at 7 days.

The animals exposed as weanlings to the highest dose level (3 $\mu\text{Ci/g}$) received radiation doses approximating those received by adults exposed to 0.5 $\mu\text{Ci/g}$ (Table I). Histologic damage was similar in nature and extent in these two groups. The radiation doses received by the prenatal or neonatally exposed animals were less than those received by the weanling animals at corresponding levels of administered dose and no histologic abnormalities were observed, even at the highest dose.

Marked age-related differences in morphologic effect were seen in the animals sacrificed at 4 months following the initial exposure. At all

levels of administered dose, the thyroid glands of the adult animals were almost completely destroyed and hemosiderin deposits were present. In the weanling animals, the response to the two lower doses consisted of moderate levels of damage and hyperplastic nodules were in evidence. At the 3 $\mu\text{Ci/g}$ dose, similar damage was seen but, for the most part, the thyroids appeared immature. An immature appearance was also seen in rats exposed to the highest dose level at birth. Small hyperplastic nodules were present in these glands, especially in rats that received the lowest dose. The animals exposed at all dose levels prenatally showed some histologic signs of damage including destructive changes with fibrotic replacement.

A marked constriction of the trachea at the level of the thyroid gland was noted grossly in the animals of the two younger age groups exposed

TABLE I. Calculated Absorbed Radiation Doses (krad) to the Thyroid of Rats Administered ^{131}I at Various Ages (Correction made for non-absorbed beta particles)

Age at Injection	Interval After Injection, days	Dose to Thyroid (krad) for Various Levels of ^{131}I Injection		
		0.5 $\mu\text{Ci/g}$	1.0 $\mu\text{Ci/g}$	3.0 $\mu\text{Ci/g}$
Adult	0-3	37	56	122
	0-7	51	64	122
Weanling	0-3	5	12	35
	0-7	9	19	52
Newborn	0-3	4	6	8
	0-7	8	8	12
Fetus	0-3	1	2	5
	0-7	2	4	10

to the higher dose levels. Histologic examination indicated that proliferation of cartilage was inhibited without histologic abnormality of the cartilage structure.

The only consistent extrathyroidal observation has been atrophy of the spleen with extensive hemosiderin deposits. This is in accord with the reduced splenic weight reported earlier and is possibly related to the total-body radiation dose.

In an experiment to differentiate between thyroid insufficiency and radiation effect, newborn animals were exposed at the three previously employed dose levels of ^{131}I , or surgically thyroidectomized, or given X-radiation to the thyroid gland. Nineteen-day fetuses were exposed by injecting the dam with 3.0 $\mu\text{Ci/g}$. Adult male rats received 1 $\mu\text{Ci/g}$ of ^{131}I , 5000 rad of X ray to the thyroid, or were surgically thyroidectomized. Appropriate control animals, including sham-thyroidectomized, sham-irradiated, sham-injected and unhandled controls were also included. At 4 months after exposure, a number of morphologic and physiologic parameters were determined (Tables II and III).

Prior exposure to large doses of ^{131}I reduced the uptake of the ^{125}I tracer dose to an extent similar to that seen in previous experiments (Annual Report, 1967); but X-irradiation of the thyroid, at the dose levels employed, failed to alter uptake. Surgical thyroidectomy had only a small effect in reducing the uptake of the tracer dose. It appears from changes in other parameters that

the thyroid gland was incompletely removed and subsequently regenerated.

As noted previously, ^{131}I -radiothyroidectomy increased extrathyroidal retention of the tracer dose of radioiodine. The surgically thyroidectomized animals, both adult and newborn, showed a moderate elevation in carcass retention.

Kidney function (clearance of ^{203}Hg -chlormerodrin from the blood) was somewhat reduced in the animals which had been surgically thyroidectomized or exposed to radioiodine but this was not sufficient to account for the levels of ^{125}I retained in the carcass. The retention of tracer iodine in the plasma was elevated in the animals thyroidectomized at birth. The percent of the tracer dose found in the protein fraction of the plasma was also increased in those treated neonatally, but was markedly reduced in the adults which received radioiodine.

Neurological deficits were apparent in the neonatally surgically thyroidectomized animals, and those exposed to the higher doses of X-radiation or radioiodine. These were absent at lower doses and in the fetuses exposed to 3 $\mu\text{Ci/g}$. Since these latter animals actually were more hypothyroid than were the surgically thyroidectomized animals, this deficit apparently does not stem from the hypothyroid state per se.

Neonatal hypothyroidism produced by the administration of radioiodine, was associated with a consistent depression of skeletal growth (Table III).

TABLE II. *Effect on Several Physiological Measures at 4 Months After Initial Treatment (Values are mean of 4-12 determinations)*

Age-Group	Treatment	Dose	Fate of Tracer ¹³¹ I Dose at 24 Hr Postadministration					Chlor-merodrin Clearance, % (b)	Incidence of Neuro-logical Deficits		
			Thyroid Uptake, %	Carcass Retention, %	Dose/ml Plasma	% Dose in Protein/ml Plasma	Conversion Ratio (a)		Audiogenic Seizures	Ataxic	Spastic
19-day fetus	¹³¹ I	3 µCi/g	7	44	-	-	-	70	0/11		
Newborn	Pooled	Controls	40	10	0.40	0.26	63	74	4/36	0/36	0/36
	¹³¹ I	0.5 µCi/g	15	36	1.20	0.55	46	66	0/11	0/11	0/11
	¹³¹ I	1.0 µCi/g	10	38	1.26	0.53	43	67	2/5	0/5	0/5
	¹³¹ I	3.0 µCi/g	3	54	-	-	21	63	1/2	1/2	1/2
	Surgical thyroidectomy		33	24	0.78	0.52	67	73	4/7	0/7	0/7
	X-ray	400 rad	40	10	0.33	0.22	66	81	0/9	0/9	0/9
	X-ray	1000	42	11	0.52	0.34	66	78	4/8	0/8	0/8
Adult	X-ray	2500	42	13	0.44	0.21	52	74	5/10	6/10	4/10
	Controls		42	9	0.49	0.35	69	76	1/6	0/6	0/6
	¹³¹ I	1.0 µCi/g	1	41	0.66	0.06	10	62	3/8	1/8	3/8
	Surgical thyroidectomy		25	38	0.50	0.29	58	68	1/7	0/7	0/7
	X-ray	5000 rad	42	15	0.56	0.37	65	75	0/7	0/8	0/8

(a) Protein-bound activity/total activity in plasma

(b) At 30 min postadministration

Growth appeared to be symmetrical with minimal, if any, alteration in the relative proportions of the body components.

Conclusions

The action of radiation on the morphology of the thyroid and trachea is dissimilar at the ages studied. In the older animals the effect was as expected--the production of cellular necrosis and resulting secondary replacement. The younger

animals responded by decreased tissue proliferation and maturation resulting in a markedly different histologic picture.

The elevated carcass retention of a tracer dose of radioiodine seen consistently throughout these studies may relate to the induced hypothyroid state rather than to a whole-body radiation effect. The reduction in kidney function, as measured by chlormerodrin clearance, did not explain this phenomenon.

TABLE III. Skeletal Effects at 4 Months After Initial Treatment

Age-Group	Treatment	Dose	Spine Length, mm	Femur Length, mm	Width at Greater Trochanter, mm	Minimum Diameter of Femur Shaft, mm
19-day fetus	^{131}I	3 $\mu\text{Ci/g}$	137	35.2	5.5	3.1
Newborn	Pooled controls		157	38.1	5.7	3.1
	^{131}I	0.5 $\mu\text{Ci/g}$	141	36.0	5.8	3.7
	^{131}I	1.0 $\mu\text{Ci/g}$	145	35.2	5.8	3.0
	^{131}I	3.0 $\mu\text{Ci/g}$	113	28.5	4.5	2.8
	Surgical thyroidectomy		151	37.8	6.2	3.5
	X-ray	400 rad	164	40.3	6.0	3.4
	X-ray	1000	157	39.0	5.6	3.1
	X-ray	2500	148	36.9	5.8	3.1
Adult	Controls		165	41.5	5.3	3.1
	^{131}I	1.0 $\mu\text{Ci/g}$	154	38.1	5.6	3.1
	Surgical thyroidectomy		170	41.7	5.8	3.2
	X-ray	5000 rad	166	41.4	5.7	3.1

AGE DEPENDENCE OF RADIOZINC METABOLISM IN THE RAT

Newborn animals retained injected ^{65}Zn more tenaciously than adults. The retention patterns are associated with age-related differences in partition between tissues.

Investigators:

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Technical Assistance:

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Joan O. Hess
C. L. Mason

These experiments with ^{65}Zn represent a continuation of our program of studies on the age dependency of radionuclide metabolism.

Observations

Following a preliminary experiment which showed substantial effects of age on ^{65}Zn retention, an experiment was performed which involved 12 adult, 12 weanling, and 20 newborn rats of both sexes. The animals were injected intraperitoneally with 0.5 μCi of ^{65}Zn , and retention determined by whole-body counting. Results are shown in Figure 1.

The newborn animals retained zinc more tenaciously than adults for the first 20 days, following which the curves became parallel. Retention by the newborn was independent of sex. The curve shown for adults in Figure 1 is a composite of male and female data, though males retained the nuclide to a somewhat greater degree than females. The rate of loss of ^{65}Zn by weanling animals initially exceeded that observed for either the adult or

newborn animals, but soon assumed a similar rate.

The ^{65}Zn contents of several tissues were determined in additional rats sacrificed at 1, 5, or 9 days after intraperitoneal injection with 0.5 μCi . These values, shown in Table I, were obtained on tissue aliquots from which total tissue content was calculated. They are expressed as percent of total-body content at time of sacrifice. The

FIGURE 1. Retention of ^{65}Zn in the Rat as a Function of Age at the Time of Intraperitoneal Injection (Range indicates standard error)

skeleton contained the greatest fraction of the ^{65}Zn at all sacrifice times in the newborn and weanling animals. The adults had substantially less activity in the skeleton than in either the muscle or liver. These are quite preliminary data, based on a small number of animals. Studies in progress should add greater precision to the pattern of partition of retained ^{65}Zn as a function of age.

Conclusions

During the first 20 days of life, ^{65}Zn is more tenaciously retained by the rat than at later time periods. In both newborn and weanling animals the skeleton contains the largest fraction of ^{65}Zn ; whereas, muscle and liver are more prominent tissues of initial deposition in the adult.

TABLE I. Tissue Distribution of ^{65}Zn Presented as Percent ($\pm\text{SE}$) of Total-Body Content on Days 1, 5 and 9 After Intraperitoneal Injection of 0.5 μCi ^{65}Zn per Rat (Two rats of each sex and from each age group were used.)

Age	Days Postinjection				
		Skeleton	Muscle	Skin	Liver
Newborn	1	39 $\pm$ 3.0	28 $\pm$ 3.4	12 $\pm$ 1.0	17 $\pm$ 2.0
	5	54 $\pm$ 0.6	16 $\pm$ 0.5	16 $\pm$ 0.5	10 $\pm$ 0.7
	9	48 $\pm$ 3.0	23 $\pm$ 2.8	15 $\pm$ 3.0	11 $\pm$ 2.1
Weanling	1	56 $\pm$ 2.5	20 $\pm$ 2.1	6 $\pm$ 2.4	11 $\pm$ 2.4
	5	61 $\pm$ 3.7	23 $\pm$ 3.8	8 $\pm$ 0.7	6 $\pm$ 0.1
	9	64 $\pm$ 1.4	21 $\pm$ 1.5	7 $\pm$ 1.3	4 $\pm$ 0.1
Adult	1	18 $\pm$ 1.6	34 $\pm$ 2.8	7 $\pm$ 1.1	28 $\pm$ 1.5
	5	19 $\pm$ 1.1	37 $\pm$ 3.7	26 $\pm$ 5.8	12 $\pm$ 1.2
	9	30 $\pm$ 1.4	36 $\pm$ 2.0	16 $\pm$ 1.6	11 $\pm$ 0.6
		Kidney	Heart	Spleen	Blood
Newborn	1	1.0 $\pm$ 0.14	0.5 $\pm$ 0.03	0.3 $\pm$ 0.04	1.2 $\pm$ 0.11
	5	1.0 $\pm$ 0.03	0.5 $\pm$ 0.04	0.7 $\pm$ 0.07	1.3 $\pm$ 0.13
	9	1.5 $\pm$ 0.26	0.5 $\pm$ 0.07	0.6 $\pm$ 0.06	1.3 $\pm$ 0.18
Weanling	1	3.7 $\pm$ 0.30	0.7 $\pm$ 0.04	1.2 $\pm$ 0.11	1.7 $\pm$ 0.10
	5	1.2 $\pm$ 0.12	0.4 $\pm$ 0.02	0.3 $\pm$ 0.06	1.2 $\pm$ 0.22
	9	1.0 $\pm$ 0.04	0.4 $\pm$ 0.02	0.3 $\pm$ 0.03	1.8 $\pm$ 0.28
Adult	1	4.7 $\pm$ 0.11	0.9 $\pm$ 0.04	1.1 $\pm$ 0.09	5.2 $\pm$ 0.33
	5	2.3 $\pm$ 0.33	0.6 $\pm$ 0.05	0.6 $\pm$ 0.09	3.8 $\pm$ 0.37
	9	1.5 $\pm$ 0.11	0.6 $\pm$ 0.06	0.4 $\pm$ 0.02	4.7 $\pm$ 0.45

RADIOIODINE IN SHEEP AND SWINE

Thyroids of miniature swine, fed 2 mCi ^{131}I 2 years previously, showed varying degrees of damage as indicated by ^{131}I uptake tests, protein-bound iodine values, and histopathologic examination.

Investigators:
R. F. Palmer
J. L. Beamer
M. T. Karagianes*

To determine the effects of thyroid ablative doses of ^{131}I in miniature swine for comparison with past studies in sheep, a group of 5-month-old female swine were given an oral dose of 2 mCi ^{131}I in 1966 (Annual Report, 1966). In 1967, tests on eight of these animals indicated partial thyroid ablation (Annual Report, 1967). Additional tests have been performed on the nine survivors and five matching controls, and the animals sacrificed for histopathologic examination.

Observations

Two years after oral administration of the 2-mCi dose of ^{131}I , all swine appeared normal with no external signs of hypothyroidism. Results following the oral administration of a 25- μCi ^{131}I tracer dose are shown in Figure 1. Three of the experimental swine handled the ^{131}I quite differently from the other six and are averaged as a separate, more heavily affected group.

Control animals showed an average thyroid uptake of 30% of the ingested dose and retained it during the 14 days of observation with a biological half-life of about 80 days. For the two groups of thyroid ablated animals, corresponding values of 23% and 10 days, and 9% and 6 days were observed. Average values for protein-bound iodine in the serum from these three groups of animals indicated the same trend of thyroid damage--3.3 $\mu\text{g}/100$ ml for the controls and 2.9 and 2.1 for the two ablated groups.

Histopathologic examination confirmed the above indications of 2 degrees of thyroid ablation among the nine experimental animals. Those three that had shown the poorest thyroid function test exhibited a much more severe damage to their thyroid tissue than did the other six (Figure 2). Damage was evidenced by decreased numbers of functional follicles, increased amounts of fibrous connective tissue, follicle collapse, increase in height of the lining follicle epithelium, and varying degrees of coloration and vacuolation of the thyroidal colloid. Thyroid tissues from the five control animals were normal.

* Bioengineering

FIGURE 1. Uptake and Retention of Ingested ^{131}I by Thyroids of Controls and Swine Fed 2 mCi ^{131}I 2 Years Previously (Number of animals shown in parentheses; range indicates standard deviation)

Conclusions

The results in swine 2 years following a single dose of 17-25 krad to their thyroids were similar to those seen in sheep following an estimated 30 krad thyroid dose from a single oral administration of ^{131}I . Both species showed similar decreases

in ^{131}I uptake and retention in their thyroids, and corresponding decreases in serum protein-bound iodine levels. The different degrees of thyroid damage seen in these swine can be attributed to biological variability in thyroid size and ^{131}I uptake and retention at the time of administration of the original 2-mCi dose of ^{131}I .

A

252X

B

252X

C

252X

FIGURE 2. Photomicrographs of Degrees of Thyroid Ablation: A. Severe; B. Moderate; C. Normal

ACCUMULATION OF ^{65}Zn BY PELAGIC CRUSTACEANS

The relative distribution of ^{65}Zn in the tissues of two pelagic crustaceans differed when the isotope was accumulated from food and from water; however, autoradiographs indicated that the actual sites of isotope accumulation are the same despite the mode of uptake. Exoskeletal adsorption is instrumental in the accumulation of zinc from seawater and probably accounts for much of the element measured in whole organisms.

Investigators:
S. W. Fowler*
J. M. Dean

Some of the reactor-produced radionuclides released into the Columbia River at Hanford reach the sea and concentrate in the marine biota. Euphausiids and shrimp, pelagic crustaceans which are important in marine food chains, are known to concentrate ^{65}Zn and to retain the isotope for long periods of time. The present study was concerned with establishing areas of tissue localization of ^{65}Zn in these crustaceans when the isotope was accumulated from water and from food. It was also hoped that the results might clarify the mechanism by which the isotope is accumulated.

Observations

Euphausiids (Euphausia pacifica and Thysanoessa spinifera) and pasiphaeid shrimp (Pasiphaea) collected from the Pacific Ocean beyond the influence of the Columbia River, were separated into different size groups and analyzed for stable zinc. The concentration

of the element in whole organisms was found to be inversely related to dry weight (Figure 1). Stable zinc analyses of several euphausiid and pasiphaeid tissues showed higher concentration in eyes and exoskeleton than in hepatopancreas and muscle. The highest concentrations found were in the cast molts.

Euphausiids were allowed to accumulate ^{65}Zn from seawater and tissues analyzed after 144, 168, and 264 hr exposures. No correlation was evident between percentage distribution of ^{65}Zn among tissues and either exposure time or whole-body ^{65}Zn accumulation. When the data were plotted against total body dry weight, an inverse relationship was evident between exoskeleton weight and ^{65}Zn , suggesting a surface area effect (Figure 2). Molts of the animals contained up to 41% of the total body burden. Highest concentrations of ^{65}Zn were found in the exoskeleton, followed by the eyes and muscle in that order.

A similar pattern of distribution was observed in studies with

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FIGURE 1. Relationship of Stable Zinc Concentration to Body Weight in Two Pelagic Crustaceans (Numbers beside each point indicate the number of organisms analyzed.)

FIGURE 2. Percentage of Total Body ^{65}Zn in Exoskeleton, Muscle and Gut, and Eyes, as a Function of Euphausiid Dry weight

Pasiphaea. Mean percentages in the exoskeleton were somewhat higher (66%), and in muscle somewhat lower (19%), than those computed for euphausiids.

Autoradiographs of euphausiids and Pasiphaea that had accumulated ^{65}Zn from seawater showed that the majority of the ^{65}Zn was located on the outer surface of the exoskeleton, between the ommatidia of the eye and in the interstitial spaces of the muscle, though not actually intra-myofibrillar.

Euphausiids were fed ^{65}Zn -labelled

Artemia nauplii daily for a period of 1 week. No relationship was found between percentage distribution of ^{65}Zn among tissue and either exposure time, total ^{65}Zn accumulation, or animal dry weight. The largest fraction of the total ^{65}Zn was contained in muscle (58%) with a lesser percentage (33%) located in the exoskeleton. Molts accounted for only 1-2% of the total body burden. Concentration of ^{65}Zn in the exoskeleton was about equal to that in the muscle, whereas when uptake was from water, the exoskeleton had a ^{65}Zn concentration nearly three times that in muscle. Both exoskeleton and muscle had ^{65}Zn concentrations almost an order of magnitude greater than the eyes. Autoradiographs prepared from euphausiids that fed on ^{65}Zn -labelled Artemia, and autoradiographs from Pasiphaea that fed on ^{65}Zn -labelled euphausiids, showed areas of isotope localization similar to those seen in animals that accumulated ^{65}Zn from water. The only difference was the lack of heavy exoskeletal surface accumulation in the animals obtaining the isotope through the food chain.

In an experiment designed to test the effect of stable zinc on ^{65}Zn uptake, euphausiids were allowed to accumulate radiozinc from solutions containing the same concentration of ^{65}Zn but varying concentration of stable zinc. Uptake of ^{65}Zn was unaffected by the external zinc concentration over the first 4 days. The amount of total zinc accumulated by the euphausiids was directly related to the external zinc concen-

FIGURE 3. Relationship Between Zinc Concentration in Water and Amount Accumulated by Euphausia pacifica (Hours indicate exposure times.)

tration (Figure 3). It would appear that zinc uptake over the 10-100 $\mu\text{g Zn/liter}$ range was the result of either a physicochemical adsorption, or ion or adsorption exchange, both of which should follow a pattern similar to that displayed in Figure 3.

Conclusions

In small pelagic crustaceans, the exoskeleton plays a major role in accumulating zinc from seawater. The process appears to be governed by physicochemical adsorption or adsorption exchange processes. The relatively high concentrations of zinc

found in exoskeleton and molts probably account for much of the zinc measured in whole organisms. This would help account for the relatively large concentrations found in smaller euphausiids and shrimp. When the animal ingests ^{65}Zn , the exoskeleton is not so important a site for accumulating the isotope although relatively

high concentrations of ^{65}Zn are still found associated with this tissue. Much of the accumulated ^{65}Zn tends to be associated with tissue surfaces rather than distributed evenly throughout tissue, regardless of whether the isotope is accumulated from food or water.

RADIOELEMENT REMOVAL STUDIES

Three reports in this section constitute a rather thorough survey of the effectiveness of DTPA in removal of internally deposited promethium from rats and miniature swine. Included in these reports are extensive data on the effect of mass of promethium deposited, over a wide range, on distribution, retention, and ease of removal; and the effect of delay in DTPA treatment. These data complement the studies on promethium removal conducted in human volunteers and described in Vol. II, Part 2, of this report.

The remaining four reports are concerned with the removal of inhaled plutonium. Of particular interest is the report of preliminary encouraging results on the removal of PuO_2 by lung washing. Another newly explored technique, that of electro-aerosol treatment, shows some promise of accelerating clearance mechanisms.

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Smith, V. H. "The Prevention of ^{147}Pm
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REMOVAL OF FIRMLY BOUND PROMETHIUM FROM THE RAT

Repetitive treatments with chelons, initiated 24 days after radionuclide injection, were quite effective in reducing tissue concentrations of ^{147}Pm in the rat. Bone burdens were reduced to half those in nontreated animals.

Investigator:

V. H. Smith

Technical Assistance:

Alison T. Clark

Since the manufacture of ^{147}Pm and its continued development for heat sources expand the possibility of human incorporation, further tests were made of the efficacy of decorporation procedures (Annual Report, 1967).

Observations

Fifty adult, female rats (Charles River CD) were intravenously injected with 31 μCi ^{147}Pm as the chloride, pH 3.0. Treatments were initiated 24 days after ^{147}Pm injection when promethium was firmly bound in tissues and repeated biweekly for 25 weeks. Results of tissue analyses, 202 days after the ^{147}Pm administration, are shown in Table I.

The chelating agents, diethylenetriaminepentaacetic acid (DTPA) and

triethylenetetraminehexaacetic acid (TTHA), were similarly effective, reducing the various tissue burdens by factors of from 2-25. There would appear to be little reason for using the theoretically stronger, but more toxic, chelon, TTHA, in preference to DTPA. Although ethylenediaminetetraacetic acid (EDTA) is more acceptable clinically, it is clearly less efficient. The zinc salt of DTPA has been suggested by Catsch⁽¹⁾ as a less toxic alternate to the calcium salt, particularly for long-term treatment. Our results suggest that it would be an acceptable alternate for promethium removal, although the grossly healthy state of all treated animals at the end of the experiment did not indicate great differences in toxicity at the treatment levels and frequency employed.

TABLE I. Concentration of Intravenously Injected ^{147}Pm Remaining in Rat Tissues After Various Removal Treatments (m% injected Pm/g tissue wet weight) (a)

<u>Treatments</u> ^(b)	<u>Liver</u>		<u>Kidneys</u>		<u>Spleen</u>		<u>Femur</u>		<u>Pancreas</u>		<u>Ovaries</u>	
Saline	41.3	A	172	A	285	A	1460	A	42.9	A	41.3	A
EDTA	11.5	B	142	A	79.5	B	1250	B	1.92	B	31.6	B
DTPA	1.84	C	73.6	B	19.1	D	677	C	2.25	B	9.73	C
TTHA	2.66	C	75.7	B	30.5	C	679	C	2.54	B	10.4	C
Zn-DTPA	2.25	C	63.0	B	36.9	C	657	C	2.30	B	13.8	C

(a) Results in the same column with the same letter are not significantly different at the 5% significance level as measured by the Duncan's New Multiple Range Test.

(b) Treatments were administered intraperitoneally as 1 cm³ of pH 7.2 solution containing 0.3 mmoles of the chelon. **Except in the case of Zn-DTPA**, all treatments were given with 1 cm³ of calcium gluconate-calcium glucoheptonate, 10% w/v (Abbot). For other details see text.

In the controls, bone retains the highest concentration of injected promethium. Chelation therapy is least effective in reducing bone deposition. The skeletal content of control rats after 200 days was about 38% of the injected promethium and represented about 95% of the body burden at that time. In the treated animals, the skeletal content represented over 99% of the body burden.

1. Catsch, A. *Experientia*, vol. 21, p. 210. 1965.

Conclusions

Of the agents tested, DTPA is the agent of choice for removal of promethium, as well as for the prevention of its deposition (Annual Report, 1967). In contrast to plutonium, promethium remains relatively accessible to chelon therapy, and the treatment of long-standing body burdens is of greater practical significance.

EFFECT OF PROMETHIUM CONCENTRATION ON DEPOSITION AND REMOVAL IN THE RAT

Deposition of intravenously administered PmCl_3 in the rat skeleton was inversely related to the mass administered. This effect was less apparent in the kidneys and absent in the liver. Prompt chelation therapy reduces skeletal deposition by about 50% at chelon:metal ratios as low as 100:1.

Investigator:

V. H. Smith

Technical Assistance:

R. L. Amster

Alison T. Clark

Promethium-143, in very low doses, has been used in hazard evaluation studies in man and may become a useful radiopharmaceutical in organ imaging and function tests. It was therefore of interest to determine the effect of mass of promethium administered on the short-term distribution of the element and on the effectiveness of removal by chelation therapy.

Observations

Groups of ten female, Charles River CD rats were injected intravenously with 0.2 ml of PmCl_3 solution at pH 3.0. Promethium-147 was neutron activated producing $^{148,148\text{m}}\text{Pm}$ which permitted radioanalyses of small masses of injected promethium. The ^{147}Pm represented at least 99% of the mass of injected promethium and 58-70% of the initial beta activity depending on the particular time injected. Three groups of rats were injected at each of the dose levels shown in Table I. Of these, one group was sham treated with physiological saline (controls); one group was treated intraperitoneally

with 0.3 mmole DTPA in 1 ml of 10% calcium gluconate-calcium glucoheptanate solution, 1 hr after promethium injection (prompt treatment); and one group received a similar DTPA treatment after 48 hr (delayed treatment). To further extend the mass range, one series of animals received $805 \mu\text{g } \text{SmCl}_3$. Samarium is chemically very similar to promethium and should act as an effective diluent. All animals were sacrificed at 7 days following promethium injection and various tissues analyzed for their promethium content.

Results are summarized in Figure 1. There is an apparent effect of mass on skeletal deposition and possibly a similar effect in the kidneys, but not in the liver. At the highest mass level (contributed by samarium) the large initial deposition in liver and the small deposition in bone is probably the result of colloid formation when the highly concentrated injectant entered the bloodstream. This was also indicated by the high deposition in spleen (3.4% of the injected dose)--17 times higher than the deposition at any other mass level.

TABLE I. $^{147,148}\text{Pm}$ Injection Levels

Beta Activity, μCi	Promethium Mass		Molar Ratio ^(a) DTPA:Pm
	ng	Relative	
0.046	0.030	1	10^9
0.43	0.28	10	10^8
4.8	3.0	10^2	10^7
33	35	10^3	10^6
400	300	10^4	10^5
8.0	805000 ^(b)	10^7 (b)	10^2 (b)

(a) Relative to 300 mmoles DTPA dose

(b) Includes 805 μg of samarium

The ability of DTPA to reduce promethium deposition (prompt treatment) and hasten its excretion (delayed treatment) is graphically illustrated in Figure 1 and summarized in Table II. There is little indication of a consistent effect of DTPA:Pm ratio on therapeutic effectiveness, except at the highest promethium (samarium) mass level, where DTPA was much less effective, which is further evidence for the probable colloidal nature of this material. There is also an indication that DTPA is less effective in removing promethium from kidney and bone at the lowest injected mass level. This would suggest that those sites which bind promethium most tightly are selectively filled so that a greater percentage of the lower-mass promethium dose is more firmly bound

and relatively less available to DTPA.

Conclusions

There is an inverse mass effect on the deposition of promethium in the skeleton but not in soft tissues. As a consequence, tracer experiments with ^{143}Pm may tend to overestimate the bone deposition to be anticipated from accidental exposures to ^{147}Pm and might lead to erroneous interpretations of excretion curves. Therapy by prompt treatment with DTPA is effective over a wide range of chelon to metal ratios. When DTPA treatment is delayed for 48 hr, promethium is removed principally from the liver, though significant removal from the skeleton also occurs.

FIGURE 1. Promethium Retention in Rat Tissues 7 Days After Injection and After Prompt and Delayed DTPA Treatment (Mean $\pm$ 95% confidence interval)

TABLE II. Relative Efficiency of DTPA Treatments

Approximate Relative Mass	Promethium Retained, as Percent of Retention in Untreated Controls ^(a)					
	Liver		Kidneys		Skeleton	
	Prompt	Delayed	Prompt	Delayed	Prompt	Delayed
10^0	7.4	22	30	43	66	72
10^1	9.6	20	46	66	45	70
10^2	6.1	33	52	83	46	73
10^3	11	--	43	--	46	--
10^4	6.2	22	48	77	43	73
10^7	58	85	38	96	56	94

(a) Retention measured 7 days following promethium injection. Prompt treatment at 1 hr, and delayed treatment at 48 hr following promethium injection, consisted of intraperitoneal injection of 0.3 mmole DTPA.

PROMETHIUM REMOVAL STUDIES IN MINIATURE SWINE

Treatment with DTPA, commencing 1 and 28 days after the intravenous administration of PmCl_3 to pigs, left about 12 and 38% of the injected dose still present at 100 days compared to 88% in nontreated animals. When the onset of the course of treatments was delayed until 78 days there was 44% retention after 155 days.

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

Technical Assistance:
Alison T. Clark
S. A. Hughes, Jr.
R. M. Oliver

The handling involved in the recovery of large quantities of ^{147}Pm from nuclear wastes for utilization in heat sources poses significant risks of accidental human exposure. The effectiveness of diethylenetri-

aminepentaacetic acid (DTPA) treatment, given promptly or delayed, was investigated to provide a basis for medical management of promethium incorporation in humans.

Observations

Hanford Miniature barrows were injected via the anterior vena cava with 557 μCi $^{147}, ^{148\text{m}}\text{PmCl}_3$ in 10 ml of pH 3 solution. Five pigs were intravenously treated with 3 g of DTPA as the neutral calcium salt in 10 cc of solution at 1 hr, and with 1 g at 24 and 48 hr after the promethium injection (prompt treatment). Five other pigs were treated with 1 g of DTPA once a week for 11 weeks commencing 28 days after the promethium injection (delayed treatment); four pigs were similarly treated commencing 78 days after the promethium injection (long delayed treatment). Prompt treatment animals were also subjected to weekly 1 g injections of DTPA starting 70 days after promethium injection. Seven pigs were injected similarly with promethium to serve as nontreated control animals for comparison of treatment effectiveness. Animals were sacrificed about 100 days after radionuclide administration except for the long delayed group which were necropsied at about 155 days. The latter were given DTPA over a treatment period comparable to the delayed treatment group. Total-body counts were made daily for two weeks, bi-weekly for two weeks, and weekly for the balance of the experiment.

Prompt treatment removed over 70% of the promethium within 48 hr (Figure 1). The continued rapid removal of promethium until about day 10 shows the protracted effect of DTPA therapy and was the rationale

underlying the weekly schedule for delayed treatment.

The sharp loss of promethium following first administration of DTPA no doubt reflects loss from soft tissue, especially liver. The later, slower loss of promethium probably reflects removal from the skeleton. This is clearly evidenced in the prompt treatment group where further accelerated removal of promethium occurred when treatments were resumed after 70 days. This further removal almost certainly occurred from bone, since soft tissues were already depleted by the earlier treatment (Table I).

A 300-day half-time for promethium retention can be calculated from those portions of the treatment curves (Figure 1) thought to represent mainly removal from bone. This compares with a retention half-time of greater than 2000 days calculated from the later portions of the retention curves for untreated animals. These data do not extend over a sufficient time to show if the rate persists with long continued treatment.

The effectiveness of DTPA treatment is clearly dependent on the time of initiation of that treatment, for soft tissues as well as for bone (Table I). In the case of delayed or long-delayed treatment, a prolonged course of weekly DTPA administration is required to reduce the radiation dose to bone. The delayed treatment data are consistent with a removal rate from bone of about 4% of the injected promethium every 30 days during the treatment period studied.

There was no grossly observable pathology associated with the multiple DTPA treatments or the levels of promethium employed in this study.

Conclusions

DTPA is effective for prompt and delayed treatment of promethium depo-

sition. Soft tissue deposits are rapidly removed and bone deposition sharply reduced by prompt treatment. With delayed treatment soft tissues rapidly lose promethium; rate of loss from bone is slow but significantly increased over that observed in untreated controls.

TABLE I. Percent of Administered $^{147,148m}\text{Pm}$ Biologically Retained After Various DTPA Treatment Schedules (Mean $\pm$ standard error)

	<u>Control Animals (a)</u>	<u>Prompt Treatment Animals (a)</u>	<u>Delayed Treatment Animals (a)</u>	<u>Long Delayed Treatment Animals (b)</u>
Liver	16 $\pm$ 0.99	0.053 $\pm$ 0.008	0.29 $\pm$ 0.05	0.71 $\pm$ 0.07
Kidneys	0.78 $\pm$ 0.05	0.047 $\pm$ 0.007	0.11 $\pm$ 0.01	0.092 $\pm$ 0.012
Spleen	0.52 $\pm$ 0.22	0.030 $\pm$ 0.004	0.022 $\pm$ 0.005	0.031 $\pm$ 0.009
Lung	0.23 $\pm$ 0.03	0.0092 $\pm$ 0.0012	0.085 $\pm$ 0.018	0.21 $\pm$ 0.08
Skeleton	55 $\pm$ 2	12 $\pm$ 1.0	38 $\pm$ 1.2	43 $\pm$ 1.2
Total body counts	88 (c)	8 $\pm$ 0.22	35 $\pm$ 1.77	56 $\pm$ 2.4

(a) Values found after 100 days

(b) Values found after 156 days

(c) Extrapolated value at 100 days (Figure 1)

FIGURE 1. Biological Retention of Promethium Administered Intravenously to Swine as Estimated by Total Body Counting

REMOVAL OF INHALED $^{239}\text{PuO}_2$ FROM RATS

The most effective agents tested were chlorothiazide (Diuril), ammonium chloride with Carbopol 941, Phenergan, and Halotestin. Lung burdens of inhaled $^{239}\text{PuO}_2$ were reduced by as much as 40% over a 21-day treatment period.

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

Continuing studies on the therapeutic removal of inhaled $^{239}\text{PuO}_2$ have involved tests of a number of pharmacologic agents which might be expected to stimulate natural physiological pulmonary clearance processes.

Observations

Thirty-five, adult, female rats were simultaneously exposed to an aerosol of $^{239}\text{PuO}_2$. The animals were randomized into groups of five,

and six of these groups were treated with one of the therapeutic agents to be tested, the remaining group serving as controls for that particular exposure. After 21 days of treatment, the animals were sacrificed and the lungs analyzed for plutonium. The more interesting results of several such experiments are shown in Table I. The most promising of the drugs tested were Diuril and ammonium chloride with Carbopol 941. Other drugs tested were less effective or detrimental.

Similar tests were conducted to determine whether these agents were effective over a shorter treatment period of 48 hr (Table II). Diuril and chlorpromazine appeared to be somewhat effective, though to a lesser degree than with prolonged treatment.

Pharmacologic agents which promote the removal of inhaled insoluble $^{239}\text{PuO}_2$ from the lung can either hasten excretion or cause translocation to other organs or tissues. In the latter case, little is gained in

TABLE I. Clearance of Inhaled $^{239}\text{PuO}_2$ from Rat Lungs After a 21-Day Treatment Period (Average values from five rats)

<u>Drugs Used</u>	<u>Daily Dose Level</u>	<u>Route</u>	<u>Lung Burden, percent of controls</u>
Sephadex G-25	10-min aerosol	Inhaled	83
Promazine HCl	10 mg/kg	Oral	83
Promazine HCl	20 mg/kg	Oral	98
Aldactone (Spironolactone)	5 mg/kg	Oral	94
Miltown (Meproamate)	20 mg/kg	Oral	115
Diuril (Chlorothiazide)	50 mg/kg	Oral	61
NH_4Cl + Carbopol 941	1 mg/ml drinking water	Oral	69
	5-min aerosol	Inhaled	
NH_4Cl	1 mg/ml drinking water	Oral	136
Carbopol 941	5-min aerosol	Inhaled	81

TABLE II. Clearance of Inhaled $^{239}\text{PuO}_2$ from Rat Lungs after a 48-Hr Treatment Period

<u>Drug Used</u>	<u>Daily Dose Level</u>	<u>Route</u>	<u>Lung Burden, percent of controls</u>
Promazine HCl	10 mg/kg	Oral	89
Promazine HCl	20 mg/kg	Oral	87
Miltown	20 mg/kg	Oral	129
Sephadex G-25	10-min aerosol	Inhaled	133
Aldactone	5 mg/kg	Oral	97
Diuril	50 mg/kg	Oral	88

reducing the hazard. Therefore, the skinned carcasses of the rats were also assayed when the last group of drugs were tested (Table III). Here it can be seen that Diamox, while significantly reducing the lung burden of the experimental animals, has increased the carcass burden by 200%. On the other hand, Halotestin and Phenergan have reduced both the lung burden and the carcass burden.

Conclusions

The tests conducted during this

reporting period indicate that Diuril, chlorpromazine, Halotestin and Phenergan show some promise for removing inhaled PuO_2 from rat lungs. Carbopol 941, a carboxy polymethylene resin, and Sephadex G-25, when the dry material is inhaled, may stimulate the removal of plutonium by acting as a "pulmonary lavage." However, a true pulmonary lavage would be more predictable and probably more effective, as described elsewhere in this report.

TABLE III. Lung and Carcass Distribution of Inhaled $^{239}\text{PuO}_2$ in Rats Treated for 21 Days with Various Agents

Drug Used	Daily Dose Level	Route	Tissue Burden, percent of controls		
			Whole Body	Lung	Carcass
Diamox (Acetazolamide)	100 mg/kg	Oral	83	72	299
Reserpine	0.1 mg/kg	Oral	108	94	378
Benadryl	25 mg/kg	Oral	92	96	189
Miltown	100 mg/kg	Oral	76	77	193
Halotestin	2.5 mg/kg	Oral	77	78	54
Phenergan	3.0 mg/100 ml drinking water	Oral	69	69	95

EFFECT OF DTPA ON INHALED $^{239}\text{PuF}_4$ IN BEAGLES

DTPA had no effect on the retention, distribution, or excretion of inhaled $^{239}\text{PuF}_4$ in beagle dogs treated over a 12-week postexposure period. The behavior of inhaled $^{239}\text{PuF}_4$ in beagle dogs was very similar to that of $^{239}\text{PuO}_2$.

Investigator:
J. V. Dilley

We previously reported whole-body counting data on the lack of effect of diethylenetriaminepentaacetate (DTPA) in removing inhaled $^{239}\text{PuF}_4$ from three beagle dogs (Annual Report, 1967). This report describes further data from that study.

Observations

Six beagle dogs were exposed to dry aerosols of $^{239}\text{PuF}_4$. Approximately 2 hr after the exposure, each of three beagles were injected intraperitoneally with 0.5 g DTPA. This

treatment was repeated after 1, 2, 4, 7, 11, 16, 18, 23, 37, 44, 51, and 58 days. Daily urine and fecal samples were collected, and at 85 days postexposure the animals were sacrificed and tissues analyzed for plutonium. Figure 1 shows the uri-

nary and fecal excretion rates for all six dogs as fit to the data by least squares analysis. There are no differences in the slopes of these curves that are unique to treated or untreated animals. Retention curves (Figure 2) were constructed

FIGURE 1. Calculated Daily Urinary and Fecal Excretion Rates of $^{239}\text{PuF}_4$ in Beagle Dogs Treated with DTPA (Dogs treated with DTPA are indicated by an asterisk. Arrows indicate treatment days.)

FIGURE 2. Retention of Inhaled $^{239}\text{PuF}_4$ in Beagle Dogs (Animals treated with DTPA are indicated by an asterisk. Arrows indicate days of DTPA treatment. Intercept of the broken lines on the ordinate indicates estimates of initial alveolar deposition.)

for each dog from plutonium analyses on all tissues at time of sacrifice and from daily excretion data. There

are no differences in the shapes of these curves that are unique to treated or untreated animals. One control (No. 448) seemed to have a relatively high normal clearance rate. This is more apparent when the amount cleared over the 85-day period is compared with the other animals, as in Table I.

DTPA had relatively little effect on the tissue distribution of the inhaled plutonium (Table II). Liver burdens appeared to be decreased, and possibly skeletal burdens, but these were an insignificant fraction of the total retained plutonium.

Conclusions

DTPA does not significantly hasten the removal of inhaled $^{239}\text{PuF}_4$ from beagle dogs. It may influence the quantity of plutonium in the liver and skeleton but, in the case of $^{239}\text{PuF}_4$, this represents a very small portion of the total amount deposited in the animal.

TABLE I. Excretion of $^{239}\text{PuF}_4$ by Beagle Dogs Treated with DTPA

Dog No.	Treatment	Body Burden, (a) μCi			Percent Cleared	
		Initial	Alveolar	Final	Total	Alveolar
434	DTPA	13.3	2.8	2.3	83	17
436	DTPA	7.7	0.39	0.29	96	24
451	DTPA	19.7	0.55	0.39	98	30
429	Control	5.4	0.41	0.32	94	21
441	Control	7.7	1.3	1.1	86	14
448	Control	3.0	0.3	0.17	94	43

(a) Estimated from clearance curves (Figure 1)

TABLE II. Distribution of $^{239}\text{PuF}_4$ in Beagle Dogs 90 Days After Inhalation (% of total retained at sacrifice)

Dog No.	Treated with DTPA			Controls		
	434	436	451	429	441	448
μCi (a)	2.299	0.293	0.388	0.325	1.095	0.170
Lung	93	89	97	94	93	82
Lymph nodes (b)	5.9	8.7	2.4	4.0	5.5	14
Liver	0.00	0.10	0.02	0.30	0.31	0.94
Skeleton	0.51	0.97	0.17	0.83	0.84	1.2
Muscle	0.10	0.46	0.07	0.22	0.09	0.45
Skin	0.03	0.11	0.39	0.20	0.05	0.73
Brain	0.21	0.01	0.01	0.13	0.01	0.55

(a) Total body burden at sacrifice

(b) Tracheobronchial lymph nodes only

REMOVAL OF INHALED $^{239}\text{PuO}_2$ BY LUNG WASHING

Over half of the plutonium deposited by inhalation in the rat lung could be removed by serial washing with saline. The amount removed depended on the amount initially deposited and the time since inhalation.

Investigator:
C. L. Sanders

Technical Assistance:
K. E. McDonald
G. J. Powers*

Pulmonary lavage has proved useful in the treatment of certain lung disorders, particularly pulmonary alveolar proteinosis. The technique of lung washing in living or killed animals offers an experimental method for the quantitative study of the deposition, phagocytosis, distribution and retention of inhaled radioactive particles as described elsewhere in this report. Also of significant interest is the possibility that pulmonary washing may offer a practical method for the therapeutic removal of insoluble radioactive particles accidentally deposited in the lungs of man.

Observations

Two groups of female rats were exposed to $^{239}\text{PuO}_2$ particles (count median diameter, $0.12\ \mu\text{m}$) by inhalation. One group had an initial mean deposition of $1.8\ \mu\text{Ci}$ (high-level group), while the other had an initial mean deposition of $0.12\ \mu\text{Ci}$

(low-level group). The lungs were dissected from the animal at intervals following inhalation and washed with 19 consecutive, 12 ml isotonic saline washes. The amount of plutonium present in the washes and that remaining in the lung at the conclusion of the washing was determined.

A maximum of 54% of lung plutonium was removed at 7 hr after exposure in the high-level group. The ability to remove plutonium by washing subsequently decreased, such that only about 10% was recovered in the washes at 14-35 days after exposure. Removal of plutonium by washing in the low-level group was highly variable, but always more effective than in the high-level group (Figure 1).

Nearly all particles found in lung washes after the first day had been phagocytized by pulmonary macrophages. Considerable pulmonary damage was observed a few weeks following inhalation in the high-level group. Only minimal changes were seen at 35 days in the low-level group. In the high-level group, high radiation doses to the lung probably resulted in pathological changes in the lungs, either resulting in obstruction of

*Radiological Sciences Department

FIGURE 1. Removal of Inhaled Plutonium from the Lungs of Rats by Serial Saline Washes (Each point represents an individual measurement).

the alveoli or sequestering of particles in the alveolar wall, thereby preventing their removal by lung washing. Foamy (histiocytic) macrophages occluded the alveoli at 20-40 days in the high-level group. Many plutonium particles were found within these macrophages.

Conclusions

The greater the amount of plutonium deposited in the lung, the earlier and more severe were the pathological alterations, resulting in less effective removal of plutonium by lung washing. The relatively effective removal at lower deposition levels encourages further study of in vivo treatments in larger animals.

EFFECT OF ELECTRO-AEROSOL INHALATION ON PULMONARY CLEARANCE OF $^{239}\text{PuO}_2$ IN RATS

Rats that had inhaled $^{239}\text{PuO}_2$ were subsequently exposed to a negatively charged aerosol, sacrificed, and lungs and trachea analyzed for plutonium. The groups treated with electro-aerosols generally retained less plutonium than their controls.

Investigator:
A. P. Wehner

Technical Assistance:
W. Skinner
M. D. Snyder

Negative air ions significantly accelerate mucociliary activity in the respiratory tract. Clinically, this should result in improved

bronchopulmonary hygiene. This has been observed empirically in the therapeutic application of electro-aerosols. However, no quantitative

studies had been conducted to investigate the effect of electro-aerosols on pulmonary clearance under controlled laboratory conditions.

Observations

Table I summarizes the experiments performed and the results obtained. Exposure to $^{239}\text{PuO}_2$ was for 15 or 20 min and was uniform for animals in a given experiment. A Küster-Barthel type electro-aerosol generator dispersed a 1% NaCl solution at a rate of 1.5 l/hr with pressurized air at 45 psi. For electro-aerosol treatment the jet was maintained at a potential of -24 kV. The resultant electro-aerosol generated a space charge potential of -6 kV/cm in the cages positioned 142 cm from the jet. Each cage contained four to six rats. The uncharged aerosol was dispersed through the grounded jet. The mass median diameter of both charged and uncharged aerosol droplets was 3.8 μm . In Experiments 1 and 2, longer term pulmonary clearance was investigated employing intermittent aerosol exposure and sacrificing the animals after 3 weeks. In Experiment 3, short-term clearance was studied with rats being sacrificed after 5, 24, and 48 hr of continuous electro-aerosol exposure.

With the exception of the group sacrificed 24 hr after plutonium exposure in Experiment 3, the mean lung burden of the electro-aerosol

treated groups was from 15-32% lower than that in groups receiving no aerosol treatment, and from 8-34% lower than in groups treated with uncharged aerosol. There was no statistically significant difference between the mean lung burdens of the two control groups in Experiments 1 and 2, showing that uncharged aerosols did not affect pulmonary clearance. The hypothesis that inhalation of negatively charged aerosols increases pulmonary clearance was tested with a one-tailed t test, with results as shown in Table I.

Conclusions

The results of this exploratory investigation suggest, but do not conclusively prove, that electro-aerosols may accelerate pulmonary clearance of inhaled $^{239}\text{PuO}_2$. This would be in good agreement with the observed stimulation of ciliary activity by negative air ions, which, according to Krueger, is caused by interaction of the electric charges with the cytochrome-linked oxidation of 5-hydroxytryptamine.⁽¹⁾ Further work is required to confirm these tentative findings.

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TABLE I. Effect of Electro-Aerosol Treatment on Clearance of Inhaled $^{239}\text{PuO}_2$ by Rats

Expt.	Treatment		No. of Rats	Plutonium Retained, μCi (a)
	Type	Duration		
1	Charged aerosol	3 1/2 hr/day for 3 weeks	10	0.26 ± 0.04 (b)
	Uncharged aerosol	3 1/2 hr/day for 3 weeks	9	0.39 ± 0.04
	None	Sacrificed after 3 weeks	10	0.36 ± 0.07
2	Charged aerosol	3 1/2 hr/day for 3 weeks	12	0.53 ± 0.05 (c)
	Uncharged aerosol	3 1/2 hr/day for 3 weeks	11	0.57 ± 0.04
	None	Sacrificed after 3 weeks	12	0.62 ± 0.06
3	Charged aerosol	Continuous for 5 hr	6	0.93 ± 0.08 (d)
	Charged aerosol	Continuous for 24 hr	6	1.09 ± 0.18
	Charged aerosol	Continuous for 48 hr	6	0.66 ± 0.06 (e)
	None	Sacrificed after 5 hr	6	1.38 ± 0.21
		Sacrificed after 24 hr	6	0.99 ± 0.09
		Sacrificed after 48 hr	5	0.80 ± 0.00

(a) Mean $\pm$ standard error

(b) Significantly different from combined controls at 0.05 level

(c) Significantly different from combined controls at 0.15 level

(d) Significantly different from 5-hr control at 0.05 level

(e) Significantly different from 48-hr control at 0.05 level

MOLECULAR AND CELLULAR LEVEL STUDIES

A rather diverse group of studies are included in this section. The first five reports are concerned with more basic biochemical studies. Some of this work, as indicated by footnotes, has received substantial support from the Battelle Institute. By combining the support available from the AEC Division of Biology and Medicine and the Battelle Institute, progress has been made on a broader front than could have been realized by separate efforts on behalf of the two sponsors.

The first two reports describe studies on the control of RNA metabolism in Escherichia coli, and represent the final phase and culmination of several years' efforts. Continuing current efforts on the elucidation of transport mechanisms and the regulation of protein synthesis in *Neurospora* are represented by the three following reports.

Two reports deal with applications of electron microscopy. One of these derives from a cooperative study with Dr. C. G. Heller of the Pacific Northwest Research Foundation, Seattle, Washington. Other applications of electron microscopy, dealing specifically with phagocytized PuO_2 particles are included in the section on Inhalation Studies.

Two reports detail current progress in the study of columnaris disease in Columbia River fish. Although supported by the AEC Division of Production, this work is closely related to programs of the Division of Biology and Medicine. The development of an effective oral immunization procedure represents a significant achievement in this area.

The two final reports in this section are concerned with thermal shock and temperature acclimation in fish. Related studies are included in Vol. 1, Part 2, of this report.

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RIBOSOMAL PRECURSORS AND RIBONUCLEIC ACID
SYNTHESIS IN ESCHERICHIA COLI*

Methods were developed to assess the level of ribosomal precursors in steady state cells. These precursors were shown to constitute a constant fraction of the total cellular ribonucleic acid (RNA) in cells exhibiting a wide range of RNA to protein ratios. When the level of ribosomal precursors was increased experimentally, the rate of RNA synthesis was reduced. These results are interpreted as offering support for the assertion that the precursors of mature ribosomes participate in the regulation of RNA synthesis.

Investigator:
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Technical Assistance:
Laura S. Winn

Our work on the regulation of ribonucleic acid (RNA) synthesis has centered primarily on questions concerning the synthesis of ribosomal RNA and of ribosomes in the enteric bacterium, Escherichia coli. Earlier reported observations (Annual Report, 1966) led us to postulate a model which suggested that a pool of immature ribosomes exists in the cell in steady state and that the size of this pool in some way regulates the

rate of polymerization of the precursors of RNA. It was of interest, therefore, to examine the relationship between the level of immature ribosomes and the rate of RNA synthesis in cells growing under various conditions.

Observations

Cultures of E. coli were grown for many generations in the presence of labeled uracil and under a variety of conditions which resulted in a fairly wide range in ratios of RNA to protein. Samples of these radio-

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

active cells were pelleted and mixed with a 40-fold excess of cells which had been treated with chloramphenicol for 30 min to produce enhanced levels of "chloramphenicol particles" which are in many ways indistinguishable from the physiological precursors of ribosomes. Extracts of these cell mixtures were prepared in high magnesium buffer. Samples of these extracts were layered on sucrose density gradients before and after treatment with ribonuclease. The gradients were centrifuged 8 hr at about $100,000 \times g$ at a temperature of 3°C . The sedimentation profiles obtained from such gradients were used to compute values for the immature ribosomes. The sensitivity of immature ribonucleoprotein particles to ribonuclease is well known and was quite apparent from comparison of the absorbency profiles before and after ribonuclease treatment. Ribonuclease treatment resulted in a considerable reduction in the amount of absorbency sedimenting at intermediate S values. Similarly, ribonuclease treatment resulted in a measurable reduction in the amount of radioactivity sedimenting at intermediate S values. Ribonuclease-sensitive radioactive material sedimenting at intermediate S values was interpreted as ribosomal precursors present in the radioactive cells. In Figure 1 these measurements are plotted as a function of RNA/protein ratios observed in cells grown under various conditions. The results show that the level of precursors was essentially a constant fraction of the total cellular RNA.

FIGURE 1. *Relative Level of Ribosomal Precursors in Steady State Cells*

These observations are not inconsistent with the notion that immature particles are involved in the control of RNA synthesis. They would seem to indicate that the level of these immature particles is regulated stringently by other factors (for example, the availability of ribosomal proteins). The possible regulatory effect of these materials was further tested by experimentally varying their concentration and examining the rate of RNA synthesis in such treated cells. For this purpose, cells were incubated for various lengths of time with chloramphenicol. Under these conditions, cells will accumulate significant quantities of immature ribonucleoprotein particles. Control cells were incubated in phosphate-free medium where such an accumulation of chloramphenicol particles does not occur. At intervals of 30 min, samples of cells were withdrawn and placed in complete medium

containing radioactive uracil. The incorporation of radioactivity by the recovering cells was followed for 20 min at intervals of 4 min. In all cases, incorporation proceeded linearly from the fourth to the twentieth minute. From these measurements, rates of RNA synthesis were determined for each sample of cells. In Figure 2, these rates are plotted as a function of the length of pretreatment of the cells in chloramphenicol. The cells incubated in chloramphenicol in phosphate-free medium essentially maintained their initial rate of synthesis of RNA for at least 60 min. Cells incubated in complete medium, however, showed a decrease in the rate of RNA synthesis which started immediately and continued for at least 120 min. It is apparent from these results that the rate of RNA synthesis was diminished in direct proportion to the increases in level of ribosomal precursors in the cells.

Extracts prepared from these cells at the conclusion of the 20-min recovery period were examined for ribonuclease-sensitive radioactivity sedimenting between 50S and 20S by the methods described above. As shown in Figure 3, the percentage of total radioactivity incorporated during the 20-min recovery period that exhibited ribonuclease sensitivity and that sedimented between 50S and 20S increased with increasing length of pretreatment in complete medium containing chloramphenicol. These cells had accumulated increased

FIGURE 2. Reduced Rate of Synthesis of RNA After Accumulation of Chloramphenicol Particles

FIGURE 3. Occurrence of Newly Synthesized RNA in Immature Ribonucleoprotein Particles

amounts of immature particles and exhibited a reduction in rate of incorporation of isotope from labeled uracil. The control cells, incubated in phosphate-free medium with chloramphenicol, did not accumulate increased amounts of immature particles and showed only a modest reduction in the rate of incorporation of labeled uracil. The percentage of total activity which was incorporated during recovery and was present in immature particles did not increase substantially with increasing length of pretreatment.

Conclusions

Less RNA synthesis occurred in cells having the greater concentration of immature ribonucleoprotein particles, and an increasing fraction of that which was synthesized was held up in the pool of immature ribosomes present at later times. The correlation is consistent with the concept of a pool of precursor which is itself implicated in the control of synthesis of materials flowing through it. These results support the idea that immature ribosomes participate in the regulation of RNA synthesis, perhaps as feedback inhibitors.

COORDINATE SYNTHESIS OF RIBOSOMAL AND NONRIBOSOMAL RIBONUCLEIC ACID IN ESCHERICHIA COLI*

The synthesis of RNA was examined in cells in which this process was inhibited by as much as 80%. Isotope from labeled uracil was incorporated randomly into the species of RNA normally present in growing cells at all levels of inhibition studied. We conclude that the inhibition caused by immature ribosomes is exerted in a coordinate manner.

Investigators:
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When cells of Escherichia coli are treated with chloramphenicol at

a concentration of 100 μ g/ml, net synthesis of protein stops but the synthesis of ribonucleic acid (RNA) continues for some time. The RNA which is synthesized under these conditions appears to consist of

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

the species synthesized by the normally growing cell. Most of the RNA which is synthesized under these conditions appears in the form of ribonucleoprotein particles (50% RNA, 50% protein) with sedimentation velocities between 50S and 20S. We have suggested previously (Annual Report, 1966) that the presence of these particles inhibits continued synthesis of RNA in the cells. In the present study, we have addressed ourselves to the question of whether this inhibition is exerted in a coordinate or noncoordinate fashion on ribosomal and nonribosomal RNA.

Observations

Cells were treated for various periods with chloramphenicol and thereby caused to accumulate various levels of immature ribonucleoprotein particles. The decrements in initial rate of RNA synthesis exhibited by these cells during recovery from chloramphenicol ranged from 0-80%. Samples of cells exhibiting various levels of inhibition were allowed to recover in the presence of labeled uracil, and the distribution of radioactivity among the classes of ribosomal and nonribosomal RNA was used as the criterion for assessing qualitatively the nature of the RNA being synthesized during the initial phase of recovery.

Results are shown in Figure 1. The panels of Figure 1 show sedimentation profiles obtained by sucrose density centrifugation of samples of RNA obtained by phenol extraction of crude extracts of cells. Panel A shows the normal distribution of

phenol-extracted bulk RNA among the three recognizable classes of RNA; namely, 23S RNA, 16S RNA, and 4S RNA. 23S RNA and 16S RNA are generally regarded as ribosomal RNA and, for purposes of the present argument, RNAs sedimenting at lower S-values are regarded as nonribosomal RNA. It can be seen from panel A that ribosomal RNA accounts for about 75% of the total extractable RNA, and that the ratio of 23S RNA to 16S RNA is in excess of 2 to 1.

Panels B through F show the distribution of uracil-2-¹⁴C incorporated during a 20-min period of continuous labeling in cells recovering from chloramphenicol inhibition and exhibiting the indicated levels of inhibition of RNA synthesis. As the level of inhibition of RNA synthesis increases, the fraction of total extractable RNA with sedimentation characteristics typical of ribosomal RNA decreases; there is a corresponding increase in the fraction of RNA which sediments between 16S and 4S. The apparent decrease in the relative amount of ribosomal RNA as compared with nonribosomal RNA could arise from a disproportionate inhibition exerted on the synthesis of ribosomal RNA, or it could arise because of a disproportionately high rate of degradation of newly synthesized ribosomal RNA, or these results could simply reflect the longer period of time required to synthesize molecules of ribosomal RNA as compared to nonribosomal RNA. Thus, either partially completed or partially degraded 23S and 16S molecules might be expected to sediment between 16S and 4S RNA.

FIGURE 1. Distribution of Uracil-2-¹⁴C Incorporated into Phenol Extractable Bulk RNA of *E. coli* Recovering from Chloramphenicol Inhibition (The density gradient profiles were analyzed with a hybrid computer. The numbers under each peak are percentages of the total area under the profile. The sum of the 23S and 16S peaks is given under the horizontal bar and is regarded as the percentage composition of ribosomal RNA.)

To eliminate this ambiguity, the experiment was repeated, except that only a 2-min "pulse" of labeled uracil was provided, followed for 18 min with excess or "chase" of nonradioactive uracil. If the material sedimenting at intermediate S values represented partially completed molecules, the chase period would depopulate this species with respect to isotope; if it represented partially degraded molecules, the 18-min chase period would allow this process to proceed essentially to completion and would increase the fraction of radioactivity appearing at this location of the gradients. The results of the "pulse-chase" experiment are presented in panels F through I of Figure 1. It is apparent that a significant decrease is seen in the fraction of newly synthesized RNA which sediments between 16S and 4S and, consequently, this material must be partially completed molecules of RNA rather than partially degraded

molecules of ribosomal RNA. It is also clear that the isotope incorporated during a 2-min pulse was distributed essentially randomly over the normal array of species present in bulk phenol extractable RNA.

Conclusions

Even at the highest levels of inhibition of RNA synthesis, the rates of synthesis of the different classes of RNA were proceeding in approximately the same ratios as were observed in the absence of any inhibition. We conclude therefore that, whatever the mechanism of inhibition of RNA synthesis by chloramphenicol particles, the effect is exerted equally on all classes of RNA that can be distinguished by the method of centrifugation on density gradients of sucrose.

TRYPTOPHAN TRANSPORT IN NEUROSPORA: TRYPTOPHAN
BINDING PROTEIN RELEASED BY COLD OSMOTIC SHOCK*

A tryptophan binding protein was released from Neurospora by cold osmotic shock. The binding protein was shown to be intrinsic to the cell membrane. Osmotic shock fluids from transport-negative mutants were shown to contain reduced levels of binding activity when compared to those of wild type strains. Cells repressed for tryptophan transport were shown to contain reduced levels of the binding component. The specificity characteristics for the binding protein are the same as those demonstrated previously for cellular transport.

Investigators:

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When cells of Neurospora were subjected to cold osmotic shock (Annual Report, 1967), a protein component was released which reversibly binds tryptophan. If the binding protein is active in tryptophan transport, it should conform to the following criteria: (1) it should be localized in the cell membrane, (2) binding should conform to the specificity characteristics established for cellular transport, and (3) binding

activity should be depressed or absent in noninduced or transport-negative mutants. The experiments described in this report were conducted in an attempt to determine the extent to which the proteins released from Neurospora by osmotic shock conform to these criteria.

Observations

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

Treatment of germinated conidia of Neurospora with ethylenediaminetetraacetic acid followed by an abrupt

osmotic transition results in the release of at least seven distinct proteins as measured electrophoretically. The methods for osmotically shocking the cells were described previously (Annual Report, 1967). Alkaline phosphatase and a tryptophan-binding protein are among the proteins which are released into the medium. Tryptophan binding was measured by gel filtration (Annual Report, 1967). Alkaline phosphatase was assayed by measuring the cleavage of p-nitrophenylphosphate at 410 mμ in a Gilford spectrophotometer at pH 8.3. Figure 1 shows the elution profile for protein-bound tryptophan and alkaline phosphatase activity following gel filtration through Sephadex G-100. The optical density in peak (I) was eluted in the excluded volume from the column and corresponds to the sharp radioactive tryptophan peak also eluted in the excluded volume. The major radioactive tryptophan peak (unbound tryptophan) was eluted in the included volume. Thus, the radioactivity associated with peak (I) could occur only by binding to protein present in peak (I). Alkaline phosphatase activity is associated with peak (II).

Various indirect lines of evidence have suggested that the proteins released by osmotic shock are localized on the cell membrane (Annual Report, 1967). To test this possibility, cell membrane preparations were prepared from a slime mutant of *Neurospora* (Slime 327) by mild, osmotic shock treatment in 0.25M sucrose tris buffer. Slime mutants of *Neurospora* do not have a rigid

cell wall and approximately 90% of the cells were lysed by this mild osmotic transition. Cell membrane fractions were isolated from the total cell lysates by differential centrifugation. The membrane preparations were washed by repeated centrifugation at 900 g. Table I shows the specific activity of several enzymes and the binding protein before and after five washes of the membrane preparation with tris-sucrose buffer (0.03M tris + 0.25M sucrose). The specific activity of alkaline phosphatase was increased more than 2-fold by the washing procedure (from 1.10 - 2.71). The tryptophan specific binding activity was also increased nearly 2-fold by this procedure. The specific activity of tryptophan synthetase (a cytoplasmic enzyme) and succinic dehydrogenase (a mitochondrial enzyme), on the other hand, were markedly decreased by the washing procedure.

When *Neurospora* is grown in tryptophan containing medium, tryptophan uptake is reduced relative to the uptake which occurs in cells grown in minimal medium (Annual Report, 1967). When the binding activities in shock fluids prepared from cells grown in tryptophan medium and minimal medium were compared, the specific binding activity in the tryptophan-grown cells was reduced approximately 10-fold relative to minimal-grown cells. Alkaline phosphatase activity was the same in the two preparations. We infer from these results that the tryptophan-binding protein is an active component of the tryptophan transport system.

FIGURE 1. Tryptophan Binding and Alkaline Phosphatase Activity in a Crude Osmotic Shock Preparation from *Neurospora* Wild Type Strain 74A [2.5 ml of a reaction mixture containing 2.9 mg/ml protein and 0.1 μ moles/ml L-[14 C] tryptophan (specific radioactivity 20 μ Ci/ μ mole) in 0.1M acetate buffer pH 6.8 was passed over an 18 $\times$ 2.5 cm Sephadex G-100 column (see text for preincubation conditions). Samples (30 ml) were collected from the column at a flow rate of 60 ml/hr; absorbency at 280 m μ , radioactivity and alkaline phosphatase activity were measured in each fraction. The OD peaks eluted from the column are identified by Roman numerals I and II. About 0.04 nmoles tryptophan were bound per mg protein added to the column. The protein occurring in peak (I) was approximately 0.5 mg.]

TABLE I. Effect of Washing on Removal of Membrane-Bound Enzymes

Treatment	SDS Extractable Protein, mg/ml (a)	Specific Activity			
		Alkaline Phosphatase (b)	Tryptophan Binding (c)	Succinic Dehydro- genase (d)	Tryptophan Synthetase (e)
Unwashed	2.35	1.11	0.5	2.80	0.032
Washed 5X	0.80	2.71	0.8	0.17	Below detection

(a) All enzymes were assayed after treatment of the membrane preparations with 0.75 mg sodium dodecylsulfate/mg protein

(b) Micromoles p-nitrophenol formed from p-nitrophenylphosphate/min/mg

(c) Nanomoles/mg protein

(d) Micromoles dichlorophenol indophenol reduced/min/mg

(e) Micromoles indole utilized/hr/mg

The tryptophan-binding protein might be expected to be absent or reduced in transport negative mutants. To test this point, binding activity in shock fluids from wild type cells was compared to that of a tryptophan transport mutant (FGSC #1117). This 4-methyl tryptophan-resistant mutant transports tryptophan at a rate which is less than 15% the rate observed for wild type strains. In partially purified preparations the tryptophan specific binding activity in the mutant shock fluids was 0.2 nmoles/mg protein as compared to 0.7 nmoles/mg protein in the wild type strain. Alkaline phosphatase specific activity in the two strains was 1.66. The level of binding activity in the mutant preparation appears higher (relative to wild type) than would be expected in view

of the differences observed in the rates of cellular transport. It is possible that this is a reflection of more than one enzymic step in the transport process. This possibility is presently under investigation.

The transport of tryptophan in *Neurospora* has been shown to be competitively inhibited by leucine, phenylalanine, and methionine but not by lysine and arginine (Annual Report, 1966). The results shown in Table II suggest that this same specificity exists for the tryptophan binding protein.

Figure 2 shows that the binding of tryptophan to the released protein is concentration dependent. There was a linear increase in the amount of L-[¹⁴C] tryptophan bound (at equilibrium) between 0 and approximately 1.6×10^{-4} M.

TABLE II. *Inhibition of Tryptophan Binding by Other Amino Acids (Amino acids were all present at a concentration of 0.25 μ moles/ml; the tryptophan concentration was 0.05 μ moles/ml.)*

Amino Acid	Tryptophan Bound, nmoles/mg protein
None	0.7
L-Leucine	0.3
L-Phenylalanine	0.3
L-Arginine	0.9

FIGURE 2. *Effect of Tryptophan Concentration on Binding (The concentration of protein added to the column in all cases was 1.68 mg/ml. The specific radioactivity of the tryptophan for each binding study was 20 $\mu Ci/\mu mole$. The column size was 14 $\times$ 2.5 cm. Three ml fractions were collected from the column at a flow rate of 60 ml/hr.)*

Conclusions

The tryptophan-binding protein released from *Neurospora* by cold osmotic shock appears to be a component of the tryptophan transport system. The protein has the same specificity characteristics as the cellular transport system. Decreased binding activity is observed in shock fluids derived from cells with depressed rates of cellular transport. The specific activity of the binding protein is reduced in mutants of *Neurospora* with low rates of tryptophan uptake. The binding protein appears to be intrinsic to the cell membrane which is the functional site of cellular transport. Attempts to purify the binding protein are presently underway.

KINETICS OF INDUCTION OF KYNURENINASE IN NEUROSPORA*

~~Kynureninase synthesis is induced by tryptophan, kynurenine, or N-formyl-kynurenine. An increased relative rate of enzyme synthesis is evident in a growing culture 10 min after introduction of the inducer. Kynureninase synthesis stops when inducer is removed from the cells and there appears to be a preferential degradation of the enzyme in the absence of inducer. The time required for synthesis of competent messenger RNA for kynureninase is 4 min. Completion of active enzyme is apparently not dependent upon the aggregation of preformed subunits.~~

Investigators:-----

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Carla J. Farrell

Tryptophan serves as the sole source of nicotinic acid in Neurospora crassa. One of the enzymes involved in the conversion of tryptophan to nicotinic acid is kynureninase. Kynureninase is present at very low levels in wild-type Neurospora but can be induced to high levels by growth in the presence of tryptophan, kynurenine, or N-formyl-kynurenine.

Observations

The sequence of events with time after addition of kynurenine to a growing culture of Neurospora are shown in Figure 1. The rate of induction, the preinduction lag, and decay of activity after removal of inducer are similar for tryptophan or N-formyl-kynurenine. Increase in enzyme occurs about 10 min after addition of inducer. Synthesis continues at a constant

rate until 30 min (at which time inducer was removed) and kynureninase activity decays after inducer is removed. Complete depletion of the intracellular pool of inducer is judged by the cessation of excretion of anthranilic acid. Using this point (45 min) as the time of inducer removal, it is noted that enzyme synthesis stops when inducer is exhausted and that there is an immediate decay in enzyme activity. This decay is greater than would be expected from dilution by growth, and may indicate a preferential turnover or destruction of kynureninase in the absence of its inducer.

In order to determine the time required for formation of messenger RNA which directs the synthesis of kynureninase, advantage was taken of the fact that the antibiotic cycloheximide inhibits protein synthesis in Neurospora but allows RNA synthesis to continue. Figure 2 shows the results of an experiment in which cycloheximide was added to a culture

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

FIGURE 1. Kynureninase Induction by Kynurenine ($0.05 \mu\text{moles/ml}$) (See text for details)

FIGURE 2. Required Time for Competent Message Formation (See text for details)

at zero time and kynurenine was added 5 min later. Duplicate samples were taken from the culture at short intervals. One sample was assayed immediately for kynureninase, the second was washed free of inducer and cycloheximide and reincubated in minimal medium for 30 min. Any messenger RNA formed during cycloheximide inhibition should be expressed as enzyme in the second, recovered, sample. The results show that messenger RNA specific for this enzyme is completed 4 min after addition of the inducer and that the amount of messenger RNA formed increases linearly with time for about 25 min, after which the rate decreases and approaches zero. This time for

messenger RNA formation is comparable to that required for synthesis of messenger RNA in bacteria which have a generation time approximately one-sixth the generation time of *Neurospora*.

Figure 3 shows the results of further examination of the effect of cycloheximide in which inducer (kynurenine) was added to the culture at 5, 28 and 58 min after addition of cycloheximide. The results show that the rate of messenger RNA synthesis decreases as a function of time, and also that a second limitation may be occurring since the total amount of enzyme-forming capacity decreases as a function of time. Incorporation of H^3 -uridine during this time period was not affected, i.e., the rate of total RNA synthesis

apparently is unchanged during the experiment.

Recent observations of inducible enzymes in bacterial systems show that in some cases the completion of a functional enzyme is dependent on the aggregation of subunits. This subunit aggregation does not require protein synthesis. Examination of *Neurospora* for protein precursors of kynureninase was carried out as shown in Figure 4. Inducer was added to logarithmically growing cells at zero time, and removed at 14.5 min. At short intervals thereafter, duplicate samples were withdrawn and one sample was assayed immediately for kynureninase (a). The second sample (b) was washed free of kynurenine and placed in medium containing cycloheximide to inhibit further protein

FIGURE 3. Induction of Kynureninase in the Presence of Cycloheximide (See text for details)

FIGURE 4. Detection of Protein Precursors of Kynureninase (See Text for details)

synthesis. If protein precursors are present in the cell during induction, the sample allowed to incubate in cycloheximide for an additional 30 min should have a greater amount of enzyme than that found in the immediately assayed sample. This difference in enzyme between the two samples would equal the amount of precursor.

There is no apparent difference in the amount of kynureninase present

under either growth condition, which indicates that kynureninase probably is synthesized de novo as an intact, functional enzyme.

Conclusions

Kynureninase is inducible in *Neurospora* with kinetics similar to those observed for most bacterial-inducible enzymes. The time required for induction is, however, much

faster in *Neurospora* if expressed as a fraction of generation time. Classically, induction in *Neurospora* has been thought to involve long lag periods; kynureninase appears to be an exception.

There are probably no protein precursors of kynureninase made during induction, and the enzymes produced by three different inducers are apparently identical. The induction process is an all or none phenomenon, since changing the concen-

tration of inducer by a factor of 10 does not produce a gradation of synthetic rate.

The characteristics of induction in this system offer a significant point of departure for the examination of the regulatory system in a higher organism, which, in the case of *Neurospora*, is easily manipulated. Our future work will include purification of the enzyme, and an attempt at genetic localization of the coding regions for this protein.

A DIRECT METHOD FOR ASSAYING KYNURENINASE*

A rapid fluorometric assay has been developed to measure kynureninase activity in cell-free extracts of Neurospora crassa. The conversion of kynurenine to anthranilic acid was measured directly in a spectrophotofluorometer. The assay can be completed in 10 min.

Investigators:
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As in the procedure described by Jakoby and Bonner,⁽¹⁾ this method involves detection of anthranilic acid produced by measuring the intensity of its fluorescence. Extracts prepared from tryptophan-induced 74A were used as a source of kynureninase. The activity was purified approxi-

mately 4-fold by ammonium sulphate precipitation and Sephadex chromatography.

Observations

The reaction mixtures contained 0.16 μ mole pyridoxal phosphate, 3 μ mole magnesium sulfate, 0.06 μ mole kynurenine, 50 μ mole tris-(hydroxymethyl)aminomethane pH 8.0, and *Neurospora* protein (50-500 μ g),

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

in a final volume of 1.2 ml. The reaction was conducted at 30 °C in the cuvette of an Aminco Bowman Spectrophotofluorometer equipped with thermal spacers. Anthranilic acid produced during the reaction was detected by measuring the intensity of its fluorescence at 409 nM. Activation was at 315 nM.

The amount of anthranilic acid produced is linear with time for about 5 min after which the rate decreased. Figure 1 shows that the velocity of the reaction is proportional to protein concentration from 0.019-0.235 mg/ml and extrapolates to zero at zero protein concentration. As shown in Figure 2, the velocity of the reaction approached a maximal value when the kynurenine concentration was 0.05 μ mole/ml. Kynurenine inhibits the reaction in concentrations greater than 0.16 μ mole/ml.

The rate of conversion of kynurenine to anthranilic acid was measured at kynurenine concentrations from 5×10^{-3} μ mole/ml to 1×10^{-1}

FIGURE 1. Effect of Enzyme Concentration on the Rate of Conversion of Kynurenine to Anthranilic Acid

FIGURE 2. Effect of Kynurenine Concentration on the Reaction Rate of Kynureninase

μ mole/ml using the direct method. A standard Lineweaver-Burke plot of the data describes a line with an intercept = 0.133, slope = 1.88×10^{-6} , correlation coefficient = 0.99. The K_m calculated from these data was 1.42×10^{-5} , which is essentially the same as that published by Jakoby and Bonner.⁽¹⁾

It has previously been reported that magnesium ions activate the enzyme at all stages of purification. The assays reported here have no magnesium requirement. Traces of magnesium present in the water could activate the reaction, however, EDTA had no effect on the rate.

Although the reaction proceeds more rapidly without pyridoxal phosphate, the amount of anthranilic acid produced is not linear with time. At a pyridoxal phosphate concentration of 0.13 μ mole/ml, the rate was constant for about 5 min. Increasing the concentration to 0.53 μ mole/ml decreased the rate to 1/3 of the value observed at 0.13 μ mole/ml.

Conclusions

The direct fluorometric assay for kynureninase can be completed within 10 min. This method produces results comparable to the more laborious procedures previously described

which require isolation of the anthranilic acid prior to measurement of fluorescence.

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SUBCELLULAR LOCALIZATION AND IDENTIFICATION OF ALPHA
EMITTERS BY ELECTRON MICROSCOPIC AUTORADIOGRAPHY

A method is described whereby the localization and identification of particulate alpha emitters within biological systems at the subcellular level can be confidently demonstrated.

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A method to localize and identify $^{239}\text{PuO}_2$ particles within the subcellular structures of the cell was required to evaluate the involvement of cellular organelles in radiation injury produced by $^{239}\text{PuO}_2$ particles that were phagocytized by rat peritoneal cells. This method was also needed to study the translocation and clearance mechanisms within the rat lung after the introduction of $^{239}\text{PuO}_2$ particles by aerosol inhalation. This method would be applicable in localizing and identifying any subcellular, particulate, alpha emitter.

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Observations

Rats were injected intraperitoneally with $^{239}\text{PuO}_2$ particles (1.4 μCi with a count mean diameter of 0.12) suspended in saline. The animals were sacrificed at intervals following injection, the peritoneal cavity washed with saline, and the cells recovered in pellets by centrifugation. The pellets were fixed in ice cold 1% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon 812. For morphological examination, the sections, obtained using diamond knives, were stained with uranyl acetate and lead citrate and photographed in an electron microscope.

For autoradiographic studies, the unstained sections were treated as described below.

A slight modification of the method described by Moses⁽¹⁾ was used. Approximately 3 ml of Ilford L-4 emulsion, melted at 45 °C, was transferred to a test tube and diluted with an equal quantity of 45 °C, distilled water. Approximately 20 drops of 0.05% sodium laurel sulfate were added to the diluted emulsion and mixed by inversion. The mixture was chilled in an ice bath for 5 min, and then warmed to 18 °C. A wire loop (~1 cm diameter) was dipped into the emulsion, the excess emulsion blotted from the loop edge with lens tissue, and the resulting film within the loop allowed to gel. The loop was lowered over a formvar-coated grid mounted on the end of a peg, so that the emulsion was flatly appressed to, the formvar film. To check the distribution of silver crystals, the grid was exposed to room light, photographically developed, and examined in the electron microscope. If the grains were unevenly distributed, a few more drops of sodium laurel sulfate were added, the emulsion reheated to 45 °C, and the above procedure repeated.

After a satisfactory emulsion was obtained, it was applied by the wire loop procedure to sections mounted on grids. The grids were stored in the refrigerator for 3-4 days in a light-tight box containing Drierite. After exposure, the grids were developed for 1 min at 20 °C in Kodak D-19, rinsed in 1% acetic acid, fixed in Kodak rapid-fixer for 5 min, and

then washed for 5 min in distilled water. The sections were stained for 20 min in lead citrate to improve contrast and remove the gelatin layer from the grids. No perceptible movement of silver grains was noted. The grids were examined in an RCA EMU-2C or 3B electron microscope.

Morphological examination showed electron-opaque particles localized in membrane-limited vacuoles of the peritoneal phagocytes (Figure 1). Dense particulate material was not seen within the peritoneal phagocytes of rats injected with saline only.

Figure 2 shows the morphological characteristics of the $^{239}\text{PuO}_2$ particles injected. It is apparent that the particles found within the peritoneal phagocytes had a shape and size similar to the injected particles. These observations strongly suggest that the particles engulfed by peritoneal phagocytes were $^{239}\text{PuO}_2$. Electron microscopic autoradiography was employed to rule out the possibility of a similarly shaped contaminant.

Alpha particles are emitted randomly in all planes and produce a typical star-like configuration of exposed silver grains in an autoradiograph (Figure 3). However, most of the alpha particles travel obliquely through the emulsion and may reduce only one or two silver grains. This results in a mass of reduced silver grains occurring over and generally obscuring the plutonium particle(s). For this reason, the precise localization of $^{239}\text{PuO}_2$ particle(s) in Figure 3 cannot be determined, though the center of the star is well within the limits of the cell.

19,800X

FIGURE 1. A Portion of a Peritoneal Mononuclear Phagocyte 1 hr after Intraperitoneal Injection of $^{239}\text{PuO}_2$ Particles [Electron-opaque structures, which have been identified as $^{239}\text{PuO}_2$ particles (see text), are localized in membrane-limited vacuoles (V).]

19,800X

FIGURE 2. Plutonium Particles Deposited on a Formvar-Coated Grid and Air-Dried (These particles were obtained from an aliquot of the $^{239}\text{PuO}_2$ injected into the rats.)

9500X

*FIGURE 3. Electron Microscopic Autoradiograph
Showing a Peritoneal Phagocyte 2 hr after Intra-
peritoneal Injection of $^{239}\text{PuO}_2$*

Conclusions

The electron-opaque particles observed within the membrane-limited vacuoles in the cytoplasm of the phagocyte in Figure 1 can be confidently identified as $^{239}\text{PuO}_2$ particles from the following facts:

- These electron-opaque particles structurally resemble $^{239}\text{PuO}_2$.
- The only particles found were localized within membrane-limited

vacuoles of the treated rats.

- Alpha emissions, characteristic of $^{239}\text{PuO}_2$, were localized within membrane-limited vacuoles as demonstrated by electron microscopic autoradiography.

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ELECTRON MICROSCOPY OF HUMAN SPERMATOGENIA

Electron microscopic examination of normal human testes has revealed the existence of four distinct types of spermatogonia in contrast to three types identified by light microscopy. With the addition of a fourth type, it now becomes possible to distinguish which cell is the most primitive and to follow the sequence of their development.

Investigators:
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Technical Assistance:
R. R. Adee

Three types of spermatogonia have been identified in the human testes by light microscopy. These are the dark type A spermatogonia (Ad), the pale type A spermatogonia (Ap), and the type B spermatogonia (B). The bases for the identification of these cells by light microscopy are the shape and staining characteristics of the nucleus, the placement of the nucleolus within the nucleus and the amount of glycogen present in the cytoplasm. Electron microscopic examination of testicular material obtained from normal, healthy men has enabled us to describe four spermatogonial types on the basis of ultrastructural characteristics. It is apparent that the Ad spermatogonia observed by light microscopy actually represents a population of two cell types. Thus, we have designated the four spermatogonial types as the

dark type A1 (Ad1), the dark type A2 (Ad2), the pale type A (Ap), and the type B (B) spermatogonia.

Observations

This study was performed on biopsy specimens from testes of normal healthy young adults that were inmate volunteers of the Oregon State Penitentiary. None of the individuals had a history of disease of the reproductive system and only those individuals having repeatable sperm counts and normal urinary outputs of 17-ketosteroids, estrogens, and total gonadotropins were selected for this study.

Tissue samples were prepared for electron microscopy by fixation in glutaraldehyde followed by a subsequent fixation in OsO_4 . After dehydration in ethanol and embedding in Epon, sections were obtained and stained with uranyl acetate and lead citrate.

The ultrastructural characteristics of the four spermatogonial types are the following:

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4400X

FIGURE 1. Representative Electron Micrograph of a Dark Type A1 Spermatogonia

Dark type A1 spermatogonia (Ad1)
(See Figure 1)

The longitudinal axis of the cell is horizontal with respect to the basal lamina. The elliptic-shaped nucleus, which contains homogeneous nucleoplasm has a longitudinal axis corresponding to that of the cell. Also, the nuclear envelope is contorted. The nucleolus is peripherally located and consists of a diffuse nucleolonema; a pars amorpha being absent. The mitochondria, with tubular cristae predominating, are frequently joined in a linear array of two or more by electron opaque bars that are morphological modifications of the endoplasmic reticulum. The cytoplasm contains pockets of glycogen and, also, glycogen lying

free in the ground substance. A previously undescribed filamentous body may be present in the cytoplasm. The Golgi apparatus is surrounded by vesicles of uniform size. There is little or no smooth endoplasmic reticulum (SER) and the isolated strands of rough endoplasmic reticulum (RER) are frequently associated with the mitochondria.

Dark type A2 spermatogonia (Ad2)
(See Figure 2)

The cell shape gives the appearance of rounding up as compared to the more squamous configuration of the Ad1 spermatogonia, although the predominant axis of the Ad2 cells is still parallel to the basal lamina. The nucleus has a coarse, granular-appearing nucleoplasm that contains

4400X

FIGURE 2. Representative Electron Micrograph of a Dark Type A2 Spermatogonia

a region of rarefaction or "vacuole." The nucleolus is peripherally positioned and is composed of a condensed nucleolonema. The mitochondria have both tubular and plate-like cristae and are frequently joined in groups of two or more by electron-opaque bars. Singular strands of RER are present and are sometimes associated with the mitochondria. SER is present in limited quantities and is associated mainly with the centrosome. The other cytoplasmic features appear similar to the Adl spermatogonia.

Pale type A spermatogonia (Ap)

(See Figure 3)

The cell shape is generally spherical with a flat face juxta-

positional to the basal lamina. The nucleus is spherical and located in the center of the cell. The nucleoplasm is homogeneous, without a "vacuole," and is lightly stained. The nucleolus is centrally located and consists of both a condensed nucleolonema and a pars amorpha. The mitochondria, with plate-like cristae, may occur in pairs, but are never found in the long chains characteristic of the Ad spermatogonia. The ground substance is less electron-opaque in the Ap spermatogonia than in the Ad's and less glycogen is present in the Ap's. Single strands of RER and SER are present in about equal amounts.

4400X

FIGURE 3. Representative Electron Micrograph of a Pale Type A Spermatogonia

6000X

FIGURE 4. Representative Electron Micrograph of a Type B Spermatogonia

Type B spermatogonia (B)
(See Figure 4)

The cell shape is cuboidal to pear-shaped with less surface area lying on the basal lamina as compared to the other spermatogonia. The nucleus is spherical and clumped chromatin is frequently peripherally located. The nucleus is centrally positioned and is composed of a pars amorphia and a nucleolonema. Multiple nucleoli may be present. The mitochondria are not joined together and the cristae are definitely plate-like. There may be a slight association of mitochondria and RER but not to the extent found in the type A spermatogonia. The Golgi complex is frequently more elaborate than found in the A's and is usually in a

perinuclear position removed from the basal lamina.

Conclusions

Four distinct types of human spermatogonia can be identified by their ultrastructural characteristics. The cell shape and nearly every cell organelle are involved in a progressive series of events leading from the most primitive to the most advanced spermatogonia. We presently consider the Ad1 spermatogonia to be the spermatogonial stem cell that perpetuates the stem cell line in the following sequence: Ad1 to Ad2, Ad2 to Ap, and Ap to the B spermatogonia which, in turn, gives rise to the spermatocytes.

COLUMNARIS EXPOSURE AND ANTIBODY DEVELOPMENT
AMONG UPSTREAM MIGRANT SALMONIDS*

Columnaris exposure and infection during upstream migration was not a problem for the spring run of chinook salmon. Sockeye, coho, and summer-run chinook salmon showed an initial heavy exposure at downstream fish ladders and a successive increase in exposure and infection during passage through upstream ladder sites. *Columnaris* exposure and infection decreased for salmon which entered the cooler Columbia River tributaries.

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*This study supported by funds from the AEC
Division of Production

We have continued our studies of the fish pathogen, Chondrococcus columnaris, using antibody production in blood sera of salmonids as a criterion of exposure. Evaluation of immune response has proven to be a more sensitive and reliable indicator of exposure than standard bacterial isolation techniques (Annual Report, 1967). A sampling program was conducted for spring and summer runs of chinook salmon (Oncorhynchus tsawytscha), sockeye salmon (Oncorhynchus nerka), and coho salmon (Oncorhynchus kisutch) to determine whether different "runs" of salmonids exhibit similar increases of antibody production and columnaris exposure during upstream migration from the estuary of the Columbia River to the spawning grounds.

Observations

Sampling sites are shown in Figure 1. Samples were taken from anesthetized jack salmon whenever possible, except on the spawning grounds and when fish were obtained from the commercial fishermen at Astoria and from the Leavenworth hatchery. Blood for agglutination tests was removed directly from the heart or dorsal aorta. Bacterial smears were taken from the gills and from external lesions, if present. Results are summarized in Table I.

The seasonal cool water temperature and minimal columnaris challenge encountered by spring-run chinook salmon at the Columbia River estuary and during upstream migration

resulted in no significant increase in number of salmon with titers or with higher titer magnitude. Minimal columnaris exposure during migration was substantiated from river water and scrapfish samples at fish ladders during passage of the salmon. Spring-run chinook salmon which entered the Nason Creek spawning grounds, a tributary of the Wenatchee River, were only lightly exposed to the pathogen after approximately 5 months in fresh water.

The sockeye migration moved through the main stem of the Columbia River during June and July and was exposed to warmer water temperatures. A maximal 90% exposure and 40% infection were observed at Wells Dam, 370 miles upstream. The effect of repetitive exposure at the ladder sites not only increased the percentage of sockeye with columnaris antibodies but also increased both average titers and range of positive titers. The adult sockeyes started their upstream migration with a higher percentage of antibodies, and the incidence of exposure and infection at the spawning grounds was lower than observed in 1967 (Annual Report, 1967).

Columnaris disease was well established in fish ladders during migration of summer-run chinook; 1.4 and 2.2 organisms per ml water were isolated at McNary and Priest Rapids fish ladders during salmon passage. The majority of summer-run chinook are tributary spawners. Since additional columnaris challenge in the cooler tributary waters is probably

FIGURE 1. Sampling Sites for Study of Columnaris Exposure and Antibody Development in Upstream Migrant Columbia River Salmonids

TABLE I. C. columnaris Exposure and Antibody Development in Columbia River Salmonids During Upstream Migration

Date	Sampling Site	Temp., °F	No. Sam-pled	Positive Isolates, percent	Infected, percent	Positive Titers, percent	All Titers, avg	Positive Titers, range
<u>Spring chinook salmon</u>								
4/9	Astoria	50	31	0	0	29	1:7	1:20-40
5/6	McNary	54	32	0	0	25	1:7	1:20-40
5/22	Rocky Reach	56	15	0	0	33	1:27	1:40-160
8/29	Nason Creek	49	25	4	0	33	1:7	1:20-40
<u>Sockeye salmon</u>								
6/26	Astoria	58	20	0	0	25	1:6	1:20-40
6/27	Bonneville	61	30	53	0	53	1:16	1:20-80
6/28	McNary	62	95	39	2	66	1:26	1:20-80
7/19	Rocky Reach	60	52	73	40	71	1:28	1:20-80
7/18	Wells	60	100	90	41	70	1:31	1:20-160
10/14	Okanogan	57	48	6	2	77	1:106	1:20-640
<u>Summer chinook salmon</u>								
7/22	McNary	65	36	25	10	44	1:17	1:20-80
7/23	Priest Rapids	62	31	60	15	84	1:39	1:20-80
7/18	Rocky Reach	60	17	88	29	88	1:52	1:20-80
7/19	Wells	62	75	90	33	92	1:41	1:20-80
<u>Coho salmon</u>								
9/8	Bonneville	51	85	23	2	23	1:6	1:20-80
9/13	McNary	68	46	41	4	41	1:10	1:20-40
9/20	Priest Rapids	61	50	-	-	60	1:48	1:20-320
11/16	Wells	57	39	79	50	82	1:73	1:20-640
11/22	Icicle Creek	40	148	10	<1	64	1:31	1:20-320

slight, the disease may not have been a problem for these fish. Unfortunately, samples were not obtained during the spawning period.

Coho salmon were more thoroughly exposed to columnaris disease than the other salmonid species; the highest columnaris concentrations

were isolated from fish ladders during September. The percentage of infected fish reached 50% at Wells Dam. The beneficial effects of cold water temperature are evidenced by the significantly lower disease exposure and infection rate at Icicle Creek.

Conclusions

Spring-run chinook salmon, which passed through the Columbia River during cool water temperatures, were not exposed to columnaris and showed no significant increase in antibody titers. Sockeye, summer-run chinook, and coho salmon entered the river when water temperatures were higher and after the disease was well established in fish ladders. The majority of these fish were exposed to columnaris during upstream migration and developed increased antibody titers. Fish which entered the cooler trib-

utary waters were protected from further exposure, and a decrease was observed in exposure, infection, and antibody development. Columnaris disease may not be a problem for salmonids which migrate during cool river temperatures, and for those that entered the cool tributary spawning grounds. The presence of agglutinating antibodies in spring-run chinook and sockeye salmon taken from Columbia River estuary prior to columnaris exposure this year suggests that these fish may have retained residual antibodies developed from exposure as juveniles.

ORAL IMMUNIZATION OF JUVENILE COHO SALMON AGAINST CHONDROCOCCUS COLUMNARIS*

Juvenile coho salmon were protected against C. columnaris disease by daily oral administration of heat-killed columnaris organisms incorporated into fish food. Ten weeks after occurrence of the pathogen in the troughs, 48% columnaris induced mortality was observed among 600 controls (average titer, 1:17) as compared to 8% mortality among 600 orally immunized fish (average titer 1:168).

Investigator:
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Technical Assistance:
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Previous studies have demonstrated that yearling rainbow trout with high columnaris agglutinating antibody production were actively immune to subcu-

taneous C. columnaris challenge (Annual Report, 1966). Field studies suggested that most river fishes and salmonids also respond to C. columnaris challenge by producing antibacterial antibodies against the disease (Annual Reports, 1964, 1965, 1967).

*This study supported by funds from the AEC Division of Production

Although individual fish can be actively immunized against columnaris disease, and a direct relationship appears to exist between production of antibodies and resistance to the disease, the only practical method for treating a large population is through oral administration of an effective vaccine.

Observations

Columnaris vaccine was administered to disease free juvenile rainbow trout (*Salmo gairdneri*) from the Natches hatchery and to juvenile coho salmon (*Oncorhynchus kisutch*) from the Leavenworth hatchery. Two types of columnaris vaccine were used. A vaccine prepared by sonic disruption of columnaris cells was fed to the rainbow trout and a heat-killed (65 °C for 1 hr) vaccine was fed to the coho salmon. Oral feeding was started in May, 2 months prior to the predictable annual occurrence of the disease in our hatchery. Control and experimental lots were closely confined in separate troughs to emphasize both columnaris-induced fish mortality and development of immune response.

Oregon Moist Pellet, a commercially available fish food, was dried overnight at 60 °C and mixed with sonically disrupted or heat-killed columnaris preparations giving a final concentration of approximately 4×10^7 cells per gram wet weight of food. Three to five feedings, daily, of the appropriate oral vaccine were based on river water temperature in the laboratory trough, weight,

and number of fish in a 1 × 1 × 4 foot trough area, following established hatchery feeding procedures. The fish were saturation fed with approximately 2.5 to 40.0 grams of vaccine-treated food daily during the 17-week test period. Controls were fed similarly with nontreated food.

Resistance to columnaris disease was not developed in juvenile rainbow trout fed sonically disrupted columnaris organisms. Seventeen weeks after the test was initiated, mortality for 600 controls was 27%; for vaccine-fed rainbow trout, 19%. Apparently the whole columnaris antigen is required to produce an immune response.

Results with the heat-killed columnaris organisms are illustrated in Figure 1. Exposure and subsequent infection of these fish were from organisms which entered the troughs from the river. Columnaris-associated

FIGURE 1. Cumulative Mortality from Columnaris Disease in Control and Orally Immunized Juvenile Coho Salmon

mortality was first observed among 600 control fish 2 months following the start of the experiment and first mortality among the orally immunized group occurred 3 weeks later. Final mortality for the controls was 48% as compared to 8% for the immunized fish.

Fewer than one organism/ml was released from these fish into the trough water when first mortality was observed. However, at the completion of the experiment at 17 weeks, approximately 900 and 300 organisms/ml were isolated from the control and immunized trough water, respectively. This constitutes a much higher exposure concentration than fish would normally encounter, even under production hatchery conditions, and is therefore a severe test of the effectiveness of the vaccine.

Average C. columnaris agglutinating titers were 1:25 for controls and 1:17 for sonically disrupted vaccine-fed rainbow trout at the completion of the test. Titers for coho salmon were 1:17 for controls and 1:168 for heat-attenuated vaccine-fed fish. The oral immunization test clearly demonstrated the relationship of high agglutinating titers and resistance to the columnaris disease.

In supporting studies, 15 control and 15 immunized yearling rainbow trout were exposed in a common trough to columnaris organisms released from juvenile rainbow trout held in a separate trough, but with the same river water supply. The resulting antibody titers as correlated with river water temperatures over a 1-year period are illustrated in Figure 2.

The average titers for previously immunized trout decreased, initially, despite the presence of approximately 15 organisms/ml in the river water at the start of the experiment. This suggests that the challenge was insufficient to increase antibody production until titers dropped to a lower level. The immunized fish followed a seasonal pattern similar to that previously observed in Columbia River scrapfish. The higher residual antibody titer observed in these trout during the winter months and the faster development of titer upon rechallenge in the spring demonstrates the value of immunization.

Conclusions

These data show oral immunization to be a practical and effective method for protecting juvenile salmonids against columnaris disease. The vaccine should be especially useful to hatcheries rearing salmonids in warm water. Fish reared in columnaris-free hatcheries may also benefit since they will, in most instances, be released to rivers where they will be exposed to the disease during seaward migration. Residual titers retained during their period in the ocean, may enhance the response of the adult fish to natural columnaris challenge during upstream migration.

The sensitivity of immune response as an indicator of exposure was further demonstrated in these studies. Columnaris infection of fishes is usually not observed in the laboratory until July; however, increase in

FIGURE 2. Columnaris Antibody Production Among Control and Immunized Yearling Rainbow Trout During a Period of Seasonally Variable River Water Temperature

titer magnitude of both immunized and control rainbow trout occurred in April.

An oral vaccine against furunculosis disease caused by the pathogen, *Aeromonas salmonicida*, is presently undergoing extensive field trials by

the Oregon Fish Commission. Incorporation of both the furunculosis and columnaris vaccines into fish food, if successful under production hatchery conditions, might help to control two of the major bacterial diseases of hatchery-reared salmonids.

THE RESPONSE OF JUVENILE SALMONIDS
TO ACUTE THERMAL SHOCK

Juvenile salmonids were acclimated at different temperatures and were tested for their response to high-temperature exposures. The temperature of acclimation and the rate of exposure had marked effects on the response of the fishes to acute thermal exposure.

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Studies to determine the resistance times of aquatic organisms to elevated temperatures are necessary for development of water quality standards and for understanding the mechanisms by which organisms respond to changes in environmental conditions. Earlier studies in the present series have been previously described (Annual Report, 1967).

Observations

Juvenile coho salmon, Oncorhynchus kisutch (average weight, 0.5 g), reared at 10, 15, or 20 °C were transferred in lots of ten to water at test temperatures varying from 25-30 °C. Curves relating geometric mean time to death as a function of test temperature were parallel for the 10, 15, and 20 °C acclimated groups but differed significantly from one another, with the fish acclimated at higher temperatures showing a greater temperature tolerance. A difference of 5 °C in acclimation temperature resulted in a 1 °C shift in the incipient lethal

temperature. This is illustrated in Figure 1 where a single curve depicts the three sets of data when the exposure temperature scale is shifted one degree for each 5 °C difference in acclimation temperature. Also shown in Figure 1 are data for 15 °C acclimated juvenile chinook salmon (Oncorhynchus tshawytscha), which show a differently sloped response curve than is shown by the coho salmon.

In the above experiment, times to loss of equilibrium were determined as well as times to death. Although each fish experiences a loss of equilibrium before death occurs, there was no statistically significant difference between these two measurements because of the wide range in time over which the two responses occur.

Juvenile coho salmon were also tested by increasing or decreasing the exposure temperature at varying rates. Results of these studies are summarized in Table I. There was a different response to a gradually increasing temperature abruptly decreased, and an abruptly increased

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FIGURE 1. A Comparison of Times to Death at Various Test Temperatures for Fish Acclimated at 10, 15, and 20 °C

TABLE I. Twenty-Four Hour Mortality in Juvenile Coho Salmon Exposed to Various Temperature Regimens

Type of Exposure	Number Exposed	Number Losing Equilibrium	Mortalities	% Mortality
10	10	10	100
20		4	20
8	4	0	0
10	9	7	70
5	2	1	20
12		1	8
5	5	3	60
5	5	5	100
25	25	24	96

temperature that was gradually decreased. These findings are reasonable in the light of attained fish temperatures which were measured by insertion of a thermistor probe in the muscle mass. Figures 2 and 3 show that exposures received with gradually increasing temperature

are greater than those received when an abruptly increased temperature is gradually decreased. When fishes were exposed abruptly to a higher or lower maintained temperature, they came to equilibrium with that temperature within 3-5 min, with 90% of the change occurring within 2 min.

FIGURE 2. Dorsal Muscle Temperature of Juvenile Coho Salmon as Related to Water Temperature Increased by 15 °C at Different Rates

FIGURE 3. Dorsal Muscle Temperature of Juvenile Coho Salmon as Related to Water Temperature Increased Abruptly by 15 °C and Cooled at Different Rates

In another experiment, juvenile coho salmon acclimated at 15 °C were repeatedly exposed to 27 °C for 30-min periods, interspersed with 30- or 60-min recovery periods at 15 °C. Results of these studies, shown in Table II indicate that the cumulative lethal effects of exposure at 27 °C are essentially eliminated during the recovery period.

Conclusions

Temperature acclimation will shift the thermal limits of behavioral and physiological responses. The absolute limits differ for species of salmonids but the overall response is similar. It is important to know the actual thermal experience of the fish tissue, which is a function of the ambient condition but not identical to it.

TABLE II. Mortality in Juvenile Coho Salmon, Acclimated at 15 °C and Cycled Between 15 and 27 °C

<u>Sequence of Exposures</u>	<u>Number Exposed</u>	<u>Mortalities</u>
20	2
20	4
20	

Observations of the reactions of intact organisms to thermal stress indicate the need for further studies of the cellular responses of the organism. It is through such studies that an understanding can be developed of the interaction between the environment and the organism

and the steps by which compensatory reactions can be incorporated into the genetic mechanism. Criteria can then be developed which are applicable to the critical thermal situations that the organism encounters in today's rapidly changing environment.

ALTERATIONS IN HEPATOCYTE FUNCTION OF THERMALLY
ACCLIMATED RAINBOW TROUT (SALMO GAIRDNERI)

There was an increased level of leucine incorporation into protein in hepatocytes from cold-acclimated fish as compared to warm-acclimated fish. The results suggest that there is a decreased rate of secretion of plasma protein from the hepatocytes of cold-acclimated fish.

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We have previously reported temperature-induced alterations in the fine structure of rainbow trout hepatocytes (Annual Report, 1965). The Golgi complex of 5 °C acclimated fish was considerably enlarged and contained more secretory particles of a lipoprotein nature than did the Golgi complex in hepatocytes of 18 °C acclimated fish. The Golgi complex is known to play a role in the intracellular transport of hepatic lipoproteins that are destined for the circulatory system. Based on morphological description alone, it was not possible to determine whether the elaborate Golgi complex of the cold-acclimated fish was related to an increased or decreased secretory activity of the hepatocyte. In an attempt to clarify hepatocyte function in trout acclimated at 5 and 18 °C, we have followed the incorporation of ¹⁴C-labeled leucine into liver and plasma proteins as a measure of protein synthesis.

Observations

Rainbow trout (Salmo gairdneri), fed a diet of Clark's pellets, were acclimated for 3 weeks at 5 and 18 °C. The fish were stunned, their livers removed, and liver protein and wet/dry weight determinations were made. For leucine incorporation studies, the fish were fasted for 16 hr, lightly anesthetized with MS-222 and injected intravenously in the dorsal aorta with 0.5 µCi of uniformly labeled ¹⁴C-leucine (specific activity 275 mCi/mmmole) per 100 g body weight. They were held at their temperature of acclimation, without feeding, until sacrificed.

There was an increased level of total liver protein in the cold-acclimated trout (Table I). There was no difference in the wet/dry weight ratio of the warm- and cold-acclimated trout livers.

Fish acclimated at 18 °C showed maximal incorporation of ¹⁴C-leucine into cold TCA-precipitable liver proteins at about 2 hr postinjection (Figure 1). In the same fish,

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FIGURE 1. Incorporation of ^{14}C -Leucine into Liver and Plasma Proteins of Warm (18 °C) and Cold (5 °C) Acclimated Rainbow Trout (Points represent the means of results on four to six fish $\pm$ standard error of the mean.)

labeled plasma proteins appeared about 2 hr postinjection and plateaued at 4 hr (Figure 1). The 5 °C acclimated fish showed insignif-

TABLE I. Total Proteins in Liver of Cold- and Warm-Acclimated Trout

	Temperature of Acclimation	
	5 °C	18 °C
Mean (mg protein/g wet liver)	113.7(a)	98.5(a)
Number of samples	12	10
Standard error of the mean	4.6	3.6
Standard deviation	16.1	11.5

(a) Significantly different, $P < 0.05$.

icant levels of labeled liver proteins at 2 hr. Peak incorporation occurred at 6 hr and levels remained higher than those of the warm fish (Figure 1). Significant amounts of labeled plasma proteins did not appear in the cold fish until 4 hr postinjection and levels increased throughout the succeeding 24 hr. If the data are plotted as the ratio of labeled plasma protein to labeled liver proteins vs. time (Figure 1), a temperature-induced alteration is apparent.

Conclusions

The time course of ^{14}C -leucine incorporation into liver and plasma proteins is delayed in the 5 °C acclimated fish but there is an increase in total protein synthesized by these fish. These results suggest that there is delayed secretory activity in the hepatocyte

MOLECULAR AND CELLULAR LEVEL STUDIES

6.45

of the cold- as compared with the warm-acclimated trout. Further studies are underway to examine the

overall synthetic response of the hepatocyte in thermally acclimated fish.

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PATHOLOGY SECTION

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President, Nutrition Foundation
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J. M. Thomas, Ph.D. - Statistics Section, Mathematics Department

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Radiation Monitors - H. E. Preston
A. L. Stinnett

PUBLICATIONS AND PRESENTATIONS

OPEN LITERATURE PUBLICATIONS

Adee, R. R., C. L. Sanders, and J. D. Berlin. "Subcellular Localization and Identification of Alpha Emitters by Electron Microscopic Autoradiography," Health Physics, vol. 15, pp. 461-463. 1968.

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PRESENTATIONS AT SOCIETY MEETINGS AND SYMPOSIA

Bair, W. J. "Research Supported by the United States Atomic Energy Commission on the Detection and Assessment of Uranium and Plutonium Body Burdens." Panel to Consider the Question of Coordination of National Research on the Detection and Assessment of Uranium and Plutonium in the Whole Body and Critical Organs, Vienna, Austria, November 11-15, 1968.

Clarke, W. J., E. B. Howard, and Patricia L. Hackett. "Strontium-90 Induced Hemopoietic Neoplasms in Swine." Sixteenth Annual Meeting of the Radiation Research Society, Houston, Texas, April 21-25, 1968.

Cline, J. F. "Uptake of ^{241}Am and ^{239}Pu by Plants." Western Society of Soil Science 1968 Meeting, Logan, Utah, June 24-29, 1968.

Cooper, P. H. and W. J. Clarke. "Marrow Hematopoiesis in Pitman-Moore Swine Following Strontium-90 Ingestion." Symposium on Myeloproliferative Disorders of Animals and Man, Richland, Washington, May 20-23, 1968.

Dilley, J. V. "Heterophile Antibody-Forming Capacity in Rats and Beagle Dogs After Inhalation of $^{239}\text{PuO}_2$ and $^{239}\text{PuF}_4$." Sixteenth Annual Meeting of the Radiation Research Society, Houston, Texas, April 21-25, 1968.

Dionne, P. J. and B. O. Stuart. "Plutonium Inhalation Model Simulates the Long-Term Burden of the Deep Lung and Systemic Organs." Sixth Annual Conference of the 1968 Institute of Electrical and Electronic Engineers, Portland, Oregon, May 22, 1968.

Fujihara, M. P. "Immunization of Juvenile Salmonids Against Chondrococcus columnaris." Annual Meeting of the Northwest Branch of the American Society of Microbiology, Richland, Washington, October 4-5, 1968.

Hackett, P. L., G. S. Vogt, C. C. Jannke, and E. B. Howard. "Hematologic and Biochemical Alterations in ^{90}Sr Induced Hematopoietic Neoplasms of Swine." Symposium on Myeloproliferative Disorders of Animals and Man, Richland, Washington, May 20-23, 1968.

Howard, E. B. and W. J. Clarke. "Induction of Hematopoietic Neoplasms in Miniature Swine by Chronic Feeding of Strontium-90." Armed Forces Institute Pathology-National Cancer Institute Symposium on Comparative Hematopoietic Neoplasia of Animals and Man, Washington, D. C., March 11-13, 1968.

Howard, E. B. and W. J. Clarke. "Induction of Hematopoietic Neoplasms in Miniature Swine by Chronic Feeding of Strontium-90." Fifty-seventh Annual International Academy of Pathology Meeting, Chicago, Illinois, March 1-7, 1968.

Howard, E. B. and W. J. Clarke. "Strontium-90 Induced Hematopoietic Neoplasms in Miniature Swine." Symposium on Myeloproliferative Disorders of Animals and Man, Richland, Washington, May 20-23, 1968.

Hsieh, J. J. C. and F. P. Hungate. " ^{131}I Diffusion Through Leaf Cuticle." American Society of Plant Physiologists, Western Section, Logan, Utah, June 25-27, 1968.

Jannke, C. C. and E. B. Howard. "Effects of Radiation on Serum Haptoglobin Levels in Experimental Animals." Thirteenth Annual Meeting Northwest Section of the Society for Experimental Biology and Medicine, Seattle, Washington, November 9, 1968.

Loftin, H. G., J. M. Dean and W. L. Templeton. "Distribution of Freshwater Fishes from Eastern Panama and Northwestern Colombia." American Association for the Advancement of Science Meeting, Dallas, Texas, December 26-31, 1968

Mahlum, D. D. and M. R. Sikov. "Skeletal Changes Produced by Administration of Plutonium-239 to Newborn, Weanling, and Adult Rats." Meeting of the Federation

of American Societies for Experimental Biology, Atlantic City, New Jersey, April 15-20, 1968

Park, J. F., W. J. Bair, and E. B. Howard. "Acute Toxicity of Inhaled Plutonium-239 Nitrate in Beagle Dogs." Annual Meeting of the Health Physics Society, Denver, Colorado, June 16-20, 1968.

Pauley, G. B. "A Critical Review of Neoplasia and Other Tumorlike Lesions in Mollusks." Symposium on Tumors in Primitive Vertebrates and Invertebrates, Washington, D. C., June 19-21, 1968.

Richardson, L. R., J. F. Park, and R. J. Olson. "The Hemogram of Rats Anesthetized with Methoxyflurane During Blood Sampling from the Ophthalmic Venous Plexus." Nineteenth Annual Meeting of the American Association for Laboratory Animal Science, Las Vegas, Nevada, October 21-25, 1968.

Sikov, M. R. and D. D. Mahlum. "Age: A Consideration in Radioisotope Exposure." Annual Meeting of the Radiological Society of North America, Chicago, Illinois, December 1-6, 1968.

Sikov, M. R. and D. D. Mahlum. "Factors Influencing Radioiodine Metabolism in the Perinatal Rat." Northwest Section of the Society for Experimental Biology and Medicine, Seattle, Washington, November 9, 1968.

Sikov, M. R. and D. D. Mahlum. "Effect of Age and Physico-Chemical Form on Plutonium Toxicity and Metabolism in the Rat." Annual Meeting of the Radiation Research Society, Houston, Texas, April 21-25, 1968.

Smith, V. H. "The Prevention of ^{147}Pm Deposition in Rats by Prompt Treatment with Chelons." Annual Meeting of the Radiation Research Society, Houston, Texas, April 21-25, 1968.

Sorsoli, W. A. and J. R. Turner. "Kynureninase Induction in Neurospora crassa." The Northwest Branch of the American Society of Microbiology Meeting, Richland, Washington, October 4-5, 1968.

Stuart, B. O. "Mathematical Model of Man for Tissue Dose Determination." Annual Meeting of Pacific Northwest Section of American Industrial Hygiene Association; Joint Meeting with Columbia Chapter of Health Physics Society, Richland, Washington, October 3-4, 1968.

Stuart, B. O. and W. J. Bair. "The Long-Term Retention, Distribution and Biological Effects of Inhaled ^{144}Ce - $^{144}\text{PrO}_2$ in Beagle Dogs." Annual Meeting of the Health Physics Society, Denver, Colorado, June 16-20, 1968.

Sullivan, M. F. "Electrolyte and Water Transport in the Rat Intestine After Exposure to 1250 R of X Rays." Twenty-Fourth International Congress of Physiological Sciences, Washington, D. C., August 25-31, 1968.

Sullivan, M. F. "Intestinal Radiation Death in Rats Fed a Sodium Deficient Diet." Sixteenth Annual Meeting of the Radiation Research Society, Houston, Texas, April 21-25, 1968

Thomas, J. M. and M. R. Sikov. "Quantitative Growth Patterns of the Neonatal Rat." Northwest Section of the Society for

Experimental Biology and Medicine, Seattle, Washington, November 9, 1968.

Ushijima, R. N., M. E. Frazier, and E. B. Howard. "In Vitro Investigations of Cells and Filtrates from ^{90}Sr Induced Leukemic Porcine Tissues." Symposium of Myeloproliferative Disorders of Animals and Man, Richland, Washington, May 19-23, 1968.

Wachholz, B. W. and T. D. Mahony. "The Influence of Pancreatic Fluid on the Morphological Response of the Intestine to X-Irradiation." Annual Meeting of the Radiation Research Society, Houston, Texas, April 21-25, 1968.

Watson, C. R. and W. J. Clarke. "Thermoluminescence Dosimetry in Bones from Swine Fed Strontium-90." Annual Meeting of the Health Physics Society, Denver, Colorado, June 16-20, 1968.

Wehner, A. P. "Effect of Medium Composition on Metabolism of a Species of Nocardia." Annual Meeting of the American Society for Microbiology, Northwest Branch, Richland, Washington, October 4-5, 1968.

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