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Pacific Northwest Laboratories
Richland, Washington 99352

AEC Research and Development Report

PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1970
TO THE USAEC DIVISION
OF BIOLOGY AND MEDICINE

VOLUME I: LIFE SCIENCES
PART 1. BIOLOGICAL SCIENCES

October 1971

BNWL-1550
Part 1

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UC-48, Biology and Medicine

PACIFIC NORTHWEST LABORATORY
ANNUAL REPORT FOR 1970
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

October 1971

BATTELLE
PACIFIC NORTHWEST LABORATORIES
RICHLAND, WASHINGTON 99352

This is the 20th Annual Report of the Biology Department. Previous volumes are: HW-25021 (1951), HW-28636 (1952), HW-30437 (1953), HW-35917 (1954), HW-41500 (1955), HW-47500 (1956), HW-53500 (1957), HW-59500 (1958), HW-65500 (1959), HW-69500 (1960), HW-72500 (1961), HW-76000 (1962), HW-80500 (1963), BNWL-122 (1964), BNWL-280 (1965), BNWL-480 (1966), BNWL-714 (1967), BNWL-1050 (1968), and BNWL-1306 (1969).

Part 2 of this volume, BNWL-1550 Part 2, covers work in the ecological sciences. Volume II of this report covers work in the physical sciences and is issued in three parts: Atmospheric Sciences, BNWL-1551, Part 1; Radiological Sciences, BNWL-1551, Part 2; and Instrumentation, BNWL-1551, Part 3.

FOREWORD

This twentieth in a continuing series of Biology Department Annual Reports marks the closing of a major part of our facilities in the 100-F Area, 30 miles north of Richland, and the beginning of our occupancy of the new five-million-dollar Life Sciences Laboratory in the 300 Area, at the northern edge of Richland. Unfortunately, about 25 of our staff will remain in 100-F to work on a study of inhaled uranium mine air contaminants, the effects of daily ingestion of radio-strontium in miniature swine and to provide care for our pigmy goat, swine, and sheep colonies. The move to our new laboratory, in part, accounts for the lateness of this Annual Report.

Again, the reports are titled to correspond with AEC budget categories. In some instances, these titles have been subdivided to facilitate organization of results.

A complete listing of the Department staff is included at the end of the report. It will be noted that only limited organizational changes occurred during the year.

The Tenth Annual Biology Symposium, Pollution and Lung Biochemistry, was held on June 2-5, 1970 under the Chairmanship of Dr. D. B. Menzel. The Eleventh Annual Symposium, Biological Implications of the Transuranium Elements, is scheduled for October 27-29, 1971. This symposia series continues to be popular and provides our staff an opportunity to meet and exchange ideas with a large number of the world's experts in a given topic area.

Late in the year the Biology Department initiated a Public Lecture series in an attempt to inform our families and the public of our research activities. We hope these lectures, given monthly, will help to disseminate the research accomplishments of the United States Atomic Energy Commission's supported laboratories.

W. J. Bair, Manager
Biology Department

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GASTROINTESTINAL IRRADIATION STUDIES

This long-continuing project has included studies on the physiologic and pathologic changes that occur after exposure of the gastrointestinal tract of rats, mice, rabbits, guinea pigs, or swine to X or gamma radiation. Recent emphasis has been placed on studies of the transport of various substances across the irradiated intestine, especially the leakage of plasma protein into the intestine. The involvement of the vascular and lymphatic systems in the gastrointestinal radiation syndrome has also been an area of special interest. Future studies are expected to emphasize those aspects of the toxicity of inhaled or ingested radionuclides that involve the gastrointestinal tract.

GLUCOSE, SODIUM, AND FLUID ABSORPTION
AND PLASMA GLUCOSE CONCENTRATION IN
THE IRRADIATED RAT

Investigator:

D. W. Baxter

Technical Assistance:

Alma L. Crosby

We have previously shown, with both in vivo and in vitro preparations, that glucose absorption is reduced in the irradiated rat by the second or third day postexposure. Increased absorption has been reported (Poppei and Erdman, *Naturwissenschaften*, 53:20, 1966) to occur at 20 hours postexposure when the everted sac technique was used. The present studies were performed to check this effect in vivo.

Adult rats weighing approximately 230 g received 0, 600, 1000, or 1500 R whole body X-irradiation. Absorption was measured in anesthetized rats by cannulating the small intestine immediately distal to the Sphincter of Oddi and again at the ileocecal junction, and injecting into the isolated loop 10 ml of 5 mg/ml glucose solution in isotonic saline. Intraluminal and plasma glucose concentrations were determined by the glucose oxidase "glucostat" method (Worthington Biochemical Corp.). Sodium concentration was determined by flame photometry.

Figure 1 shows intraluminal glucose concentration after a 30-min test period in control and X-irradiated animals at various times postexposure. Control animals were able to reduce the glucose concentration from 5.0 to 0.038 mg/ml in 30 min; irradiated animals were only slightly less efficient at day 1 (0.143 mg/ml). From 2 to 4 days postexposure the radiation effect was increased and became dose dependent.

Sodium concentration did not change at any time in these studies; therefore, sodium absorption was directly related to fluid transport. As shown in Figure 2, control and irradiated animals could absorb approximately 4 ml of test solution in 30 min at day 1. A radiation effect was seen at days 2 and 3 but with no apparent dose dependency. Recovery was occurring by day 4 after 600 to 1000 R but did not occur in animals which received 1500 R.

Plasma glucose concentration is shown in Figure 3. The rise in concentration is apparent on day 1 and proportional to radiation dose by day 3. Comparing Figures 1 and 3, it is evident that glucose absorption is inversely proportional to plasma glucose concentration.

Further studies were performed to investigate the cause for the rise in plasma glucose concentration. It was found that each step in the surgical preparation of the rats prior to perfusion resulted in a slight increase in glucose concentration, and that the total procedure increased plasma

FIGURE 1. Intraluminal Glucose Concentration ($\pm$ SE) After a 30-min Absorption Period in Control and Irradiated Animals at Various Times Postexposure

FIGURE 2. Intraluminal Fluid Volume ($\pm$ SE) After a 30-min Absorption Period in Control and Irradiated Animals at Various Times Postexposure

FIGURE 3. Plasma Glucose Concentration ($\pm$ SE) After a 30-min Absorption Period in Control and Irradiated Animals at Various Times Postexposure

glucose four-fold (Figure 4). Intestinal perfusion with a saline solution caused no additional increase, and perfusion with glucose solution caused only a slight additional increase. In nonirradiated animals there was no difference between animals perfused with glucose solution and those just anesthetized. These data indicate that the radiation effect on plasma glucose concentration is a stress-related phenomenon

and bears little, if any, relationship to intestinal function. The effect of radiation is to reduce net sodium and fluid absorption and to eliminate the ability of the small intestine to reduce intraluminal glucose concentration to values approaching zero. Plasma glucose concentration is related to surgical stress and independent of glucose absorption.

FIGURE 4. Plasma Glucose Concentration ($\pm$ SE) as Affected by Various Stages of the Surgical Preparation and Perfusion in 1500 R Irradiated Animals at 3 Days Postexposure and in Unirradiated Control Animals (Number of animals shown in parentheses.)

STUDIES OF THE MECHANISM OF PLASMA
PROTEIN-LEAKAGE INTO THE IRRADIATED
BOWEL

Investigators:

M. F. Sullivan and B. W. Wachholz

Technical Assistance:

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

We previously reported (Annual Report, 1969) the excessive leakage of either ^{131}I -labeled polyvinylpyrrolidone (PVP) or ^{51}Cr into the irradiated intestine. The appearance of either labeled protein or protein substitute in the gut lumen could imply direct leakage from blood to lumen or an indirect route, such as from blood to an extravascular space and then to the gut lumen. To explore these possible mechanisms, ^{51}Cr -labeled plasma protein was determined in the blood and in excreta for several days following exposure of the abdominal region of rats, mice, and swine to X or gamma radiation.

The lower body of rats and mice, posterior to the xiphoid process, was exposed to 1000 X ray, 2 days prior to intravenous injection of ^{51}Cr . The lower body of swine was bilaterally exposed to 2500 R ^{60}Co gamma rays, 3 days prior to ^{51}Cr injection. Blood withdrawals and excreta collections were made daily from swine;

separate groups of rodents were employed for excreta collections and blood analyses.

Fecal and urinary excretion and blood retention of ^{51}Cr in control and irradiated rats are shown in Figure 1. Four days after 1000 R (2 days after $^{51}\text{CrCl}_3$ injection) there was more than a three-fold increase in the quantity of ^{51}Cr appearing in the feces. The quantity of ^{51}Cr excreted in the urine of irradiated and nonirradiated animals did not differ significantly. The effect on whole body retention was, therefore, largely a reflection of the effect on fecal excretion.

After 1 day, about 8% of injected ^{51}Cr was still present in control blood, while only half that amount remained in the blood of irradiated animals, demonstrating the increased permeability of the irradiated vasculature. Between the second and fourth days after injection, the amount of ^{51}Cr excreted each day in the feces exceeded the total quantity present in the blood indicating storage of ^{51}Cr at a site other than the blood. That this radiation-induced vascular permeability is not species specific is apparent from Figure 2, which compares the effect of irradiation on ^{51}Cr retention in the blood of mice, rats, and swine. In our earlier study, we demonstrated that swine were unusually resistant to intestinal irradiation injury. Their vascular system is apparently also more resistant to damage from irradiation.

FIGURE 1. Effect of 1000 R Lower-Body X-Irradiation on the Retention and Excretion of Intravenously Injected $^{51}\text{CrCl}_3$ by Rats (Number of animals indicated in parentheses.)

To study the recovery characteristics of the injury responsible for plasma protein leakage, rats were exposed twice weekly, for several total time periods, to 500 R lower-body X ray. Two days after the last exposure of each group they were injected with $^{51}\text{CrCl}_3$. Daily excreta collections were made for 4 days.

The fecal excretion data shown in Figure 3 indicate that the injury responsible for protein leakage is, to some degree, cumulative. This suggests the use of ^{51}Cr excretion as a diagnostic aid in indicating intestinal injury during protracted radiotherapy exposures.

ERRATA

This page replaces Page 8 of BNWL-1550 Part 1.

FIGURE 2. Effect of Lower-Body Irradiation on the Retention of Intravenously Injected $^{51}\text{CrCl}_3$ in the Blood of Mice, Rats, and Swine (Number of animals indicated in parentheses.)

FIGURE 2. Effect of Lower-Body Irradiation on the Retention of Intravenously Injected $^{51}\text{CrCl}_3$ in the Blood of Mice, Rats, and Swine (Number of animals indicated in parentheses.)

FIGURE 3. Effect of Repeated Lower-Body Irradiation on the Fecal Excretion of Intravenously Injected $^{51}\text{CrCl}_3$ by Rats (Average values $\pm$ standard error of the mean for six animals.)

MOVEMENT OF CHROMIUM-51 IN THE
LYMPHATIC SYSTEM OF RATS AFTER
ABDOMINAL IRRADIATION

Investigator:

B. W. Wachholz

Technical Assistance

Alma L. Crosby and Michael L. Smith

The appearance of increased protein in the feces following irradiation has been previously reported from this and other laboratories and has been generally attributed to in-

creased capillary permeability. It is not clear, however, whether protein enters the lumen of the gut by direct leakage from the vasculature or if other mechanisms are involved.

To determine the source of the leaked protein, groups of rats were bile duct-cannulated, thoracic lymph duct-cannulated, or sham-cannulated immediately prior to abdominal X-irradiation (1000 R) or sham-irradiation. Two days later, ^{51}Cr was injected intravenously. Most of the groups were given 0.45% saline to drink to compensate for electrolyte and fluid loss, as well as to reduce clotting in the cannulae of the

thoracic duct cannulated animals. The experimental groups are shown in Table 1.

Table 2 shows the distribution of the cumulative excreted and/or secreted ^{51}Cr during the period of 9 days post-administration. As expected, there was an increase in the fecal excretion of ^{51}Cr following X-irradiation. Less than 3% of the urinary ^{51}Cr was protein-bound. The quantity of ^{51}Cr removed with the bile was unaffected by irradiation, but a 22% decrease occurred in the amount removed with the lymph following irradiation.

The increased fecal excretion of ^{51}Cr in the thoracic duct cannulated animals is paralleled by the decrease in the quantity appearing in the lymph. In the absence of further studies, the exact significance of these observations is not clear.

Distribution studies in irradiated and nonirradiated animals show a major ^{51}Cr shift in the liver, skin, muscle, gastrointestinal tract (including contents), and blood (Table 3). The decreased ^{51}Cr activity in the skin, muscle, and blood at 4 days after irradiation (2 days after ^{51}Cr injection)

TABLE 1. Experimental Groups

Surgery	Irradiation	Drinking Fluid	Code	Number of Rats
Sham	0	H ₂ O	NC-NX (H ₂ O)	10
Sham	1000 R	H ₂ O	NC-X (H ₂ O)	6
Sham	0	NaCl	NC-NX	4
Sham	1000 R	NaCl	NC-X	4
Bile duct cannulated	0	NaCl	BC-NX	7
Bile duct cannulated	1000 R	NaCl	BC-X	6
Thoracic duct cannulated	0	NaCl	TDC-NX	6
Thoracic duct cannulated	1000 R	NaCl	TDC-X	6

TABLE 2. Cumulative Chromium-51 Excretion and/or Secretion
(Percent of total excretion and/or secretion)

	NC-NX (H ₂ O)	NC-X (H ₂ O)	NC-NX	NC-X	BC-NX	BC-X	TDC-NX	TDC-X
Urine	91.3 ± 0.4	70.4 ± 1.6	91.0 ± 0.9	61.2 ± 6.4	91.8 ± 0.4	83.4 ± 1.9	51.7 ± 0.1	50.6 ± 2.4
Feces	8.7 ± 0.4	29.6 ± 1.6	9.0 ± 0.9	38.8 ± 6.4	5.0 ± 0.3	12.7 ± 1.6	9.3 ± 1.2	19.1 ± 2.6
Bile					3.2 ± 0.3	3.9 ± 0.9		
Lymph							39.0 ± 1.9	30.3 ± 1.5

TABLE 3. Distribution of Intravenously Injected ^{51}Cr at 2 Days Postinjection

Tissue	Percent of Injected Dose (a)	
	Control	1000 R
Liver	7.5	14.3
Kidneys	2.5	2.4
Spleen	0.8	0.6
Heart	0.2	0.1
Lungs	0.6	0.5
Reproductive organs	0.8	0.7
Adrenals	0.02	0.02
Stomach (incl. contents)	0.1	0.2
Small Intestine (incl. contents)	0.7	0.9
Cecum	0.4	1.1
Large Intestine	0.3	0.5
Skin	9.3	6.2
Bone	18.6	17.1
Muscle	11.3	7.8
Blood Cells	0.2	0.1
Blood Plasma	0.7	0.3
Whole Blood	0.9	0.4

a. Average values from 4 rats

is about the same as the increase seen in the gastrointestinal tract (primarily in the cecum) and in the liver.

During the period from 96 to 120 hours postirradiation, the quantity of ^{51}Cr excreted in the feces of sham-irradiated animals was two-thirds of that present in the blood, while the quantity excreted in the feces of irradiated animals was 2-1/2 times that present in the blood. This suggests that extravascular sources of ^{51}Cr contribute to the gut contents.

CARDIOVASCULAR RESPONSE IN THE IRRADIATED RAT

Investigator:

D. W. Baxter

Technical Assistance:

Alma L. Crosby

These studies were undertaken to determine changes in cardiac output, organ blood flow, and vascular resistance during the period of the intestinal radiation syndrome, in the hope of clarifying possible relationships between functional changes and radiation-induced alterations of the cardiovascular system.

Adult female rats weighing approximately 250 g were exposed to 1250 R lower-body X-irradiation (250 kvp, 30 ma, 0.25 mm Cu, 1.0 mm Al). The dose rate was approximately 47 R/min. Controls were sham-irradiated and pair-fed.

Cardiac output distribution was measured by injecting 3 to 5 mCi $^{86}\text{RbCl}$ in 0.3 ml of 0.9% NaCl into a femoral vein, sacrificing the animal at 45 seconds postinjection, and determining the percent of the injected isotope in each of the following tissues: stomach, small intestine, cecum, colon, liver, spleen, kidney, and heart. Cardiac output was measured in separate groups of animals by injecting 1 to 3 mCi $^{86}\text{RbCl}$ in 0.3 ml of 0.9% NaCl into the abdominal vena cava, sampling arterial blood from a cannula in the abdominal aorta, and

calculating cardiac output by standard indicator dilution methods. Changes in organ vascular resistance were estimated by dividing the percent of the cardiac output delivered to an organ by the organ weight, as percent of total body weight. If this ratio was larger in irradiated animals than in pair-fed, sham-irradiated controls, and the difference was greater than could be explained by any change in percent body weight, then vasodilation or a decrease in vascular resistance was inferred.

Cardiac output (Figure 1) decreased from a control value of 259 ml/min/kg to a low value of 119 ml/min/kg on the fifth day postexposure. Although cardiac output also fell in the pair-fed, sham-irradiated animals, the difference between the two groups at

5 days was significant ($p < 0.01$). There was no significant difference between the two groups on day 7.

Blood flow to the stomach, small intestine, cecum, spleen, and kidney (Table 1) decreased through day 5 and increased thereafter. These changes reflect those of the cardiac output (Figure 1) which was probably the dominant factor in determining the volume flow to these tissues. In some tissues, however, the decrease was modified by local changes in vascular resistance. Decreased resistance, as indicated by a rise of the ratio of percent cardiac output to percent body weight, was apparent on day 5 in both the heart and liver (Figure 2). This changing ratio was not the result of a loss in tissue weight. The changes in resistance

FIGURE 1. Cardiac Output in 1250 R Lower Body X-Irradiated and Pair-Fed, Sham-Irradiated Animals at Various Times Postexposure (Number of animals shown in parentheses; standard error shown by bar.)

TABLE 1. Organ Blood Flow at Various Times Postexposure in 1250 R Lower Body X-Irradiated (X) and Pair-Fed, Sham-Irradiated (S) Animals. (Average values from 8-13 animals expressed as ml/min/kg.)

Organ		Days Postirradiation				
		0	1	3	5	7
Stomach	X	-	73	44	27	49
	S	55	75	76	58	50
Proximal, Small Intestine	X	-	158	80	76	96
	S	113	134	123	125	131
Distal, Small Intestine	X	-	127	50	43	71
	S	107	108	90	92	95
Cecum	X	-	119	62	45	69
	S	106	138	87	87	98
Colon	X	-	72	57	43	87
	S	74	73	46	58	57
Liver	X	-	31	38	42	32
	S	34	33	43	58	57
Spleen	X	-	80	57	24	62
	S	60	68	66	59	64
Kidney	X	-	594	403	326	344
	S	535	656	485	517	552
Heart	X	-	175	146	161	141
	S	157	220	198	160	210

of these two organs paralleled the changes in cardiac output and prevented the decreased blood flow seen in other tissues. The heart and liver under normal conditions, are known to have the capacity for autoregulation, i.e., decreased vascular resistance occurs in the face of decreased blood pressure. These tissues apparently retained their capacity for autoregulation in spite of the radiation dose.

Supralethal lower-body X-irradiation leads to reduced cardiac output and decreased blood flow to most abdominal tissues. Since early radiation death is thought to be caused by loss of gastrointestinal function, and since absorption is sensitive to decreased blood flow, the present data suggest that the cardiovascular response to radiation may play a role in the impaired function of the small intestine and other radiosensitive tissues.

FIGURE 2. Ratio of Organ Blood Flow to Organ Weight in 1250 R Lower Body X-Irradiated and Pair-Fed, Sham-Irradiated Animals at Various Times Postexposure (Number of animals shown in parentheses; standard error shown by bar.)

LABELING OF THE INTESTINAL MUCOSAL
CELL BY INTRALUMINALLY ADMINISTERED
³H-THYMIDINE

Investigator

*B. W. Wachholz and A. Hopper**

Intestinal cell kinetics have often been studied in small animals by the intravenous or intraperitoneal injection of ³H-thymidine. This method is not practical for large animals or humans because of the large and potentially hazardous quantities of ³H required. We, therefore, initiated studies utilizing low thymidine doses and an intraluminal route for its administration.

Isolated intestinal segments of rats were injected at 24 and 72 hours after 900 R X-irradiation given either to the whole body or to abdomen only. Forty-five minutes later the animals were killed and mucosal tissue sections were taken from the isolated segment and from adjacent segments. They were weighed, and used either for radiochemical measurements of their ³H activity or for squash preparation. The uptake of the ³H by the intestinal mucosa of these rats is shown in Table 1.

There was a 60:1 ratio in the ³H-thymidine activity observed between mucosal segments within and adjacent to the tied-off segment of the ileum, both at 24 and 72 hours postinjection,

indicating a high differential in uptake between the isolated segment receiving the ³H and segments on either side. Twenty-four hours following 900 R lower-body or whole-body X-irradiation, there was a 79 and 87% drop, respectively, in the labeling, within the tied-off segment; indicative of the decreased mitotic activity following irradiation. The data indicate essentially no difference between the whole-body and the partial-body irradiated animals with respect to this parameter. At 72 hours postexposure, the return toward normal mitotic activity was apparent, with the labeling index about 1.5-3 times that at 24 hours.

TABLE 1. Uptake of ³H-Thymidine by Intestinal Mucosa

24 Hours Postirradiation	Average cpm/mg tissue	
	Injected Segment	Adjacent Segment
Nonirradiated Control	1681	28
900 R to abdomen	352	17
900 R to whole body	212	17
72 Hours Postirradiation		
Nonirradiated Control	2128	35
900 R to abdomen	585	30
900 R to whole body	633	55

* Summer Professor (Norcus)

Limited studies of a similar nature were conducted in mice, guinea pigs, hamsters, and miniature swine, with similar results.

It is apparent that the proliferative activity of the intestinal mucosa can be evaluated by the intraluminal injection of ^3H -thymidine as well as by intravenous or intraperitoneal injection. Those squash counts which have been completed show similar re-

lationships. Although further development of the technique is necessary, this method appears applicable to mucosal cell kinetic studies. This procedure may be useful for labeling human intestine by remote isolation, injection, and biopsy of intestine loops, as an aid in the diagnosis of intestinal cellular disorders, perhaps including cancer.

EFFECTS OF IRRADIATING SKIN

Investigator:

D. D. Mahlum

Technical Assistance:

Joan O. Hess

Rats of the Fischer strain, exposed as newborns, weanlings, or adults, to 1, 2.5, 4, and 10 krad of beta radiation from ^{204}Tl plaques, over skin areas of 0.01, 0.5, or 1.0 cm^2 are being observed for development of tumors. Previously reported differences in tumor incidence (Annual Report, 1969) persist at 22 months postexposure (Table 1). Animals exposed as newborns and weanlings continue to show a much higher tumor incidence than animals exposed as adults. Male rats continue to show a higher incidence than females although this difference has become less marked as the animals have aged. Tumors have now developed in all groups of male animals exposed to the smallest field size (0.01 cm^2)

indicating that any threshold for area exposed must be smaller than 0.01 cm^2 . The larger field sizes, however, show a higher incidence and a shorter latency.

Transplantability characteristics seemed of interest in the characterization of these tumors. Tumors were removed from the skin, minced, and small sections implanted subcutaneously in both Fischer and CD strain rats. Tumors did not take in CD recipients, but did in some of the Fischer animals (Table 2). Second passage transplants again failed to grow in CD recipients; however, they grew in all Fischer strain animals and at a rate much faster than observed for the initial transplant.

TABLE 1. Incidence of Rat Skin Tumors Approximately
22 Months After Exposure to ^{204}Tl Plaques

Age at Exposure	Dose, rads	Male ^(a)			Female ^(a)		
		Field Size, cm ²			Field Size, cm ²		
		0.01	0.5	1.0	0.01	0.5	1.0
Newborn	1,000	1/5	4/6	2/5	0/7	1/8	2/6
	2,500	3/10	4/6	3/5	0/6	4/5	1/7
	4,000	1/6	6/10	4/6	0/10	1/5	1/6
	10,000	1/10	6/8	8/12	0/5	0/6	1/4
Weanling	1,000	0/3	2/9	3/4	0/8	0/5	1/6
	2,500	1/5	4/4	3/6	0/5	0/5	1/8
	4,000	2/10	2/6	2/5	1/8	2/10	2/3
	10,000	1/6	2/5	3/7	0/6	2/8	0/8
Adult	1,000	1/8	0/10	1/13			
	2,500	2/12	1/10	1/10			
	4,000	1/10	1/11	2/6			
	10,000	0/11	1/7	1/10			

(a) Numbers listed are sites showing tumors/total sites irradiated.

TABLE 2. Transplantability of Skin Tumors Induced by
 ^{204}Tl --Irradiation of Fischer Rats

		Time After Transplantation (Days)					
		20		30		90	
		M	F	M	F	M	F
First Passage	Fischer Newborns	-	-	-	-	3/3	3/9
	CD Newborns	-	-	-	-	0/10	0/9
	Fischer Adults	-	-	-	-	3/11	3/12
	CD Adults	-	-	-	-	0/12	0/12
Second Passage	Fischer Adults	6/6	-	6/6	6/6		
	CD Adults	0/12	-	0/12	-		

These radiation-induced tumors appear to contain strain specific antigens which will permit growth

only in animals of the strain in which the tumor originated.

RADIATION EFFECTS ON CHYLOMICRON SYNTHESIS AND CAPILLARY
PERMEABILITY IN MICE

Investigators:

J. C. Hampton and B. Rosario

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

or whether they may cross the damaged epithelium, unchanged, by diffusion.

Mice were subjected to 1500 R of X rays and then given 0.25 ml of safflower oil by stomach tube 30 min prior to sacrifice at 1, 2, 3, 3.5, and 4 days postirradiation. Nonirradiated mice served as controls and specimens of liver, ileum and jejunum were examined. Absorption and transport of lipid were most severely affected in the ileum where epithelial damage was clearly established by day 2 and became extreme by day 4. Crypts in the jejunum showed the same kinds of damage and to the same degree as those in the ileum, but the jejunal villus epithelium was still intact and functional through day 4, although at a

significantly diminished level beginning on day 3. Ribosomal depletion in epithelial cells was virtually complete in the ileum, and well established in the jejunum, by day 4. Livers of the mice appeared normal until the third day postexposure, at which time lipid accumulation sharply diminished and glycogen depletion increased, becoming nearly complete by day 4.

Although cell proliferation in the ileum and jejunum ceases after exposure of 1500 R of X rays, the absorptive cells on jejunal villi do not show the severe damage, morphological and functional, seen in the ileum. Thus, lipid absorption remains quite high in jejunum well into day 4 when the absorptive cells begin to show evidence of radiation damage. The reasons why ileal epithelium is more sensitive to ionizing radiation than that in the jejunum are still unknown.

The fine structure of liver cells, particularly cytoplasmic organelles, was not greatly altered by irradiation and, on this basis, one would assume that metabolic pathways would be intact and able to synthesize and store glycogen and to remove and metabolize lipids (chylomicrons) derived from plasma. Electron micrographs suggest that liver function is impaired since lipid and glycogen stores were greatly diminished by days 3 and 4. Careful examination of animals in a repeat experiment revealed that the mice were eating and drinking during this period, so starvation is not a ready explanation for these observations. Further work using autoradiography and H^3 -palmitate

and H^3 -leucine feeding as a more sensitive indicator of gut and liver function is in progress. These experiments should help to clarify the absorptive and transport pathways of the ingested materials, particularly if such materials are in a form that is different from normal and therefore has gone undetected in earlier work.

Capillary permeability: Radiation has been shown to affect capillary permeability in the small bowel of the mouse, as determined by the distribution of small molecular weight tracers injected intravenously. Some capillary endothelial cells become extremely "leaky" after exposure to 1500 R, allowing the tracer (m.w. 40,000) to diffuse through the cell cytoplasm rather than following the more controlled route of pinocytosis and passage through minute "gap junctions" between cells. The present studies were undertaken to determine whether vascular beds in organs other than small bowel were similarly affected and whether only certain types of capillaries sustained damage while others might be more radiation resistant.

Eight organs from more than 20 mice are being surveyed in this study; results are very preliminary. The distribution of tracer (horseradish peroxidase) in nonirradiated mice has been quite uniform from animal to animal and among organs of the same animal. The distribution in irradiated mice has been nonuniform, the brain being the only tissue in which reaction product can be routinely detected. Careful study of many more specimens will be necessary for the assessment of permeability changes.

Larger vessels, arterioles in particular, seem to be affected by 1500 R X rays. The internal elastic lamina in some vessels became highly permeable to the tracer (Figures 1

and 2), a result which was not anticipated. Because of this finding, the scope of our observations will be extended to all vessels present in the tissues examined.

11,700X

FIGURE 1. A Small Artery from a Control Animal Which had Received an Intravenous Injection of Horseradish Peroxidase 5 min Prior to Sacrifice [The endothelium (E), elastic lamina (EL) and muscle (M) layers are normal and unstained by the HRP reaction product.]

11,000X

FIGURE 2. A Small Artery from a Mouse Killed 1 Day After 1500 R X rays [The endothelial lining (E) appears damaged and slightly separated from the elastic lamina (EL) which is heavily stained by reaction product as are elastic elements (EE) outside the vessel. This suggests damage and permeability changes at an early interval after irradiation.]

CELLULAR REGULATORY MECHANISMS

The basic cellular and molecular level studies included in this project are directed toward the elucidation of the mechanisms involved in regulation of enzyme synthesis and growth in eucaryotic organisms. The ultimate goal is to apply this information to an investigation of the primary effects of ionizing radiation. Present studies are concentrated on the cellular control mechanisms in a fairly simple, but representative, eucaryotic cell, Neurospora crassa. Progress has been made in understanding the role of the gene in the transcriptional and posttranscriptional control of enzyme synthesis. Work is described that shows how the absence or presence of a simple alkaline earth metal ion can alter the activity and/or specificity of an enzyme, and how the molecular composition of the cell membrane, one of the crucial organelles of the cell, affect its properties and metabolism.

REGULATION OF TRYPTOPHAN BIOSYNTHETIC
ENZYME IN NEUROSPORA

Investigator:

J. R. Turner

Technical Assistance:

Marilyn J. Hooper

Addition of either indole acrylic acid (IA) or 3-amino-1,2,4-triazole (3AT) to log phase cultures of Neurospora causes the induction of anthranilate synthetase (Aase), phosphoribosyl transferase (PRTase), indole glycerol-phosphate synthetase (IGPase) and tryptophan synthetase (TRPase).

Table I shows the ratio of the relative rates of synthesis of these enzymes during induction, to the relative rates of synthesis in uninduced cells. These relative rates were

PUBLICATIONS: CELLULAR REGULATORY
MECHANISMS

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TABLE 1. Relative Rates of Synthesis of Enzymes During Induction and in Uninduced Cells

<u>Enzyme</u>	<u>Induced Synthesis/ Uninduced Synthesis</u>
AAse	7.5
PRTase	5.2
IGPase	3.0
TRPase	12.5

determined after 60 min of induced synthesis. Three of the activities (AAse, PRTase, IGPase) were synthesized in a coordinate fashion after 60 min in the presence of IA or 3AT. That is, a plot of any one activity versus any other activity results in a straight line indicating that an increase in the quantity of one of these activities is accompanied by a proportional increase in the other two.

Induction of TRPase was not coordinate with the other three enzymes. This is shown in Figure 1. Specific activities of all four enzymes were plotted as a function of time after addition of inducer (Panel A, IA; B, 3AT; C, no addition). A single line drawn through points depicting AAse, PRTase and IGPase activities was the same; the slope of the line for TRPase, on the other hand, was greater regardless of the inducer used. In the absence of inducer (Panel C), all points for the four enzymes cluster around a single horizontal line, in-

dicating no change in specific activity as a function of time. The implication of these data is that unlinked genes, as represented by each of these four enzymes, can be controlled coordinately (AAse, PRTase, IGPase) and that all the enzymes which function in this pathway are induced at the same time although not to the same extent.

A series of regulatory mutants have been isolated from *Neurospora* in which the tryptophan-mediated feedback inhibition of anthranilate synthetase is altered. The mutations do not confer auxotrophy but result in excretion of anthranilic acid, an intermediate of tryptophan biosynthesis. Figure 2 shows the excretion of anthranilic acid by mutant #92814 in the presence of kynurenine, tryptophan, or with no additions. Anthranilic acid excretion in the presence of kynurenine is identical to wild-type; however, in the presence of tryptophan or in minimal medium, excretion does not occur until about 3 hr. The failure to excrete anthranilic acid in the presence of tryptophan is not due to altered transport of tryptophan since a large intracellular pool of this amino acid is formed. The level of anthranilate synthetase in the mutant is the same as wild-type under all conditions examined.

The isolation of what appears at present to be a regulatory mutant in *Neurospora* represents quite a milestone in the study of enzyme regulation in this organism. Utilizing

FIGURE 1. Enzyme Specific Activity as a Function of Time After Addition of Indole Acrylic Acid (A), 3-Amino-1,2,4-Triazole (B), or with no Addition to Minimal Medium (C)

these regulatory mutants, we will be able to elucidate in the mechanism of gene transfer in eucaryotic cells.

Studies along this line are presently underway.

FIGURE 2. Excretion of Anthranilic Acid by Mutant 92814 in the Presence of Kynurenine ($0.1 \mu\text{mole/ml}$) ●—●; Tryptophan ($0.5 \mu\text{mole/ml}$) ○---○; Minimal Medium Δ—Δ

REGULATION OF KYNURENINASE SYNTHESIS IN NEUROSPORA CRASSA

Investigators:

J. R. Turner and H. Drucker

Technical Assistance

Marilyn J. Hooper and Louise C. Neil

Conformational Aspects of Kynureninase Regulations

Two forms of the enzyme kynureninase (Kyn I and Kyn II) were isolated and purified from *Neurospora*. The two forms of the enzyme differ in kinetic properties and in reaction to pyridoxal phosphate. Table 1 shows K_m values for Kyn I and Kyn II at two ranges of substrate concentrations of kynurenine and 3-OH kynurenine. The K_m for Kyn II, with respect to kynurenine and 3-OH-kynurenine is the same at both substrate concentration ranges but is about 2-fold lower for 3-OH-kynurenine than for kynurenine. The values of K_m for kynurenine show an approximate 2-4 fold difference between the two enzymes, with Kyn I appearing to have a greater affinity for this substrate. The K_m for Kyn I with respect to kynurenine at both concentration ranges was the same. However, with respect to 3-OH-kynurenine, the K_m for Kyn I (at the higher concentration range) was increased by about 5-fold, indicating an apparent decrease in substrate affinity at higher concentrations. This observation appeared, at first sight, to resemble a "negative cooperative effect." However, we found that as

the affinity decreases, the reaction velocity increases. Thus, if this is an example of allosteric modulation, it is clear that changes in the binding sites of an enzyme for its substrate need not be accompanied by a commensurate change in catalytic activity.

Pyridoxal phosphate was previously shown to be a cofactor for kynureninase activity. Our results show that Kyn I and Kyn II differ in response when preincubated for 12 hr with $10^{-5}M$ pyridoxal phosphate and assayed on kynurenine. Kyn I was inhibited by 10% while Kyn II showed an approximate 2-fold increase in activity. A symptom of pyridoxal phosphate deficiency in mammals is the decreased ability to degrade tryptophan characterized by an increased excretion of kynurenine, OH-kynurenine, xanthurenic acid and other metabolites of tryptophan. This differential response of Kyn I and Kyn II to pyridoxal phosphate may be a reflection of the regulatory system in *Neurospora* which promotes channeling of excess tryptophan to anthranilic acid rather than to nicotinic acid.

Mechanism of Kynureninase Induction

During induction of kynureninase in *Neurospora*, the specific activity in crude cell extracts is increased by about 100-fold. Figure 1 shows the DEAE elution of activity from induced cells; Figure 2 (Panel A) shows the profile of kynureninase from noninduced cells; and Figure 2 (Panel B) shows purified Kyn I on an identical DEAE column. It is clear that noninduced cells have only Kyn I at very low

TABLE 1. Kinetic Constants for Kyn I and Kyn II

Substrate:	Km Values, mole/l			
	Substrate Concentration			
	1-5 μ M		5-20 μ M	
	Kyn I	Kyn II	Kyn I	Kyn II
3-OH-kynurenine	1.6×10^{-5}	2.6×10^{-5}	9.5×10^{-5}	2×10^{-5}
Kynurenine	1.5×10^{-5}	6.4×10^{-5}	2.3×10^{-5}	4×10^{-5}
	V_{max} values, μ moles/hr			
3-OH kynurenine	0.016	0.031	0.109	0.045
Kynurenine	0.013	0.008	0.023	0.040

FIGURE 1. DEAE Chromatography, Kynureninase from Induced *Neurospora*: (—), Protein; (----), Kynureninase Activity

FIGURE 2. DEAE Chromatography, Kynureninase [Panel A, uninduced wild-type, dialyzed 50-70% $(\text{NH}_4)_2\text{SO}_4$ precipitate. Panel B, purified Kyn I from Induced wild-type. (o—o), protein; (●---●), kynureninase activity.]

levels of Kyn II and that induction results in the *de novo* production of Kyn II and increased synthesis of Kyn I. Induction of these two activities is therefore similar to a promoter response (Kyn I) and an operator response (Kyn II) when expressed in terms of the bacterial induction sys-

tem. When Kyn I or Kyn II were mixed with homologous ^{14}C -labeled enzyme at pH 9.0 and fractionated by sucrose density centrifugation, a peak with decreased activity (relative to the native enzyme at pH 7.0) was observed. The molecular weight, however, corresponds to that of the native enzyme.

When Kyn I and Kyn II were both mixed with ^{14}C -labeled enzyme, an additional inactive sedimenting peak with a molecular weight two times that of the native enzyme was observed (Figure 3). These results suggest that a hybrid of Kyn I and Kyn II was formed which gave rise to an inactive enzyme twice the molecular weight of the native enzymes.

These results and the fact that uninduced cells contain no detectable levels of Kyn II suggest that induction of kynureninase in *Neurospora* consists of two events; the promotion of Kyn I synthesis by inducer, followed by de novo synthesis of Kyn II. The latter event may be triggered either by the inducer or by products of Kyn I action on the inducer.

FIGURE 3. Sedimentation Profile of ^{14}C Kyn I and ^{14}C Kyn II at pH 9.0 in Gradient of 5-20% Sucrose

AMINO ACID ACCUMULATION AND ENZYME
LOCALIZATION IN MEMBRANE VESICLES
PREPARED FROM CELL MEMBRANES OF
NEUROSPORA CRASSA

Investigators:

W. R. Wiley, Laura S. Winn, and
and R. B. Sanders*

In an attempt to gain some insight into the molecular architecture and functioning of Neurospora cell membranes, we have directed our efforts to the isolation, purification and characterization of membrane vesicles.

Membrane vesicles were prepared from spheroplast formed by enzymic removal of cell wall material in the presence of an osmotic stabilizing agent (i.e., NaCl, LiCl or sucrose). The vesicles were partially purified by low speed differential centrifugation and by high-speed centrifugation on discontinuous sucrose gradients containing equal volumes of 20, 30, 40, 45, and 55% sucrose. Arginine and lysine were accumulated 20- to 40-fold by the partially purified vesicles. The apparent K_m for arginine uptake was shown to be $1.82 \times 10^{-5}M$ which is approximately 7-fold higher than that observed for whole cells.

Figure 1 shows that arginine accumulation activity is associated with membrane fragments equilibrating at a

* % activity relative to crude membrane vesicle preparation

FIGURE 1. Activity Associated with Membrane Fragments Equilibrating at Various Densities Following Discontinuous Sucrose Gradient Centrifugation

density of approximately 1.2 following discontinuous sucrose gradient centrifugation at $106,000 \times g$ for 11-15 hr. Alkaline phosphatase and amino peptidase, two enzymes which are localized on the cell membrane, also equilibrated at a density of 1.2.

Table 1 shows a comparison of the rates of arginine accumulation in spheroplast, crude membrane vesicles, and vesicles equilibrating at density of 1.2. There was no further purification of the membrane vesicles by the discontinuous gradient centrifugation process; the specific transport activities were essentially the same for all three preparations. This is not surprising since the membrane vesicles were washed five times before they were added to the sucrose gradient.

* Battelle Institute Summer
Professor - Fy 1970

TABLE 1. Arginine Uptake by Spheroplasts and Membranes from *N. crassa* WT 74A

Preparation	Rate of Arginine Uptake, (a) nmole/mg protein/6 min
Isolated spheroplasts	0.329
Crude membrane vesicles	0.232
Membrane vesicles equilibrating at a density of 1.2 (gcm ⁻³)	0.229

a. The external arginine concentration was $3.56 \times 10^{-5}M$. The rate of uptake was calculated from time course studies which were linear between 1 and 6 min uptake.

The results shown in Figure 1 and Table 1 suggest that arginine transport, aminopeptidase and alkaline phosphatase are intimately associated with membrane vesicles. Arginine uptake by vesicles was not enhanced by the presence of glucose or D-lactate in the uptake medium. Therefore, the energy source for driving the uptake is presently unknown.

It is anticipated that future studies with these subcellular structures will permit elucidation of the mechanism of nutrient translocation. In addition, we hope chemical and physical analysis of the membrane vesicles will permit us to explore the molecular architecture of cell membranes.

INDUCTION AND MOLECULAR BASIS OF PROTEOLYTIC ENZYME ACTION

Investigators:

*H. Drucker and S. Borchers**

Technical Assistance:

Louise C. Neil

Induction of Proteolytic Enzymes

N. crassa produces protease when grown on a bovine serum albumin-(BSA) salts medium. Sucrose at a concentration of from 0.5 to 2% represses protease production. Addition of a substance isolated from maximally induced cultures relaxes the sucrose repression and protease is secreted at about 50% the rate of maximally induced cells. The nature of the inducer substance is not known. It is heat stable, is not a simple amino acid or peptide since casamino acids, tryptone, and peptone do not substitute. The factor is not the most generally recognized promoter substance, cyclic 3'5' AMP, since sucrose derepression was not reversed by the addition of 3'5' cyclic AMP to the growth medium. Figure 1 shows that the inducer substance is made by cells grown on BSA salts after 2 hours growth (before detectable amounts of protease are observed) and is maximal in effect from cultures grown 4 to 6 hours. At this time, the term "inducer" substance may not be valid since no differentiation between a "promoter" or inducer effect can be made.

* Battelle Institute Summer
Student - Fy 1970

FIGURE 1. Rate of Protease Production, Protease Units (PU/ml hr⁻¹) Plotted as a Function of Time of Incubation [The cells were grown on BSA-medium, harvested at the intervals indicated and placed in sucrose (repressing) medium.]

When cells grown on 1% sucrose are transferred in log phase to 1% BSA, no growth or protease production occurs. When a heat labile factor isolated from protease-induced cells is added, induction of protease occurs at the same rate and reaches the same level as in cells grown from conidia on BSA-salts. This is not an activation of inactive protease in the sucrose-grown cells, since the heat labile factor does not cause an increase in the protease activity of cell extracts. The substance involved in the phenomena is large molecular weight (not retarded) by Sephadex (G-25) and would appear to be a protein. Again, the mode of action of this material is not known. Present results suggest that the factor may

be a rather general growth promoting substance. Studies are presently underway which hopefully will permit a clarification of these questions.

Conformational Aspects of Proteolytic Enzyme Action

In our studies on the molecular basis of proteolytic enzyme actions, we examined the changes in molecular weight of the neutral protease thermolysin with varying calcium concentrations. The results showed that calcium binding sites with a $pK_{Ca^{2+}}$ of 4.1 are involved in autodigestion. This is the same $pK_{Ca^{2+}}$ as for sites involved in loss of catalytic activity, conformational change, and change in the kinetics of hydrolysis of the synthetic substrate furylacryloylglycine leucinamide at non-denaturing temperatures. Autodigestion occurs at temperatures above 50°C and calcium concentrations below $10^{-4}M$. It proceeds in an all-or-none fashion (no intermediates) until 77% of the enzyme is digested (Figure 2). The products of autodigestion are of molecular weight 6000-10,000, implying that 3-5 bonds are hydrolyzed. This means a great deal of specificity occurs in the protease, a random autodigestion could yield up to 35 peptides, given the specificity and amino acid composition of the protein. We postulate that calcium plays a specific stabilizing role in thermolysin catalysis. At non-denaturing temperatures, enzyme- $X+Ca^{2+} \rightarrow$ enzyme-Y, where Y and X differ in kinetic parameters, i.e., Y is conformationally "open." Thus, increasing the temperature results in enzyme-Y

FIGURE 2. Elution of Thermolysin from Sephadex G-200 Column After 2 (●—●) and 12 min (○—○) at 66°C

unfolding at a high rate, therefore increasing the availability of specific sites for autodigestion.

Further studies on the physical and chemical properties of the autodiges-

tion products of this enzyme are expected to provide information that will permit us to confirm or eliminate this model.

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

The aim of this project has been to obtain the kind of information needed to establish appropriate radionuclide-exposure limits for the rapidly growing infant or child, or for the pregnant female. The need for this information arises from age-dependent differences in the metabolism of radionuclides and from the greater intrinsic radiosensitivity of the immature organism. Reports are included on the metabolism and effects of ^{131}I , ^{144}Ce , and ^{239}Pu ; and on the quantitation of such functional parameters as prenatal growth, development of liver function, and susceptibility to audiogenic seizure, in rats.

COMPARATIVE EFFECTS OF ^{131}I AND X RAY ON THE RAT THYROID

Investigators:

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

Technical Assistance:

Joan O. Hess

We have been engaged in a study to determine whether the effects of relatively large doses of ^{131}I are due to direct radiation damage to the thyroid, to indirect effects resulting from the induced hypothyroid state, or to radiation effects on the surrounding tissues and the whole body (Annual Report, 1969).

As part of this study, experiments were performed to compare the effects of X radiation of the thyroid to those produced by radioiodine. Neonatal animals as well as the adults were used for this comparison. We consistently found that X rays were less effective than ^{131}I when the endpoint used was the uptake of a test dose of ^{125}I at 4 months after the original exposure. This was in sharp contrast to reports by Doniach and others, which indicated that the relative biological effectiveness of X rays on the thyroid was greater than that of ^{131}I . Differences in endpoints used to evaluate the damage (they used the increase in weight produced by administration of a goitrogen) were thought to be a possible explanation for the discrepancy in results. Accordingly, an experiment was performed to use both of these

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endpoints, ^{125}I uptake and weight increase following administration of the goitrogenic agent aminotriazole (ATA).

Adult rats of the CD strain were exposed to 0.1 $\mu\text{Ci/g}$ of ^{131}I or 1000 R X rays to the thyroid. Two weeks later half of the animals of each exposure group were given ATA in the drinking water for a period of 4 weeks, while the others received tap water. At the end of this 4-week period, all animals (ten per subgroup) were given a test dose of 0.5 μCi of

^{125}I and killed 24 hr later. The thyroid glands were excised, weighed, and the radioactivity determined by gamma counting.

At the doses employed neither X ray nor ^{131}I administration affected weight or ^{125}I uptake of the nonstimulated thyroid gland (Table 1). Exposure to X radiation decreased the response of the gland to ATA as measured by weight differences. This was about the same magnitude of effect as that produced by administration of radioiodine, which delivered approximately the same radiation dose to the thyroid. Administration of ATA increased the uptake of ^{125}I in control animals. The magnitude of this increase was significantly less in the rats exposed to X rays and no increase was found in those that received ^{131}I .

It is thus apparent that goitrogen stimulation (ATA) increases the sensitivity of measurement, regardless of which endpoint is used. Although ATA

stimulation was required to produce a measurable effect at the radiation doses used in this experiment, ^{131}I beta particles were as effective, or more effective, than X rays, in accord with our previous findings.

DISTRIBUTION AND EFFECT OF ^{239}Pu IN THE FETOPLACENTAL UNIT OF THE RAT

Investigators:

M. R. Sikov and D. D. Mahlum

Technical Assistance:

Joan O. Hess

As little as 6.25 μCi of ^{239}Pu administered to pregnant rats was shown to produce excess prenatal death (Annual Report, 1969). We have extended these studies to determine the threshold dose for the lethal effect and to establish the overall dose-response curve.

Mortality by 14 days of gestation, following injection of ^{239}Pu at 9 days, was reasonably representative of the overall mortality pattern at earlier or later times. Accordingly, deaths to this time were used in preparing Figure 1. Injection of 1.0 μCi or less did not significantly increase prenatal mortality above the control level of 11%; doses of 3 through 12 μCi all produced approximately 60% mortality.

TABLE 1. Effect of Exposure of Thyroid Gland to X Rays or ^{131}I on the Subsequent Uptake of ^{125}I and on the Response to Aminotriazole (ATA) (Mean $\pm$ S. E.)

Radiation Treatment	ATA	Thyroid Weight, mg	Thyroid Uptake of ^{125}I , %
None	-	26 $\pm$ 2.1	14 $\pm$ 0.9
None	+	66 $\pm$ 4.3	45 $\pm$ 2.8
1000 R X ray	-	20 $\pm$ 1.1	15 $\pm$ 1.1
1000 R X ray	+	40 $\pm$ 3.5	23 $\pm$ 3.4
0.1 $\mu\text{Ci/g}$ ^{131}I	-	24 $\pm$ 2.2	16 $\pm$ 1.2
0.1 $\mu\text{Ci/g}$ ^{131}I	+	34 $\pm$ 3.1	10 $\pm$ 1.8

FIGURE 1. Fetal Mortality at 14 Days of Gestation Following Intravenous Injection of the Dam with ^{239}Pu at 9 Days of Gestation

The membranes, placentas, and fetuses were analyzed for ^{239}Pu content and the measured concentrations used to calculate average daily radiation doses to these structures (Figure 2). Doses to the placenta and fetus were similar; those to the membranes were about four times as high.

Autoradiographic studies of the distribution of plutonium showed moderately high activity in the yolk sac. There was less activity in the trophoblast, although localized hot spots were associated with the giant cells. There was much less activity in the amnion and allantois. Activity throughout the embryo was primarily diffuse, but with increased concentrations in the neural groove, in the splanchnic mesoderm, and in the somites, particularly in the outer portion or dermatome.

The cumulative radiation dose to

the embryo or placenta after injection of ^{239}Pu at 9 days of gestation would not seem sufficient to account for the observed mortality, even considering the elevated local concentrations, which may deliver substantial doses to some areas of the fetal membranes. We have estimated that doses of 75 to 150 rads may be received by the villous visceral splanchnopleure during the first 2 days following injection of 12 μCi . The importance of this structure to the nutrition of the embryo during this time would suggest that doses of this magnitude might account for the observed mortality. It should be recalled that in other experiments (Annual Report, 1969), injection of 50 μCi of ^{239}Pu at 15 or 19 days of gestation failed to cause an increase in fetal mortality even though doses of 1000 rad/day were received by portions of the membrane.

FIGURE 2. Estimated Average Radiation Dose Rates to Fetoplacental Unit Following Intravenous Injection of the Dam with ^{239}Pu at 9 Days of Gestation

STRAIN DIFFERENCES IN THE RESPONSE OF THE RAT TO ^{144}Ce

Investigators:

D. D. Mahlum and M. R. Sikov

Technical Assistance:

Joan O. Hess

Carcinogenicity of ^{144}Ce in rats was shown to be dependent on age at time of administration (Annual Report, 1969); the incidence of osteogenic

sarcoma was greatest in animals exposed at the time of weaning. Attempts to transplant this tumor in the strain of origin (CD strain) were successful in only a few cases probably because of genetic heterogeneity. Additional studies were undertaken to compare the effects of this radio-nuclide in the original strain (CD) and in animals of Fischer origin (CDF strain). The CDF strain was selected to provide animals of greater genetic homogeneity in which innate transplantability could be better tested.

Weanling rats of the CD and CDF strains were injected intravenously with 0, 0.5 or 1 $\mu\text{Ci/gram}$ of ^{144}Ce .

Substantially fewer tumors have developed in the CDF animals than in the CD strain. The comparison in Table 1 is based on the administered dose of radionuclide. Radiation dose will not be strictly proportional to administered dose because of strain differences in growth rate and ultimate size of organs. Animals are being analyzed at intervals to establish radionuclide concentration curves from which the radiation dose can be calculated.

Tumors developing in the injected animals have been minced and transplanted into animals of the same strain as well as of the reciprocal strain. Results from these studies are not yet available.

Liver function was determined at intervals after injection using ^{131}I -rose-bengal to measure parenchymal function and ^{198}Au colloid to measure the function of the Kupffer cells. Rose-bengal clearance was not significantly affected in either strain by the administration of ^{144}Ce , although there was a tendency toward decreased clearance in the animals receiving

the highest dose. In the animals of the CD strain, the uptake of gold in the spleen was elevated by 1 $\mu\text{Ci/g}$ of ^{144}Ce , with concomitant slight decrease in incorporation in the liver; little defect was seen in the rats of the Fischer strain.

The osseous response in the two strains was different. Radiographs obtained 6 months after administration of ^{144}Ce , in the CD strain, at the 1 $\mu\text{Ci/g}$ level, showed two of 18 animals with spontaneous fractures of the long bones and two with tumors. In the CDF animals no skeletal alterations were noted at this time at any dose level. As shown in Table 2, administration of ^{144}Ce in the CD strain decreased femur strength as early as 1 month postadministration. A less marked effect was seen in the CDF strain.

TABLE 1. Effect of Strain on Incidence of ^{144}Ce -Induced Bone Tumors in the Rat

^{144}Ce Dose, $\mu\text{Ci/g}$	Tumor Incidence ^(a)	
	CD Strain	CDF Strain
0	0/12	0/18
0.5	0/24	0/18
1.0	8/18	2/18

(a) Number with tumors at 1 year postinjection/total injected.

TABLE 2. Effect of Strain on ^{144}Ce -Induced Weakening of Femurs in the Rat (Average of 6 rats $\pm$ S.E.)

Time After Injection	^{144}Ce Dose, $\mu\text{Ci/g}$	Breaking Strength ^(a)	
		CD Strain	CDF Strain
1 month	0	13 $\pm$ 1.1	
	0.5	12 $\pm$ 0.5	
	1.0	5.9 $\pm$ 0.8	
8 months	0	11 $\pm$ 0.5	10 $\pm$ 0.7
	0.5	8.5 $\pm$ 0.2	9.9 $\pm$ 1.0
	1.0	6.1 $\pm$ 0.9	7.7 $\pm$ 0.8
10 months	0	11 $\pm$ 0.7	8.4 $\pm$ 0.2
	0.5	8.6 - 9.0 ^(b)	
	1.0	2.9 - 3.1 ^(b)	7.9 $\pm$ 0.3

(a) Breaking Strength, $S = \frac{W}{A}$, where

W = weight in grams required to break bone,
 l = length of bone between supports in cm,
and A = cross sectional area of bone in cm^2 .

(b) Individual values for two animals.

PRENATAL GROWTH OF THE RAT

Investigators:

*M. R. Sikov and J. M. Thomas**

Despite recent interest in the development of mathematical relationships to describe the kinetics of growth in the rat, published equations describing fetal rat growth have been derived or fitted using the data of Stotsenburg obtained in 1915 (Anat. Rec. 9, 667). During the course of recent studies requiring calculation of radiation dose, we have obtained the weight and size

of the fetus and several fetal organs and structures at several stages of development. These data have been tabulated to provide a more adequate description of the growth of the prenatal rat and published growth models have been tested for their ability to describe these data. The following is a brief summary of material published in Growth, 34, 1-14 (1970).

The data were initially examined using a published cube root model which predicts a linear relationship between gestational age and the cube root of body weight. Our data as well as the earlier data were plotted and linear and cubic equations fitted by least squares techniques (Figure 1).

FIGURE 1. Prenatal Rat Growth Data from Present Study (solid lines) and from Stotsenburg (dashed lines) Plotted as Linear Regression of the Cube Root of Weight (right) and as a Cubic Regression (left)

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It is visually apparent that the data are better represented by a cubic equation than by a linear equation. The size of the prenatal rats in our laboratory is markedly different from that of the rats observed in 1915.

Overall growth data were summarized using three published growth models and several statistical weighting schemes, made possible by the availability of measures of dispersion, which were not available for the earlier reported data. Use of a cube root model resulted in the poorest fit to the data and lack of meaning for both the resulting intercept and asymptotic values, although it allowed ease of determination of growth rate differences. A weighted Gompertz expression gave a satisfactory fit and reasonable approximations for ovum weight and ultimate adult weights. However, both weighted and unweighted logistic equations gave fitted curves with smaller residual sum of squares than any of the comparable Gompertz curves, but did not provide good estimates for egg weight or mature body weight. Specific growth weight during the prenatal period was more adequately explained by the logistic model.

DEVELOPMENT OF LIVER FUNCTION IN THE NEONATAL RAT

Investigators:

M. Kashima, D. D. Mahlum and
M. R. Sikov*

Technical Assistance:

Joan O. Hess

We previously found age-related differences in the hepatic deposition of ^{144}Ce and ^{239}Pu in the rat. It was felt that some of these differences could be ascribed to hepatic immaturity. Studies were, therefore, undertaken to evaluate the function of the parenchymal and Kupffer cells in the young rat.

Neonatal (0, 1, 6, and 13 days of age) and adult rats were injected with a tracer dose (0.5-5 μg of colloidal ^{198}Au and the deposition in the liver and spleen at 10 min after injection used as a measure of reticuloendothelial function. The livers of the 0- and 1-day-old animals removed smaller fractions of the dose than did those of the older age groups (Table 1). No differences in hepatic deposition were found between the adults and the 6- or 13-day-old rats. The uptake by the spleen was significantly greater in all suckling groups than it was in the adults.

Since the use of tracer levels or gold maximizes uptake by the liver

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TABLE 1. Effect of Age on ^{198}Au Incorporation into Liver and Spleen

Age, days	No. of Rats	Percent of Administered Dose (Mean $\pm$ Std. Error)	
		Liver	Spleen
0	3	75.0 $\pm$ 1.0	1.9 $\pm$ 0.0
1	9	78.0 $\pm$ 6.0	2.3 $\pm$ 0.4
6	4	86.0 $\pm$ 4.0	2.7 $\pm$ 0.3
13	4	90.0 $\pm$ 3.0	3.1 $\pm$ 0.4
Adult	5	88.0 $\pm$ 2.0	0.9 $\pm$ 0.1

in the neonate, additional experiments will be performed in which non-radioactive colloid (carrier) will be used to increase the mass of material injected. This should permit an even better evaluation of RES function in the neonate.

Other neonatal and adult rats were injected with tracer amounts of ^{131}I -rose bengal. The ratio of the concentration in the blood at 20 min to that at 5 min was used as an index of parenchymal cell function. (Blood samples were obtained from the same adults at both times but from different animals in the neonatal groups.) The rate of clearance was significantly reduced in 4-day-old rats but approached adult values in 13-day-old rats (Table 2). Again it may be possible to widen the differences between neonate and adult by injecting larger amounts of test material.

It is obvious that both the reticuloendothelial and liver parenchymal cell functions are less in the new-

born than in the adult. The magnitude of this difference may account for part, though probably not all of the differences between newborn and adults in the deposition of ^{144}Ce and ^{239}Pu .

TABLE 2. Influence of Age on the Clearance of ^{131}I -Rose Bengal from Rat Blood

Age, days	No. of Rats	Blood Level at 20 min as Percent of Blood Level at 5 min (Mean $\pm$ Std. Error)
4	5	47.0 $\pm$ 5.0
13	6	33.0 $\pm$ 5.0
Adult	4	27.0 $\pm$ 2.0

EFFECT OF BIOCHEMICAL INTERVENTION ON AUDIOGENIC SEIZURE SUSCEPTIBILITY INDUCED BY PRENATAL IRRADIATION

Investigators:

D. D. Mahlum and M. R. Sikov

Technical Assistance:

Joan O. Hess

The incidence of audiogenic seizures (AS) is increased by a prenatal exposure of rodents to ionizing radiation, presumably due to lesions of the cerebral motor cortex. Our observation of increased AS susceptibility in animals neonatally exposed to

^{239}Pu (Annual Report, 1967), or ^{131}I (Annual Report, 1968), led us to undertake studies examining possible mechanisms for this decrease in cortical threshold.

We hypothesized an effect mediated through decreased levels of gamma amino butyric acid (GABA), an inhibitor of neuronal firing. If correct, the incidence of audiogenic seizures should be reduced by increased brain levels of GABA. Since GABA does not freely penetrate the blood brain barrier, we first tested the effect of amino oxyacetic acid (AOAA) which elevates GABA levels *in situ* by inhibiting GABA transaminase.

Pregnant rats were exposed to 185 R of X or gamma rays after 15 days of gestation. The offspring were tested for susceptibility to AS at 4 to 5 weeks of age. Seizure-susceptible rats were injected with either AOAA or saline, and tested at several subsequent times. Results are shown in Figure 1. Most of the rats injected with saline continued to show seizures while those injected with AOAA showed a marked and continuing reduction in seizure susceptibility. The data shown in Figure 1 were obtained with AOAA doses of 20 mg/kg; similar but less consistent effects were seen at lower doses.

FIGURE 1. Effect of AOAA (20 mg/kg body weight) on Audiogenic Seizure Susceptibility (Numbers of animals shown in circles)

A similar study was performed in which prenatally irradiated animals were treated with AOAA or GABA for a 2-week period starting at birth. These animals were tested for AS susceptibility at several times between 1-1/2 and 11 months of age. Neither GABA nor AOAA was effective under these conditions in reducing seizure susceptibility. Efforts to repeat the observations of Figure 1 were in one case successful and in another, unsuccessful. Further studies are required to clarify the situation.

EFFECTS OF RADIOSTRONTIUM IN MINIATURE SWINE

Initiated in 1958, this project seeks to define the biological effect of daily ingestion of ^{90}Sr in miniature swine. Nearly 800 female Pitman-Moore miniature swine, representing three generations, have been exposed to ^{90}Sr at feeding levels ranging from 1 to 3100 $\mu\text{Ci/day}$. About 150 animals with 9 to 12 years of exposure are currently on experiment, not including an additional 40 animals, not part of the main experiment, which are being used for in-depth studies of certain dosimetric, metabolic, and pathologic parameters. The very interesting leukemogenesis studies, which have evolved from this project, are now included in a separate project, "Radiation-Induced Leukemogenesis."

RADIOSTRONTIUM CHRONIC TOXICITY STUDY

Investigators:

W. J. Clarke, Patricia L. Hackett,
and H. A. Ragan

Technical Assistance:

Glenda S. Vogt, Martha C. Perkins,
Darlene H. Hunter, and
Eugenia T. Edmerson

Observations of sows and their offspring fed 1, 5, 25, 125, 625, and 1300 μCi ^{90}Sr /day have been compiled over the past 12 years. The original dams were put on the ^{90}Sr regimen at 9 months of age and bred to control boars to produce the F_1 generation. These offspring received ^{90}Sr exposure in utero and from the dam's milk, and at weaning were fed 1/4 of the dam's ^{90}Sr level, 1/2 at 3 months of age, and the full amount at 9 months of age. The F_2 generation was derived from the F_1 sows and was treated in a comparable manner.

Mortality Data

At present, 98 of the swine from the original study are alive. This number includes 39 controls, thirty-one 1 μCi offspring, fifteen 5 μCi offspring, two 25 μCi sows and 11 of their offspring. Cumulative mortality data (Figure 1) show that differences between control and ^{90}Sr -fed adults are apparent after 1 year in the 625 μCi /day level, after 5 years in the 125 μCi /day group, and perhaps after 10 years in the 25 μCi /day level. Cumulative values for these sows were calculated from 9 months of age when ^{90}Sr feeding was initiated

PUBLICATIONS: EFFECTS OF
RADIOSTRONTIUM IN MINIATURE SWINE

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and include the 1 and 5 μCi dams which were not fed ^{90}Sr for their complete lifespan.

Cumulative mortality (Figure 2) in the weaned offspring of 1 and 5 μCi /day animals has not significantly exceeded control values up to the

FIGURE 1. Cumulative Mortality of Swine Fed ^{90}Sr from 9 Months of Age

present time. Mortality in offspring of 25 μCi sows was not greater than that of the controls for 9 years and was even less for the first 5 years of life. Curves for the 125 μCi and 625 $\mu\text{Ci/day}$ offspring reflect the development of hematopoietic disorders such as radiation-induced pancytopenia, neoplasia and metaplasia, which occur at an earlier age in the offspring than in their respective dams.

Tumor Incidence

The incidence of tumors (other than hematopoietic and bone tumors which have been compiled previously) in the adults and offspring of the chronic ^{90}Sr program is shown in Table 1. Uterine tumors, namely leiomyomas, were common in all groups. Carcinomas and sarcomas constituted 6 of 22 tumors occurring in sows and their offspring fed 25 μCi $^{90}\text{Sr/day}$, while

FIGURE 2. Cumulative Mortality in Weaned Offspring of Dams Fed ^{90}Sr

TABLE 1. Tumor Location and Incidence in Swine Fed ^{90}Sr (Hematopoietic and bone tumors excluded)

Tumor Location	⁹⁰ Sr Feeding Level And Generation									
			1 μCi		5 μCi		25 μCi		125 μCi	625 μCi
			Adult	Offspring	Adult	Offspring	Adult	Offspring	Adult	Offspring
Organ	No.	Control								
Uterine	51	16	6	9	2	4	8	4	2	
Ovarian	3	1					1	1		
Hepatic	5	1	1				1	2		
Intestinal	3	1					1	1		
Pituitary	1	1								
Adrenal	2	1						1		
Thyroid	1			1						
Brain	2						1			1
Pancreatic	1						1			
Total/level		21	7	10	2	4	13	9	2	1

Tumor Incidence	Age	No.							
4 YEARS	1								1/2(50.0)
5	1								1/10(10.0)
6	5	2/76(2.6) (a)		2/53(3.8)					1/4(25.0)
7	6	1/69(1.4)	2/20(10.0)		1/6(16.7)	1/24(4.2)		1/25(4.0)	
8	22	9/48(18.7)	5/10(50.0)	3/42(7.1)	1/5(20.0)			4/22(18.2)	
9	13	3/22(13.6)		2/38(5.3)		1/22(4.5)	6/10(60.0)	1/13(7.7)	
10	13	2/11(18.2)		2/25(8.0)			6/8(75.0)	3/7(42.9)	
11	7	3/9(33.3)		1/3(33.3)		2/12(16.7)	1/2(50.0)		
12	1	1/1(100.0)							

(a) Number of tumors/number of animals (percentage)

2 of 21 tumors in control animals fell in this category.

A meningioma, detected in a 625 μ Ci offspring, was observed earliest (at 4 years of age), but the peak incidence for all types of tumors occurred at 8 years of age in the treatment levels that permitted survival to this time.

"Leukemic Pool" Swine

In order to ensure a source of porcine material for viral and immunological studies on leukemic animals, twenty-one 125 μ Ci/day offspring are being maintained on this ^{90}Sr regimen, which has previously produced hematopoietic neoplasia and metaplasia in 72% of the treated animals. At the present time the ages of these offspring range from 12 to 23 months, the six oldest being in the experimental period of high probability for the onset of neoplasia.

Studies on Intermediate ^{90}Sr Feeding Levels

In an effort to minimize the time interval necessary to produce a leukemic animal, two new feeding levels of ^{90}Sr were added. These levels, 250 μ Ci and 313 μ Ci/day, are intermediate to the ^{90}Sr regimen known to produce a hematopoietic crisis (625 μ Ci), and the treatment previously resulting in the highest incidence of neoplasia (125 μ Ci/day offspring).

Four sows were put on a ^{90}Sr regimen of 313 μ Ci/day at 10 months of age, farrowed after 8 months of feeding, and killed at 34 months of age.

Platelet and neutrophil values were depressed and one adult exhibited signs of a hematopoietic disorder (i.e. a terminal leukocyte value of 117,000 with many immature granulocytes, including myeloblasts, and a low leukocyte alkaline phosphatase score).

Offspring of these 313 μ Ci/day dams survived the 78 μ Ci/day feeding level from weaning to 3 months of age, but platelet and neutrophil values were severely depressed by feeding 156 μ Ci/day and the first animal died after a month of this regimen. Of the 15 offspring, only two animals survived over 6 months of age. All of the animals exhibited symptoms of the hemorrhagic syndrome except one that was killed after 10 months. Myeloid metaplasia was observed in one animal, while foci of extramedullary hematopoiesis occurred in seven animals.

A feeding regimen of 250 μ Ci ^{90}Sr /day was initiated in six 31-month-old sows. Farrowing occurred after 6 experimental months and the dams were killed after the offspring had been weaned. Terminal blood samples from these sows revealed an abnormal hemogram (decreased erythrocytes and cellular alkaline phosphatase, and increased numbers of immature basophils) in one of the six animals.

Fifteen female offspring of the 250 μ Ci/day animals survived a ^{90}Sr regimen of 62 μ Ci/day from weaning to 3 months of age. The first death occurred after 2 months of the 125 μ Ci/day regimen and all animals were removed from ^{90}Sr feeding at 6 months of age when a marked thrombocytopenia was observed.

FIGURE 3. Comparison of Cellular Values from Young ^{90}Sr -Fed Swine and Control Swine

In Figure 3 the graduated feeding levels, age at first death, and mean cellular levels in offspring of the 250 μCi and 313 $\mu\text{Ci/day}$ animals are compared with data derived from the 125 μCi and 625 $\mu\text{Ci/day}$ offspring. It is evident from these studies that the acute toxic feeding level of ^{90}Sr in young animals, as evidenced by the development of the hemorrhagic syndrome and pancytopenia, may be closer to the "optimum leukemic dose" of 125 $\mu\text{Ci/day}$ than was originally believed.

Leukocyte Alkaline Phosphatase Scores

Studies on alkaline phosphatase activity in leukocytes of the ^{90}Sr -fed swine were continued because of their previously assessed value in classifying hematopoietic disorders.

An earlier observation that a depressed cellular enzyme level, concurrent with lowered neutrophil values in sows fed 313 $\mu\text{Ci } ^{90}\text{Sr/day}$, responded to the stimulus of gestation by exhibiting a higher LAP activity per cell with no increase in numbers of neutrophils, required confirmation in pregnant control sows. These values were assessed in swine from the breeding herd and the results indicate that although LAP scores show an initial depression in

enzyme activity they are somewhat increased in value in the later period of gestation (Figure 4). Apparently ^{90}Sr feeding does not alter the ability of the neutrophil to synthesize enzymes although the number of cells produced is restricted.

LAP scores were also obtained for controls and 250 $\mu\text{Ci/day}$ offspring from birth to 6 months of age and compared with other blood constituents that are changing rapidly in this interval (Figure 5). Neutrophil and LAP patterns in control swine are very similar except for the perinatal values. Evidently, the cells have a low enzyme activity until weaning (1.5 months of age) and then respond to the environmental stresses appearing at this time. Changes in gamma globulin fractions of the serum proteins also reflect this post-weaning adaptation. It is interesting to observe that the offspring of the ^{90}Sr -fed animals follow these trends in a qualitative fashion but not to the same degree. The reciprocal relationship between extra- and intra-cellular enzyme activities in blood of very young animals, as exemplified by serum and neutrophil alkaline phosphatase levels (Figure 5), would seem to be a productive area of investigation for early toxic effects in perinatal swine.

FIGURE 4. Neutrophil Values and Cellular Alkaline Phosphatase Scores in Control Sows and Sows Fed 313 µCi ⁹⁰Sr/Day During Gestation

FIGURE 5. Cellular and Serum Constituents in Controls (—●) and Offspring of Dams Fed 250 μCi $^{90}\text{Sr}/\text{Day}$ (---○)

DOSIMETRY STUDIES

Investigators:

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

A previous Annual Report (1968) described Gompertz curves that represented skeletal growth and ^{90}Sr accumulation in miniature swine ingesting ^{90}Sr daily from birth to nearly 7 years of age, and the derivation from these curves of equations expressing skeletal ^{90}Sr concentration versus age. An exponential curve was also derived to describe skeletal ^{90}Sr concentration versus age for parent animals that started daily ingestion of ^{90}Sr at about 9 months of age.

The ^{90}Sr concentrations measured in the skeletons of the thirty-five 1, 5, 25, or 125 $\mu\text{Ci/day}$ offspring animals that have died or been sacrificed in the past 2 years fit the previously derived equations, except that for animals living past 10 years of age, the equation predicts ^{90}Sr concentrations 15 to 20 percent higher than those measured. This is not surprising, considering that the equations were derived with data from animals no more than 7 years old.

The previously derived concentration equation well describes the skeletal ^{90}Sr concentrations measured in 18 parent animals that died or were sacrificed during the past 2 years, except again, for the animals living

past 10 years of age, who show concentrations 15-20 percent lower than predicted. The skeletal ^{90}Sr concentration versus age equations will be modified when sufficient new data are available from animals still on the experiment.

The ^{90}Sr concentrations in the skeletons of fifteen 313 $\mu\text{Ci/day}$ offspring animals that died between 140 and 411 days of age agree well with those predicted by the concentration equation derived for the 125 $\mu\text{Ci/day}$ animals, modified to correct for the higher ^{90}Sr level fed. Concentrations peak at about 250 days of age and decrease nearly to the predicted plateau value by 411 days of age.

UTERINE-MILK EXPOSURE STUDY

Investigator:

Beatrice J. McClanahan

Technical Assistance:

*Martha C. Perkins and
Darlene H. Hunter*

The purpose of this study is to determine the effect of ^{90}Sr exposure at different stages of pre- and postnatal development on the subsequent production of lymphopoietic and hematopoietic disorders. The heat periods of 20 sows were synchronized and part of the animals were fed 125 $\mu\text{Ci } ^{90}\text{Sr/day}$ until apparent equilibrium body burdens were obtained. They all were then bred and the litters switched at birth to provide a

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total of 47 animals in the following ^{90}Sr exposure groups; uterine exposure only, uterine + milk exposure, uterine + milk + subsequent radioisotope feeding exposure, milk exposure only, and milk + subsequent radioisotope feeding exposure. Except for these alterations, the same protocol was employed as that used in the 125 $\mu\text{Ci/day}$ chronic toxicity study.

Table 1 describes the treatments and the number of animals originally started in each treatment group. The numbers in parentheses represent the number of animals that have subsequently died. The castrate male, whose exposure to ^{90}Sr was intra-uterine only, died at 1.3 years of age with acute pancreatic necrosis, an event that was not associated with radiation exposure. The two males that were exposed by milk + feeding died at 1.8 and 2.0 years of age.

They both had severely depressed platelet values and died from a hemorrhagic syndrome. The two animals, one male and one female, whose exposure to ^{90}Sr began at conception and continued through nursing + feeding after weaning, died at 1.5 and 1.9 years of age. The older pig, a female, died of acute myelogenous leukemia; histologic examination of the other animal's tissue has not been completed.

The surviving animals of this study appear to be in good health. At this time, the two groups receiving 125 μCi $^{90}\text{Sr/day}$ have only slightly depressed platelet, neutrophil, and lymphocyte values. However, they have entered the critical period for development of leukemia, as evidenced by our past experience with 125 $\mu\text{Ci/day}$ animals. At this dose level of ^{90}Sr , most of the leukemia cases in the F_1 offspring developed at 2-4 years of age.

TABLE 1. Experimental Design of Uterine-Milk Exposure Study

	Mode of ^{90}Sr Exposure				
	Uterine	Uterine + Milk	Uterine + Milk + Feeding	Milk	Milk + Feeding
Castrated Males	3 (1) ^(a)	5	5 (1)	6	4 (2)
Females	7	3	4 (1)	4	6

(a) Deceased animals in parentheses.

RADIATION - INDUCED LEUKEMOGENESIS

The objectives of this project are to characterize the leukemias induced in the miniature swine of our ^{90}Sr -toxicity project; in particular, to determine whether this disease has a virus etiology. This will be accomplished by isolating viruses from leukemic pigs, identifying the viruses, adapting them to tissue culture systems, and eventually passing them to immunologically-depressed, unirradiated animals in an attempt to transmit the leukemias. The project is also concerned with the detection of humoral factors in plasma and tissues from these leukemic animals responsible for the induction or regulation of hemopoiesis.

VIRAL STUDIES ON RADIOSTRONTIUM-
INDUCED LEUKEMIA OF MINIATURE SWINE

Investigators:

M. E. Frazier and J. R. Pratt

Technical Assistance:

B. Rosario and Jean Stearns

A number of diverse factors are known to play a part in the etiology of leukemia in both animals and man. If a virus plays a role in the radiostrontium-induced leukemia of miniature swine, we hope to gain insight into the interaction of virus and radiation.

Primary cultures were prepared from the tissues of leukemic swine. About 1-5% of the cells exhibited nuclear inclusions similar to those observed in adenovirus-infected cells. Stable porcine cell lines inoculated with fresh plasma from these leukemic animals began to develop similar inclusions after 60-90 days in culture (Figure 1). Rarefied zones inside the nuclear membrane were present in about 5-10% of the cells. Expended medium from these cultures produced similar slowly progressing cytopathological effects (CPE) in monolayer cultures of a stable porcine embryonic kidney (PEK) cell line. After serial passage in PEK cells, the agent was able to produce complete CPE (Figure 2). The agents causing this

PUBLICATIONS: RADIATION-INDUCED
LEUKEMOGENESIS

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320X

FIGURE 1. Pk-15 (a Stable Porcine Cell Line) 60 Days After Inoculation with Fresh Serum from a Leukemic Swine (The rarefied zone inside the nuclear membrane is present in about 5% of the cell population. Hematoxylin and eosin.)

cytopathology were isolated and characterized as adenoviruses, based on chemical, physical, and serological determinations. Adenovirus 2, and adenovirus 4 were isolated from two different leukemic animals; a swine adenovirus unrelated to the human strains except for common complement fixing antigens was isolated from two other leukemic swine.

In addition to the adenovirus, electron micrographs show "C" type particles associated with cell cultures from leukemic swine (Figure 3). The "C" type particles were separated from the adenovirus by sucrose and cesium chloride density gradient techniques. Adenovirus infectivity banded at a level indicating a density of 1.35 gm/cm^3 ; the "C" type particles

192X

FIGURE 2. PEK Cells Fixed at 100 Hours Illustrate the Nuclear Inclusions Developing After Infection with Adenovirus [(a) Intranuclear inclusions showing a rarefied zone inside the nuclear membrane. (b) Inclusions with the rarefied zone as well as formation of basophilic granules in the nucleus. (c) The nucleus and several basophilic inclusions in the nucleus are surrounded by halos or rarefied areas. (d) A cell near death shows the honeycombed central mass. Hematoxylin and eosin.]

FIGURE 3. Electron Micrograph Displaying "C" Type Particles Present in Cell Cultures from Animals with Myelogenous Leukemia

FIGURE 4. Budding "C" Type Particles Transferred from Cell Cultures of Leukemic Animals to a Normal Porcine Cell Line (PEK)

formed a wider band at a calculated density of 1.08 to 1.12 gm/cm³. The "C" type particles from this preparation were transferred to the PEK cell line and subsequently produced typical "C" type particles, including the budding process seen in Figure 4. The infectivity of this agent is apparently low since it takes about 15 days before virus particles can be detected in the cell. The presence of "C" type particles show no evidence of having transformed the PEK cells after 4 months in culture.

Adenoviruses have also been seen in association with human leukemia, where they have generally been regarded as insignificant passenger viruses. This

is probably the case in swine as well, but adenoviruses have induced malignant lymphomas in hamsters. It is possible that the adenovirus may enhance the leukeomogenic process by any of several mechanisms.

We suspect that the leukemogenic agent in our swine is the "C" type particle. The specific gravity of this agent is very close to that observed for the feline leukemia virus. Employing immunodiffusion and complement fixation tests we will compare the "C" type particles from leukemic swine with known leukemogenic viruses. We will also attempt to transmit leukemia, with our virus preparations, to newborn immunosuppressed swine.

THE EFFECT OF RADIOSTRONTIUM-INDUCED
LYMPHOID LEUKEMIA FILTRATE ON PORCINE
THYMIC AND SPLENIC CULTURES

Investigators:

M. E. Frazier and J. R. Pratt

Results of a preliminary study (Annual Report, 1968) indicated that there was a factor associated with tissue homogenates from swine with ^{90}Sr -induced lymphocytic leukemia that would increase the growth rate of cultured splenic cells from normal newborn pigs. This was concluded

from experiments comparing the effect of lymphocytic leukemic homogenates (LLH) with homogenates from normal swine. More recent studies indicate that control cultures, with no homogenate present, undergo cell division at least as fast as those with added LLH. This would suggest that there is a factor associated with normal swine tissues that inhibits the cell division of splenic or thymic cultures from newborn pigs. This factor suppressing cell division appears to be diminished or absent in the tissue homogenates of animals afflicted with lymphocytic leukemia (Figure 1). The addition of phytohemagglutinin to the

FIGURE 1. Spleen Cell Culture Indicating Inhibiting Effect of Various Addenda

cell cultures also inhibits cell division. This supports the idea that the mechanism of derepression of cell division in this system is different from that observed with normal leucocyte cultures.

We are currently trying to characterize the factor in normal homogenates that is responsible for suppressing cell division. To do this, we are utilizing homogenates made from individual tissues rather than the mixtures used in the past. Once the best source of this factor is found, attempts will be made to purify and identify the compound.

COMPARATIVE STUDIES ON LONG-TERM CELL CULTURES FROM NORMAL AND LEUKEMIC SWINE

Investigators:

M. E. Frazier and J. R. Pratt

Technical Assistance:

Jean Stearns

Virus-like particles, morphologically similar to viruses causing leukemia in other species, have been observed in electron micrographs of cell lines from leukemic miniature swine that were chronically exposed to ^{90}Sr . These particles have not been found in porcine cells of non-leukemic origin. In an attempt to determine if the "C" type virus par-

ticles affect culture characteristics, long-term cell cultures from "normal" and leukemic swine have been compared.

Stable cell lines from the tissues of three normal and three leukemic animals displayed unusual growth characteristics in vitro. These cells formed multilayered colonies of epithelial-like cells (Figure 1). As these colonies increased in size, the central portions developed foci of aggregated multilayered cells. Only the stabilized porcine embryonic kidney (PEK) cell line formed complete monolayers and displayed contact inhibition. The lack of contact inhibition shown by the other cell lines

FIGURE 1. Stabilized Cell Culture Obtained from Kidney Tissue of a Miniature Pig with Myelogenous Leukemia (These cells grow in multilayered colonies with numerous mitotic figures indicating at least a degree of transformation.)

from both normal and leukemic animals is usually taken as an indication that a cell line is transformed.

Experiments were conducted to determine the percentage of cells capable of growing from a single cell. The plating efficiency of PEK cells was about 10%, that of the other cell lines ranged from 15% to 30%. There were no significant differences in plating efficiencies between cell lines from leukemic and nonleukemic animals.

The ability of a cell to grow and divide in soft agar is usually taken to indicate that the cell has lost contact inhibition and therefore been transformed. A normal cell when placed in semisolid agar will remain a single cell. In our experiments the PEK cells would not grow in soft agar. However, the remaining cell lines, from both normal and leukemic animals, did grow in the agar suspension. Approximately 30% of the cells seeded in these experiments formed colonies in semisolid agar. The viability of the cells forming such clones was confirmed by isolation and transfer into regular growth medium.

Chromosome counts did indicate major differences between normal and leukemic animals with modal counts for "normal" cell lines of 37 chromosomes. The cell lines from leukemic animals had modes ranging from 31 to 33 chromosomes. The cell lines studied were all subdiploid (the normal porcine count is 38) with approx-

imately 1 to 5% of the chromosome exhibiting polyploidy.

Attempts to produce tumors by inoculation of the cells into hamster cheek pouches were unsuccessful. In one experiment, small lesions grossly resembling tumors were present in a hamster inoculated with cells containing the "C" type particles. Histologically, these lesions were granulomatous without any mitotic figures and the center of the lesions were necrotic.

The presence or absence of "C" type particles had no discernible effect on the cultural characteristics of the cells studied. The absence of observable "C" type particles in cells from normal swine may be attributed to a concentration factor. If this is not the case, replication of the virus is not essential for porcine cell cultures to display some of the characteristics of a transformed cell. Either the PEK cells are the only "normal" cell line utilized in these experiments or the observations of lack of contact inhibition typical of porcine tissues. In the latter case, the soft agar procedure cannot be used as a primary criteria of cellular transformation. On the other hand, if loss of contact inhibition is associated with transformation, repression of the oncogenic potential of so-called normal cells could have been altered by in vitro manipulations resulting in the expression of the oncogene without production of virus particles.

INHALATION STUDIES

This project is concerned with determining the consequences of inhaling plutonium and other alpha emitters - the biological responses and the dose relationships. It is concerned with establishing parameters by means of which the hazards of human exposure may be recognized and evaluated. And, it is concerned with the development of effective therapy procedures for removing inhaled radionuclides from the body. A variety of investigations are included in this project, ranging from basic studies of lung biochemistry to life-time studies of retention and toxicity in beagle dogs. No report is included on the long-term toxicity study, now in its twelfth year, because no important changes have occurred since last year's report.

Several major long-term studies of biological effects have developed from this project and are now separately funded. These include: "Low-Level Plutonium Inhalation Studies in Beagles," and "Inhalation Hazards to Uranium Miners - Biological Studies."

COMPUTER SIMULATION OF THE RETENTION
AND TRANSLOCATION OF INHALED $^{239}\text{PuO}_2$
IN BEAGLE DOGS

Investigators:

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

Data are now available on the tissue distribution of plutonium in beagle dogs up to 10 years after single inhalation exposures to aerosols of sub-micron particulates of $^{239}\text{PuO}_2$. The initial translocation model that was fitted to shorter-term data has been extended using hybrid computer facilities. The fractional uptake constants and biological elimination rates were determined by refitting the model to the current data by minimizing the integral of a normal error-squared function to the build-up curve for each organ and for the total body. Figure 1 is a block diagram of the long-term simulation based on the translocation and elimination of insoluble plutonium deposited in the pulmonary lung. The majority of this material is avidly retained and follows a 3-year retention half-time in the alveolar lung. Translocation is principally to the thoracic lymph nodes (85%) and abdominal lymph nodes (5%), with more than half being retained in these lymph nodes indefinitely and 40% released to blood with a half time of 1 year. Figure 2 shows the long-term retention and accumulation of plutonium in the

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FIGURE 1. Long-Term Dynamic Simulation of Inhaled $^{239}\text{PuO}_2$ (Half-times in parentheses)

FIGURE 2. Long-Term Tissue Burdens of Plutonium After Inhalation of $^{239}\text{PuO}_2$

principal tissues. Ten percent of the plutonium initially deposited in the pulmonary lungs remains at 10 years after exposure. Based on the available data, the model predicts that at 15 to 20 years after exposure the tracheobronchial lymph nodes, liver, and skeletal burdens plateau at 50, 20, and 8% of the plutonium initially deposited in the pulmonary lung. Total accumulated absorbed doses at fifteen years in the beagle dog as determined by this model following an initial alveolar deposition of 1 μCi $^{239}\text{PuO}_2$ are ~ 4000 rads, 1 megarad, 600 rads, and 80 rads for the pulmonary lung, tracheobronchial lymph nodes, liver, and skeleton, respectively.

METABOLISM OF INHALED ^{239}Pu CITRATE AND ^{239}Pu NITRATE

Investigators:

J. E. Ballou and J. F. Park

Technical Assistance:

W. G. Morrow

The lung clearance and translocation of inhaled ^{239}Pu was reported earlier for dogs exposed to lethal amounts of $^{239}\text{Pu}(\text{NO}_3)_4$ (Annual Report, 1967). Those animals died due to progressive pulmonary damage so it is questionable

if the results represented normal clearance and translocation kinetics. The present investigation was designed to describe the clearance and translocation of less toxic amounts of ^{239}Pu inhaled as the nitrate or citrate salts.

Twenty-eight beagle dogs were exposed to aerosols dispersed from a Lauterbach generator containing ^{239}Pu in either 0.27N HNO_3 or 0.08M citrate buffer, pH 3.5. The aerosols were generated as droplets with AMAD of 1.45 μm , σ_g 2.75, for the citrate, and 0.91 μm , σ_g 3.56, for the nitrate. The dogs deposited from 1.6 to 11.4 μCi and retained from 0.87 to 8.1 μCi when sacrificed in groups of two at 1, 3, 7, 14, 30, 62, and 100 days postexposure.

Initial deposition and thoracic clearance was estimated by external counting. Long-term clearance from the thorax ($T_{1/2} \sim 120$ days) was the same for the two compounds. Early clearance during the first 6 days was more rapid for the citrate. Later clearance, however, as illustrated by retention in the total animal and lung (Figure 1), was not appreciably different.

Plutonium reached a higher concentration and peaked earlier in the blood of the citrate dogs (Figure 2). Sixty-two days after exposure, the concentration in blood approached a value of 3×10^{-4} percent of body burden/ml for both groups (Figure 3). Earlier values determined by Bair using higher levels of $^{239}\text{Pu}(\text{NO}_3)_4$ agree well with this study.

FIGURE 1. Total Body and Lung Retention of Inhaled Pu Citrate and Pu Nitrate

FIGURE 2. Early Buildup of ^{239}Pu in Blood After Inhalation of ^{239}Pu Citrate or ^{239}Pu Nitrate

FIGURE 3. Retention of ^{239}Pu in Blood After Inhalation of ^{239}Pu Citrate or ^{239}Pu Nitrate

Plutonium-239 was detected in all of the 40 individual tissues analyzed for each dog. The tissues with highest ^{239}Pu concentration are shown in Tables 1 and 2. The concentration in lung exceeded that in all other tissues except tracheobronchial lymph nodes. Data are not yet complete for animals sacrificed after 100 days or for hematological and histopathological studies, which will permit an evaluation of the biological effects in these animals.

Inhaled ^{239}Pu citrate was cleared from the lung and translocated to the skeleton and liver more rapidly

than inhaled ^{239}Pu nitrate. After 2 weeks the differences in early lung clearance were no longer apparent and the distribution of plutonium in lung, skeleton, and liver was about the same for both compounds. The plutonium distribution was qualitatively similar to that seen in earlier studies with acutely toxic ^{239}Pu nitrate doses except for more rapid mobilization from the lung to the skeleton in the low-level studies. This difference in pulmonary clearance may be due to radiation effects in the acute toxicity studies.

TABLE 1. Concentration of ^{239}Pu in Tissues After Inhalation of Citrate, as Percent of Concentration in Lung

Tissue	Days Postexposure					
	1	3	7	14	30	62
Lung	100	100	100	100	100	100
Skeleton	0.74	5.3	7.1	6.0	14	30
Liver	0.46	4.9	2.9	10	4.0	21
Tracheobronchial Lymph Nodes	11	28	29	68	91	158
Turbinates	10	23	38	21	18	18

TABLE 2. Concentration of ^{239}Pu in Tissues After Inhalation of Nitrate, as Percent of Concentration in Lung

Tissue	Days Postexposure					
	1	3	7	14	30	62
Lung	100	100	100	100	100	100
Skeleton	0.15	0.60	2.1	4.3	20	17
Liver	0.08	0.35	1.3	9.1	11	10
Tracheobronchial Lymph Nodes	10	13	22	72	127	113
Turbinates	9.2	7.0	3.7	15	43	38

IN VITRO STUDY OF CANINE LUNG TUMORS

Investigator:

M. E. Frazier

Technical Assistance:

J. R. Pratt

Canine lung tumors were grown in culture to determine the feasibility of employing in vitro methods to investigate the nature of the host/tumor relationship.

Cytological and histochemical stains were used to help identify the nature of inclusion or secretory activity. Stained preparations revealed two main cell types; large fibroblast-like cells with normal cytoplasmic nuclear ratios and a few vacuoles (Figure 1), and cells similar to those observed in lung washings (Figure 2). The latter were usually clumped and stained darkly; they were PAS positive indicating an increased mucoprotein or lipid content. Vacuoles showed some PAS activity. The stains specific for nucleic acids (Feulgen, acridine orange,

120X

FIGURE 1. Primary Cell Culture from Lung of a Beagle Dog with Bronchiolar Adenocarcinoma [Arrows indicate cells with vacuoles. (10-day culture hematoxylin eosin)]

120X

FIGURE 2. Primary Cell Culture from Lung of a Beagle Dog with Bronchiolar Adenocarcinoma [Fibroblast-like cells are interspersed with cells that appear to be of tumor origin. Tumor cells are indicated by arrows. (10-day culture hematoxylin eosin)]

and methyl green pyronin Y) revealed no inclusion bodies or unusual areas of nucleic acid activity. Phase contrast microscopy also showed two main cell types during the first few weeks. After 40 to 60 days, small fibroblast-like cells usually take over, even if the cells are not cloned. Cytoplasmic vacuoles tend to disappear about the same time.

The ability of the tumor cells to grow and divide was compared to normal embryonic dog lung cell cultures. The cloning or plating efficiency of the normal cells was approximately 0.5%. About 60% of the lung cells from dogs with tumors were capable of producing clones of cells under the same conditions (Table 1). A high efficiency of clone formation from diluted cell suspensions is considered to be one criteria of a tumor cell.

The cultured tumor cells were able to continue growth and cell division

in a semisolid agar medium (Table 2). This, together with phase microscopy observations, indicated that the cells lacked normal contact inhibitory mechanisms, fulfilling another criteria for tumor cells.

Animal inoculation studies remain incomplete. Approximately 10^6 tumor cells were injected into hamsters treated at 3-day intervals with cortisone to suppress the immune response. Nodules resulted that increased in size from approximately 1 mm to 7-10 mm during a 24-day period, after which time they began to regress. In all cases, cytotoxic antibodies were present in the animals with regressed tumors. Future transmission studies will utilize X-irradiated animals injected with min-u-sil (a ground glass preparation), which should result in more nearly complete suppression of the immune response.

TABLE 1. Plating Efficiency of Lung Cultures from Beagle Dogs

Dog Number and Diagnosis	Number of Cells/ 0.1 ml added	Plating Efficiency ^(a)		
		0.1 ml/plate	0.2 ml/plate	0.3 ml/plate
Dog 273 - Bronchiolar Adenocarcinoma	46	0.60		0.68
Dog 278 - Mixed-Cell Carcinoma	27	0.49		0.57
Control Dog 1	40	0.005	0.004	0.004
Control Dog 2	26	0.002	0.004	0.003

(a) Plating efficiency is the number of cells that grew into colonies containing more than 20 cells during the 10 days of the experiment divided by the number of cells added per plate.

TABLE 2. Growth of Lung Cultures from Beagle Dogs
in Semisolid Medium

<u>Dog Number and Diagnosis</u>	<u>Percent of Cells Capable of Dividing and Producing Multicellular Colonies in Semisolid Agar(a)</u>
278 - Mixed Cell Carcinoma	55
273 - Bronchiolar Adenocarcinoma	70
CD1 - Normal	0
CD2 - Normal	0

(a) Represent averages of experiments with 25, 50, 75, 100, and 500 cells per culture dish. The values for each dilution were based on ten culture dishes at each cell dilution level.

A preliminary experiment using the indirect fluorescent antibody test indicated that there were antibodies to the tumor present in homologous dog serum. It is not clear whether the antibody is complete or only the blocking type.

These experiments have shown that the tumor cells can be grown and

studied in vitro, and that there is a host response to the tumor. Attempts are currently underway to grow tumor cells from lung washings. If this is possible, it will allow a variety of nondestructive experiments dealing with the nature of the hosts immune reaction to the tumor.

CARCINOGENESIS FROM INTRAPERITONEALLY
DEPOSITED PuO₂ PARTICLES

Investigators:

C. L. Sanders and Thelma A. Jackson

Technical Assistance:

F. T. Faubert and G. J. Powers

A study is in progress involving about 500 rats, with the objective of determining the carcinogenic response to PuO₂ administered by inhalation or intraperitoneal injection. The results reported here are concerned with two of the groups of rats given an intraperitoneal injection of about 2.5 μ Ci ²³⁸PuO₂ or

2.5 μ Ci ²³⁹PuO₂ particles. The ²³⁸PuO₂ particle size was about 0.02 μ m count median diameter and for ²³⁹PuO₂ about 0.12 μ m (Figures 1 and 2). From 30-51% of PuO₂ was retained in the omentum. Approximately 50% of the smaller ²³⁸Pu was translocated to the skeleton; about 2% of the ²³⁹Pu was found in the skeleton. A significant amount of PuO₂ was also cleared into mediastinal lymph nodes and lymphatics. Both groups treated with PuO₂ exhibited a moderate to severe anemia and lymphopenia, and decreased body weights compared to control rats. Of the 108 control rats (72 given an intraperitoneal injection of saline and 36 untreated), 14 have been

FIGURE 1. Cumulative Tumor Incidence in Rats Following Intraperitoneal Administration of 2.5 μ Ci ²³⁹PuO₂ (CMD, 0.12 μ m)

FIGURE 2. Cumulative Tumor Incidence in Rats Following Intraperitoneal Administration of 2.5 µCi $^{238}\text{PuO}_2$ (CMD, 0.02 µm)

autopsied up to 520 days after start of the study; 11 of these exhibited tumors, all of mammary origin.

Mammary carcinomas were predominant in animals given $^{238}\text{PuO}_2$; sarcomas accounted for 9% of the tumors with one bile duct carcinoma and one melanoma also being observed in this group. In animals given $^{239}\text{PuO}_2$, the mesenchymal tumors predominated with mesotheliomas accounting for 30% and sarcomas 36% of tumors; 2 osteogenic sarcomas were also observed in this group. Mammary carcinomas arose earlier following $^{238}\text{PuO}_2$ than $^{239}\text{PuO}_2$. No difference in mammary fibroadenoma incidence was detected. The median induction time for all tumors following $^{238}\text{PuO}_2$ was about 300 days and about 360 days

for $^{239}\text{PuO}_2$. The abdominal sarcomas and mesotheliomas were characterized by production of a large volume of bloody ascites fluid and a severe anemia. These tumors mostly arose around small fibrotic nodules containing most of the omental PuO_2 contents and spread by implantation and infiltration throughout the abdominal cavity. Further studies will be required to determine if carcinogenic differences observed between $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ were due to particle size or to specific activity. The study does demonstrate the ability of PuO_2 to elicit a wide variety of tumors, particularly those arising from mesothelial cells and other mesenchymal tissues. Induction of mesenchymal tumors with PuO_2

appears to result from "hot spots" of high plutonium activity, whereas mammary tumor formation may be more indirectly related to plutonium-induced hormonal imbalance.

DISTRIBUTION OF INHALED METAL OXIDES IN PULMONARY ALVEOLI

Investigators:

*C. L. Sanders, R. R. Adee,
Thelma A. Jackson, G. J. Powers,
and A. P. Wehner*

The phagocytosis of inhaled $^{239}\text{PuO}_2$ by alveolar macrophages and Type I alveolar epithelium is described elsewhere in this report. The present studies were undertaken to compare the distribution in pulmonary alveoli of inhaled nonradioactive metal oxides. Hamsters were exposed for 60 days, 4 hr per day, to an aerosol of NiO,

or for a single 4-hr exposure to a Cr_2O_3 aerosol (Table 1).

The count median diameters of the two oxides in the alveoli ranged from 0.13 to 0.17 μm . No differences in particle size were found among the cellular locations within the same animal. Macrophages contained 80-90% of deposited particles. A larger amount of NiO was free in the lumens and less was present in Type I epithelium than was the case for Cr_2O_3 (Table 1). Differences in particle distribution may be due to the different duration of exposure as well as to physico-chemical differences between particles. Although quantitatively different, the distribution of the non-radioactive oxides was qualitatively similar to that seen for PuO_2 of comparable particle size.

Neither PuO_2 , NiO, or Cr_2O_3 was found in Type II alveolar epithelium (granular pneumonocytes). This observation supports the view that lamellar inclusions within granular pneumonocytes, thought to be responsible for surfactant production, have an intracellular origin, and are not formed by phagocytosis of surface active material present in the alveolar lumens.

TABLE 1. Distribution of Deposited Particles in Hamsters Exposed 60 days, 4 hr per day to NiO Aerosol and 4 hr to Cr_2O_3 Aerosol

Aerosol	Dust Concentration in Chamber, $\mu\text{g}/\ell$	Amount of Dust Deposited in Lung, mg	Distribution of Deposited Particles, Percent (mean $\pm$ standard error)		
			Macrophages	Lumens	Type I Epithelium
NiO	60	10.2	80.0 $\pm$ 5.4	19.5 $\pm$ 5.5	0.5 $\pm$ 0.2
Cr_2O_3	~ 750	0.6	91.2 $\pm$ 1.6	4.3 $\pm$ 0.7	4.2 $\pm$ 0.8

REMOVAL OF PLUTONIUM AND MACROPHAGES BY LUNG WASHINGS

Investigators:

C. L. Sanders and Thelma A. Jackson

Inhaled PuO_2 particles are phagocytized and concentrated within alveolar macrophages. Significant amounts of lung plutonium within macrophages were removed in earlier studies by saline lung washings (Annual Report, 1968). Other than in macrophages, inhaled PuO_2 particles deposited in the alveoli have been found only in Type I alveolar epithelium.

Female rats were exposed to an aerosol of $^{239}\text{PuO}_2$ particles with an estimated deep lung deposition of about 30 nanocuries. Their lungs were washed in situ, with 19-10 ml portions of saline, following cannulation of the trachea. The number of alveolar macrophages and the amount of plutonium removed in each wash were determined. Multiple washings of the lung were found to yield statistically more reliable data than a single washing. Results are summarized in Figures 1 and 2.

Nanocurie amounts of plutonium appear to exhibit a significant cytotoxic effect on macrophages as evidenced by their reduced numbers in washings at 150 days; studies up to 250 days show a continuing decline in macrophage numbers (Figure 1). The subsequent biological effect may be manifested in a depressed bacteri-

cidal action in the lung and increased susceptibility to pulmonary infections. No age related depression of alveolar macrophage numbers was seen in non-exposed animals.

Pulmonary washing is an effective method for removal of large amounts of inhaled $^{239}\text{PuO}_2$, when performed during the first month following exposure, but is relatively ineffective when performed after 5 months. The residence half-time for PuO_2 in macrophages appears to be less than 60 days.

FIGURE 1. Influence of Inhaled $^{239}\text{PuO}_2$ Particles on the Removal of Alveolar Macrophages by Serial Pulmonary Washings (Time represents the period following exposure and n is the number of animals.)

FIGURE 2. Removal of Inhaled $^{239}\text{PuO}_2$ by Serial Pulmonary Washings (Time represents the period following exposure and n is the number of animals.)

METABOLISM OF ^{145}Sm AND ^{147}Pm IN RATS

Investigator:

J. E. Ballou

Technical Assistance:

W. E. Morrow

Samarium has been selected as a non-radioactive substitute for promethium in exploratory studies of promethium inhalation in man. Preliminary studies were therefore initiated to establish the metabolic equivalence of samarium and promethium in rats.

Animals were injected intravenously with ^{145}Sm or ^{147}Pm chloride solutions in citrate buffer, pH 4.5. They were sacrificed nine animals for each radionuclide after 14 days. During this time 40 to 47% of the injected dose was excreted, mainly in the feces, and about 50% was retained, mostly in the liver and skeleton. Differences in retention and excretion of the two radionuclides were demonstrated at the levels of significance shown in Table 1.

These very preliminary results suggest that samarium may be deposited more in liver and less in bone than is promethium. These differences noted are small and could be due to minor physical-chemical variation in the injectant solutions. Further studies are in progress to compare the clearance and translocation kinetics of inhaled oxides of samarium and promethium.

TABLE 1. Disposition of ^{145}Sm and ^{147}Pm 14 Days After Intravenous Injection as Citrates in Rats

	Percent of Injected Dose ^(a)		
	^{145}Sm	^{147}Pm	Significance of Difference
Liver	16.6	13.3	P < 0.05
Femur	1.06	1.41	P < 0.01
Carcass residue	31.8	34.9	
Urine ^(b)	7.5	6.7	
Feces ^(b)	32.1	40.7	P < 0.01

(a) ^{145}Sm dose, 2.48 μCi , ^{147}Pm dose, 3.89 μCi .

(b) 14 day accumulation

CHOLINE INCORPORATION INTO LECITHIN

Investigator:

E. G. Tombropoulos

Technical Assistance:

A. Jacqueline Clary and J. G. Hadley

We reported previously that the incorporation of (Me- ^{14}C) choline into lecithin by lung mitochondrial and microsomal preparation was stimulated by Ca^{++} , and cytidine triphosphate (CTP), and that the Ca^{++} , and CTP stimulation was additive. Since the so called CDP-choline pathway is known to be inhibited by Ca^{++} , these data suggested that we had uncovered a second pathway for the incorporation of choline into lecithin. Additional support for this conclusion was obtained by measuring the effect of Ca^{++} on the rates of incorporation of choline and phosphorylcholine (an intermediate in the synthesis of lecithin from choline). The rates of incorporation of methyl-labeled choline

into lecithin in the presence of Ca^{++} were approximately four times higher than in its absence. This trend was completely reversed when phosphorylcholine was used as the methyl donor. Ca^{++} -stimulated choline incorporation was tenfold higher than the rate of incorporation via the CDP-choline pathway.

These results tended to confirm our original conclusion that there were two pathways for the methylation of lecithin. They also suggested that the major route for lecithin synthesis by mitochondrial and microsomal lung preparations was not the classical CDP-choline pathway.

Diglycerides are the acceptors of phosphorylcholine (which, in turn is derived principally from CDP-choline) in the de novo synthesis of dipalmitoyl lecithin (DPL). Using this information, it was possible to make a direct comparison of the relative contributions of exchange and CDP-pathways to the synthesis of DPL. The results of such an experiment are shown in Table 1. When dipalmitate was added to the incubation medium, the rate of choline incorporation was stimulated. The addition of lecithin, however, caused a

TABLE 1. Factors Affecting the Incorporation of ^{14}C from (1, 2- ^{14}C) Choline into Lecithin (nmoles/mg protein)

Addition	Subcellular Fraction	
	Mitochondrial	Microsomal
None	0.20	0.05
Dipalmitin	0.31	0.21
Dipalmitoyl-Lecithin	0.75	0.75
CTP	1.19	0.66
CTP + Dipalmitin	0.68	0.29
CTP + Dipalmitoyl-Lecithin	1.3	0.90

four- to fifteen-fold increase in choline incorporation, suggesting that exchange was occurring at a rate 4-15 times higher than de novo synthesis.

In conclusion, both particulate

fractions of the lung incorporate choline into lecithin by the CDP-choline pathway and by reactions which do not conform to parameters established for the CDP-choline pathway.

LOW LEVEL PLUTONIUM INHALATION STUDIES IN BEAGLES

This project is concerned with a specific experiment to determine the long-term effects in beagle dogs of low-level inhalation exposure to plutonium dioxide. The experiment will extend previous and continuing studies in our laboratory on the effects of higher level exposures. The initiation of the project is proceeding as outlined in last year's report, with no results to justify a report this year.

INHALATION HAZARDS TO URANIUM MINERS - BIOLOGICAL STUDIES

This project will determine the biological effects of daily exposures to controlled levels of potentially carcinogenic uranium mine air contaminants in large and small experimental animals. The continuing experiments involve exposure of hamsters and dogs to radon daughters and uranium ore dust. A closely related project, supported by the National Institute for Environmental Health Sciences, HEW, supplements these studies to include exposures to diesel exhaust fumes and cigarette smoke. The HEW and AEC projects were integrated into one experimental design, at the request of the funding agencies, and the results of the combined projects are described in this report. A closely related project, "Uranium Miners Exfoliative Pathology," compares lung washing samples from dogs exposed to laboratory-produced uranium mine atmospheres with similar samples from uranium miners.

LIFESPAN EXPOSURES OF HAMSTERS TO URANIUM MINE AIR CONTAMINANTS

Investigators:

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Technical Assistance:

H. G. Steele, J. C. Gaven,
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The initial hamster studies are nearly completed in which 102 animals per group inhaled for 6 hours daily one of the following: 30 WL of radon daughters, 600 WL of radon daughters, 600 WL of radon daughters with 15 mg/m³ uranium ore dust, or laboratory air (controls). These exposures continued over a period of >20 months in lifespan studies; during this time a variety of lung pathology has developed. Diffuse vesicular emphysema was found in several hamsters of the 600 WL and 600 WL with uranium ore groups. Inflammatory cell and fibrin exudation was found in animals of the 600 WL group sacrificed at 8 to 10 months of exposure. Similar but more severe exudation occurred in the 600 WL with uranium ore group animals at 2 to 5 months and 10 to 13 months of exposure with parallel fibrosis and epithelial changes. Both groups showed macrophage proliferation paralleling fibrosis and hyalinization, and occasional hyperplasia or metaplasia of the bronchiolar epithelium. In the

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PUBLICATIONS: INHALATION HAZARDS TO URANIUM MINERS - BIOLOGICAL STUDIES

BAIR, W. J. "Inhalation of Radionuclides and Carcinogenesis," pp. 77-101. In: M. G. Hanna, Jr., P. Nettesheim and J. R. Gilbert (eds.), *Inhalation Carcinogenesis*. CONF-69001. CFSTI, Springfield, Va. 1970.

STUART, B. O., D. H. WILLARD, and E. B. HOWARD. "Uranium Mine Air Contaminants in Dogs and Hamsters," pp. 413-427. In: M. G. Hanna, Jr., P. Nettesheim and J. R. Gilbert (eds.), *Inhalation Carcinogenesis*. CONF-691001. CFSTI, Springfield, Va. 1970.

600 WL with uranium ore group these changes were more frequent and severe, with epithelial hyperplasia of the bronchioli, and alveolar septal adenomatous lesions with scattered anaplastic cells and metaplasia of bronchiolar epithelium in animals showing advanced fibrosis after about one year of exposure (Figure 1). Emphysema, fibrosis, hyalinization, and

FIGURE 1. Lung of a Hamster Exposed to 600 WL with Uranium Ore Dust for 16 Months (1. Area of adenomatoid proliferation of alveolar epithelium. Anaplastic changes of the proliferating epithelium can be found in occasional cells.)

epithelial alterations were not present in control animals. Mortality, body weight, and hematology in these exposure groups show no significant difference from controls. Radiochemical analyses for long-lived alpha emitters in the lungs of hamsters inhaling 600 WL with carnotite uranium ore revealed nonequilibrium ratios for $^{230}\text{Th}/^{234}\text{U}$ of 1.2 to 2.0.

Sampling instrumentation was fabricated to measure the fractional attachment of radon daughters to condensation nuclei or larger particles, because this parameter may determine the pulmonary deposition site and absorbed dose. Percent unattached radon daughters ranged from <5% in chambers with uranium ore dust to 20 to 40% without ore dust; corresponding condensation nuclei counts were 3000 to 20,000 and 32,000 to 110,000/cm³, respectively.

Daily lifespan exposures of six new groups of hamsters were begun in the fall of 1970, as outlined in Table 1. The radon daughter levels were increased to 1200 WL and a group exposed to uranium ore dust alone was included to define the contribution to lung pathology of the carnotite dust. Two additional groups (supported by HEW) are exposed to another potentially carcinogenic uranium mine contaminant, diesel engine exhaust, with and without 1200 WL of radon daughters plus ore dust.

A three-cylinder diesel engine of the type used in uranium mines produces exhaust during a continuous cycling program in which engine speed and load are varied to simulate mine operating conditions. Figure 2 shows the parallel chamber diesel exhaust exposure systems. The exhaust gases are automatically diluted with air to maintain

TABLE 1. Experimental Design for Hamster Studies

Group	Number of Animals	Inhalation Exposure
1	102	Controls (laboratory air)
2	102	1200 WL radon daughters
3	102	1200 WL radon daughters with uranium ore dust (carnotite; 15 mg/m ³)
4	102	Uranium ore dust (carnotite; 15 mg/m ³)
5	102	Diesel engine exhaust
6	102	Diesel engine exhaust plus 1200 WL radon daughters with uranium ore dust

50 ppm CO in response to an infrared photometer CO monitoring and control system.

FIGURE 2. Diesel Engine Exhaust Dilution System for Inhalation Exposure of Hamsters (Exhaust passes from a surge tank through an orifice meter; individually controlled diluted exhaust fractions are introduced at the top of each chamber.)

CHRONIC EXPOSURE OF BEAGLE DOGS TO URANIUM MINE AIR CONTAMINANTS AND/OR CIGARETTE SMOKE

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Technical Assistance:

*J. C. Gaven, W. Skinner, S. W. Hall,
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V. Daniels, Jr.*

Sixty-nine beagle dogs are continuing to receive daily inhalation expo-

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sure in three groups of 20 each to 600 WL of radon daughters with 15 mg/m³ uranium ore dust, to the same levels of radon daughters and ore dust with concurrent smoking of ten cigarettes over a 16-hour period each day, and to cigarette smoking only; the fourth group consists of nine control dogs that receive sham smoking only. These animals have received 22 months of exposures to date. Routine examinations of all dogs include physical examination, thoracic radiography, hematology, and clinical chemistry. No changes significantly different from controls have appeared in respiratory rates or volumes, tidal volumes, hematology and clinical chemistry. After 15 months of exposures, small focal densities have appeared in the lungs of two dogs in each of the three exposure groups; no significant changes have appeared in the radiographs of the control dogs. One dog from each group was sacrificed 6 months after beginning exposures. The most marked changes occurred in the lungs of the dog exposed to 600 WL radon daughters with uranium ore. Bronchial and bronchiolar epithelium showed both hyperplasia and metaplasia. Metaplastic lesions were scattered and focal, tending to involve smaller airways. Figure 1 shows bronchiolar epithelial hyperplasia and metaplasia, with moderately severe atypia of epithelial cells. Vesicular emphysema and fibrosis of alveolar septae were diffusely distributed throughout the lung. Figure 2 shows an example of metaplastic epithelium overlying a fibrotic nodule at the periphery of the lung. Perivascular and peribronchiolar accumulations of macrophages and lymphocytes

FIGURE 1. Bronchiolar Epithelial Hyperplasia and Metaplasia in a Dog Exposed to 600 WL Radon Daughters with Uranium Ore Dust; Note Loss of Normal Polarity of Cells

FIGURE 2. Metastatic Epithelium Overlying a Fibrotic Nodule in the Lungs of a Dog Exposed to 600 WL Radon Daughters with Uranium Ore Dust

were present throughout the lungs, and involved nearly all of the smaller bronchioles and vessels. The epithelium of the involved bronchioles frequently showed proliferation. The

lungs of the dog exposed to both 600 WL radon daughters with ore dust and daily cigarette smoking showed changes very similar to those described above. The lungs of the animal exposed to cigarette smoke only did not exhibit diffuse fibrosis. Peribronchiolar and perivascular lesions were much less severe than those described above; epithelial alterations appeared to be in the early stages of development. No significant lesions were found in the control dogs.

Two of the four dogs in a previous study that received daily inhalation exposures to a pitchblend uranium ore alone for 6 months were sacrificed 2 years later. The lungs of one of these dogs contained consolidated nodules of a papillary structure, consisting of fibrous tissue inner stroma covered with anaplastic bronchial epithelium (Figure 3). This anaplasia and pleomorphism with no relationship to the basement membrane is classified

FIGURE 3. Focal Areas of Lesion from Lung of Dog Exposed by Inhalation to Pitchblend Ore, Showing Infiltration of the Supporting Stroma by Cells Showing Varying Degrees of Anaplasia and Large Bizarre Nuclei

as a bronchiolo-alveolar carcinoma. Radiochemical analyses for long-lived alpha-emitting radionuclides in tissues from these two dogs showed lung and tracheobronchial lymph node ratios of $^{230}\text{Th}/^{234}\text{U}$ ranging from 2 to 13 and

2 to 20, respectively. $^{230}\text{Th}/^{234}\text{U}$ ratios in the skeleton, liver, kidney, and spleen ranged from 8 to 18. ^{210}Po and ^{210}Pb levels were greater than ^{234}U levels in all tissues.

URANIUM MINERS EXFOLIATIVE LUNG CYTOLOGY

Investigator:

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Technical Assistance:

K. E. McDonald

Various techniques for obtaining representative cellular material from the lungs of live dogs are being tested. These methods include intra-bronchial injection and retrieval of saline. Methods involving multiple injection of large volumes (100-200 ml) appear to yield the greatest number of cells. Comparisons are being made to determine the extent to which actual lung histology is reflected in the materials obtained by washing.

Cell populations obtained by washing lungs from normal dogs consist primarily of macrophages with a few neutrophils, and cellular debris. Washings from a dog with an advanced bronchiolo-alveolar cell carcinoma bore large numbers of macrophages, neutrophils and cellular debris. Malignant cells appeared in clumps or singly, but were not abundant. A likely sequence followed by tumor cells destined to appear in a bronchial washing is illustrated by Figures 1-3. In Figure 1, single and

clumps of cells appear to be detaching from the tumor mass. Similar cells appear in the lumen of a small bronchus (Figure 2) and in bronchial washings (Figure 3).

FIGURE 1. Bronchiolar Alveolar Cell Carcinoma of Beagle Induced with Plutonium [Cells appear to be detaching from tumor mass. (Hematoxylin eosin)]

FIGURE 2. Small Bronchus Some Distance from Area Shown in Figure 1. [Cellular exudate and malignant cells are present in lumen (Hematoxylin eosin)]

FIGURE 3. Malignant Cells Present in Bronchial Wash Sediment (Note similarity to cells in Figures 1 and 2.)

SPACE NUCLEAR SYSTEM STUDIES

Space vehicles containing nuclear reactors or radionuclide power sources present special hazards in case of early abort or upon return from orbit. These problems are principally concerned with the biological effects of intensely radioactive small insoluble particles. We have studied and previously reported on the deposition and retention kinetics of inhaled particles, and are retaining a number of dogs for observation of long-term effects. No important results were obtained during 1970.

REMOVAL OF INTERNAL EMITTERS

This project is concerned with the development of methods which will hasten the removal, prevent the deposition, or otherwise decrease the damage potential from ingested or absorbed radionuclides. Associated with this effort is the development of information on the absorption and tissue distribution of radionuclides to help in understanding and predicting their behavior, and to serve as a basis for recommendations for the treatment of exposed persons. Studies relating to the removal from the lung of inhaled radionuclides are covered in the Inhalation Studies project.

THE EFFECT OF DTPA DOSE ON PLUTONIUM
REMOVAL FROM RATS

Investigator:

V. H. Smith

Technical Assistance:

Michael L. Smith

PUBLICATIONS: REMOVAL OF INTERNAL
EMITTERS

SMITH, V. H., R. L. AMSTER, and
J. M. THOMAS. "Biological Disposi-
tion of Promethium After Intravenous
Intramuscular and Subcutaneous Ad-
ministration to Swine." *Radiation
Res.*, vol. 43, p. 249. 1970.
(Abstract)

It has been suggested that the dose level of diethylenetriaminepentaacetic acid (DTPA) commonly used in treating humans might be reduced without appreciable loss in effectiveness of plutonium removal and with considerable decrease in the risk of undesirable

side effects. Results of giving various levels of Ca- or Zn-DTPA to rats, 1 hr after the intravenous administration of plutonium citrate (prompt treatment), are shown in Figure 1.

FIGURE 1. Removal of Intravenously Injected Plutonium Citrate from Rats as a Function of Prompt Treatment with Various Levels of Chelating Agent [Each point represents the average of data from ten rats.)

While single doses >0.5 mmol/kg of Ca-DTPA and >1 mmol/kg of Zn-DTPA are relatively equivalent in their ability to remove plutonium from bone and liver, lower doses are much less effective in removing plutonium from either liver or bone.

Starting on the sixth day after plutonium administration (delayed treatment) and giving twelve doses of DTPA over a 6-week period produced the results shown in Figure 2. Almost maximally effective removal of plutonium from bone is achieved at treatment levels as low as 0.01 mmol/kg, for either Zn- or Ca-DTPA; however, higher dose levels are more effective in removing plutonium from the liver. A dose of 0.03 mmol/kg is commonly used in man.

Our data suggest that in DTPA treatment for removal of monomeric plutonium the dose should be as high as can be tolerated in the prompt treatment

situation, and should not be decreased below the presently used level in the delayed multiple treatment situation. The reason for the latter recommendation is the greater efficiency of larger DTPA doses to remove plutonium from the soft tissues. The desirability of maintaining relatively high treatment levels is a strong argument for the use of the less toxic Zn-DTPA in preference to Ca-DTPA.

REMOVAL OF PLUTONIUM, CERIUM, AND ZINC FROM RATS BY TREATMENT WITH Ca- AND Zn-DTPA

Investigator:

V. H. Smith

Technical Assistance:

R. D. Castro and Michael L. Smith

FIGURE 2. Removal of Intravenously Injected Plutonium Citrate from Rats as a Function of Delayed Treatment with Various Levels of Chelating Agent (Each point represents the average of data from ten rats.)

The zinc salt of diethylene-triaminepentaacetic acid (DTPA) was shown by Catsch [Experientia 21, 210 (1965)] to be 10 to 30 times less toxic than the calcium salt, which is currently used for removal of plutonium from humans. We have also found Zn-DTPA to be less toxic than Ca-DTPA when administered chronically to immature rats (Annual Report, 1969). If the zinc salt were as efficient for the removal of radionuclides as is Ca-DTPA, then its greater margin of safety would make it a preferable therapeutic agent.

Figure 1 shows the results of an experiment comparing the effectiveness of Zn-DTPA at various treatment levels for removal of plutonium, cerium, and zinc from the liver and bone of rats. A single intraperitoneal treatment was administered 1 hr (prompt treatment) after intravenous injection of $9.6 \mu\text{Ci } ^{238}\text{Pu}$ + $2.9 \mu\text{Ci } ^{144}\text{Ce}$ - ^{144}Pr + $1.1 \mu\text{Ci } ^{65}\text{Zn}$ in a pH 4.6 citrate buffer solution. Animals were sacrificed at 7 days. The Zn-DTPA had little effect below concentrations of about 0.1 mmol/kg; little additional effect was obtained with concentrations above 1 mmol/kg.

Particularly, in the case of zinc removal, there appears to be an exponential response to Zn-DTPA concentration which is reasonable as the removal of zinc-65 occurs by isotopic exchange.

Comparative studies with Zn-DTPA and Ca-DTPA showed them to be about equally effective in removing plutonium or cerium when administered promptly at levels above 1 mmol/kg. At lower doses, Ca-DTPA was more effective in removing plutonium and cerium from the liver. This effect is shown in Figure 2 which summarizes

FIGURE 1. Effect of Prompt Treatment Level of Zn-DTPA on the Removal of ^{238}Pu , ^{144}Ce , and ^{65}Zn from Rats

an experiment in which the DTPA concentration was kept at 0.5 mmol/kg while the associated metal moiety was varied from 100% zinc to 100% calcium. The removal of plutonium and cerium from bone was not significantly influenced by the choice of metal. Removal of zinc-65 was very dependent upon isotopic exchange, which was a much more efficient process than elemental exchange for the calcium in Ca-DTPA. If it were not for the former property, then one would expect greater removal by Ca-DTPA since it has a lower stability constant than the zinc salt, and this appears to be reflected in the soft tissue results for plutonium and cerium retention. The effect of calcium in increasing removal from liver seems to occur almost maximally with as little as 0.05 mmol/kg.

When treatment was delayed, Zn-DTPA was found to be as effective as Ca-DTPA at all dose levels. Figure 3 shows the results on removal of plutonium, cerium, and zinc from bone and liver of rats given 12 spaced treatments over a 6-week period with various doses of Zn- or Ca-DTPA commencing 6 days after radionuclide administration. Results for kidneys, spleen, and muscle were qualitatively similar to those shown for liver.

From these data we conclude that Zn-DTPA, because of its lower toxicity and generally equivalent removal capability, should be favored over Ca-DTPA for the treatment of humans accidentally contaminated with plutonium or other heavy metal radionuclides.

FIGURE 2. Comparative Effects of Zinc and Calcium and Mixtures thereof on the Effectiveness of DTPA (0.5 mmol/kg) in Removal of Deposited ²³⁸Pu, ¹⁴⁴Ce, and ⁶⁵Zn from Rats

FIGURE 3. Effect of Delayed Treatment with Various Levels of Zn- or Ca-DTPA on the Removal of Deposited ^{238}Pu , ^{144}Ce , and ^{65}Zn from Rats.

ORAL THERAPY WITH CHELATING AGENTS

Investigator:

V. H. Smith

Technical Assistance:

Michael L. Smith

An orally effective chelate for removal of internally deposited plutonium and other radionuclides would be highly desirable from medical management and health physics points of

view. The absorption of diethylenetriaminepentaacetic acid (DTPA) and similar polyaminopolycarboxylic acids from the gut is too little for reasonable therapeutic effectiveness unless massive doses are given. Such massive doses are contraindicated because of toxicity to the gut. It was thought that the action of aspirin on the stomach might enhance the absorption of DTPA and result in effective oral chelate treatment at a safe dose level.

Female rats were given 5 μCi ^{238}Pu intravenously as the pH 4.6 citrate.

FIGURE 1. Effect of Various Oral Treatments on the Decorporation of ^{144}Ce from Rats as Measured by Total Body Counting (Intraperitoneally administered Ca-DTPA at 1.2 mmol/kg level included for comparison purposes; other chelates given per os at 3.0 mmol/kg and aspirin at 5.5 mmol/kg. Each point represents the average of eight rats except for the aspirin + Ca-DTPA group where only one rat survived beyond the fourth day.)

Rats given 200 mg of aspirin suspended in gum acacia, by gavage, 1 hr, 1, 5, 13, 17, and 20 days after the radionuclide, excreted 18% of the plutonium in the feces and 5% in the urine. When 0.9 mmol of Zn-DTPA at pH 4 was given in similar series of oral treatments, 9% of the plutonium was excreted in the feces and 57% in the urine. Combined treatment eliminated 18% in the feces and 65% in the urine. The aspirin, therefore, seemed to have an effect of its own on fecal excretion of plutonium, aside from any enhancement of the effect of DTPA.

A similar experiment was performed, involving injection of 10 μCi $^{144}\text{CeCl}_3$, pH 3.0, for counting convenience, and

including Ca-DTPA and Mn-DTPA in the treatments. Results of total body counts are shown in Figure 1. Aspirin alone had no effect on retention of cerium-144; the oral DTPA salts were about equally effective. The combination of Ca-DTPA with aspirin proved unexpectedly toxic with only one of eight rats surviving the first two treatments. The rats receiving Mn- or Ca-DTPA were distressed also by the treatments suggesting that some impurity in the DTPA used for the first two treatments may have been responsible.

The use of aspirin to augment oral chelate treatment does not appear to offer particular promise.

FINE STRUCTURAL EFFECTS OF TEMPERATURE IN FISH

Investigators:

J. C. Hampton and R. R. Adée

Planning for future power development must be based, in part, upon accurate knowledge of limits to which river water temperature can be raised and still permit sound management of the environment and maximum use of natural resources. It is known that trout can adapt to temperatures as high as 25°C and that temperatures above that level are lethal, even when exposure is limited to a few minutes. Neither the process of adaptation nor the mode of death at temperatures greater than 25°C is understood. This study began as an attempt to understand the reason(s) trout die when exposed to temperatures of 25°C + and then to apply knowledge and experience gained to experiments dealing with adaptation. It was decided to study organs which are directly exposed to the environ-

ment, on the basis that the first observable effects might be found in tissues which are directly in contact with water and sensitive to temperature variation and concentration of gases.

Gills, swim bladders, and lateral line organs were prepared for light and electron microscopy from rainbow trout, some of which were kept at water temperatures of 15° and 21°C (ambient river temperature) and others which were abruptly transferred to water at 30°C. The higher temperature proved to be lethal within 5 minutes for 6- to 8-inch fish and 20 min for fingerlings. Extreme capillary dilatation was a consistent finding in all three tissues from fish subjected to 30°C (Figures 1 and 2). There was evidence of hemorrhage into the connective tissue stroma of the swim

FIGURE 1a. Swim Bladder from a Trout Maintained at Ambient River Temperature of 21°C [Subepithelial capillary plexus (C) shows no dilatation.]

1a

520X

FIGURE 1b. Swim Bladder from a Trout Fixed About 5 min After Transfer to Water at 30°C [Note extreme dilatation of capillaries (C).]

1b

520X

FIGURE 1. The Swim Bladder is Composed of a Lining Epithelium (E), Subepithelial Capillary Plexus (C), Dense Connective Tissue (CT), and an Adventitia (A) Composed of a Loose Network of Connective Tissue Fibers Covered by a Layer of Mesothelial Cells; the Latter Are not Shown in the Pictures.

2 a

520X

FIGURE 2a. Gill from a Fish Maintained at River Temperature of 21°C [Capillaries (C) are normal.]

2 b

520X

FIGURE 2b. Gill Fixed About 5 min After Transfer to Water at 30°C [Note dilated capillaries (C).]

FIGURE 2. The Gills, at This Level of Organization, Show Finger-Like Extensions Covered with Epithelium (E) Which Surround a Connective Tissue Core Containing Blood Capillaries (C).

bladder but not of gills or lateral line organ. Since no obvious discontinuities in vascular endothelium were observed, no statement can be made as to whether hemorrhage occurred by rhexis or by diapedesis in the swim bladders. Capillary beds were severely congested by blood cells and, at the electron microscope level, the capillary endothelium showed greatly increased pinocytic activity.

Findings in the lateral line organs were inconsistent in that vacuolization of sensory cells was found in some of the 30° fish but not in others. This may be a sampling problem, since the organs appear to be larger and better developed cranially than cau-

dally; we intend to standardize the locations from which the lateral line organs are sampled during future experiments.

It is clear that the vascular system promptly responds to abrupt changes in water temperature. This response will be studied in future experiments dealing with acclimation, the primary purposes being to find a clear-cut tissue response that can be used as an accurate and convenient indication of impending lethality and to help define limits of the river environment within which people should work to insure optimal management of natural resources.

DEVELOPMENT AND EVALUATION OF BLOOD IRRADIATORS

Investigators:

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Technical Assistance:

Linda G. Smith

A program was initiated last year to develop small, implantable irradiators capable of chronically irradiating blood. The five irradiators tested to date have utilized the photon emitters ^{55}Fe (6 keV) or ^{125}I (35 keV) as source materials in devices that were implanted in the thoracic aorta of dogs. These devices are basically tubular in shape with the ends sutured directly to the cut ends of the aorta. Two basic designs have been used, one having knitted dacron as the complete blood interface inside an epoxy tube, the other having the dacron only as end pieces for suturing and the central portion of the irradiator lined with a graphite-benzalkonium-heparin (GBH) coating on the inner surface of the epoxy tube. In both styles the source material was applied around the out-

side of the epoxy with shielding material around this to protect the surrounding tissue from excessive radiation.

Blood samples taken from the dogs following implantation were submitted to routine hematology and surveyed for the presence of any source isotope in the blood. All dogs were sacrificed 5-8 months following implant to determine changes occurring in the materials-blood interface region, and histological sections are being studied.

Significant observations made to date include: (a) Irradiators capable of significantly irradiating blood, but which emit no appreciable radiation to surrounding tissues, can be made small enough for easy implantation. This was demonstrated primarily by dosimetry measurements and

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verified by the absence of gross effects in tissues adjacent to the implanted irradiators. (b) Radiation exposures less than 0.5 rad/day to beagle blood are insufficient to produce early lymphopenia. No significant change in lymphocyte levels was observed in either dog with an implanted ^{125}I irradiator producing an average exposure of this magnitude. (c) Source encapsulation is a greater problem than anticipated. Of the five irradiators implanted, only the one with saran film and silastic supplementing the epoxy barrier showed no leakage. Epoxy alone is insufficient and special attention will have to be given to the problem of developing a barrier sufficiently thin to maximize

dose to blood but adequate to contain the isotope. (d) Of the five irradiators implanted, one having GBH coated dacron end pieces showed less obstructive growth in the lumen than the other four. This observation, if verified, could benefit not only this project but others involving artificial blood vessels.

In reviewing available photon emitters, none fully satisfied requirements of multiyear half-life, energy in the range of 10-30 keV, and reasonable cost. Irradiators now being constructed will utilize the beta emitter ^{204}Th and appropriate shielding materials to minimize bremsstrahlung radiations to surrounding tissue.

1970

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(1) Transferred to Animal Care

(2) Terminated

(3) Transferred to Cellular and Molecular Biology Section

(4) Transferred to another Department

(5) Leave of absence

NUTRITION AND FOOD TECHNOLOGY SECTION

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(1) Terminated

(2) Transferred to Battelle Development Corp.

(3) Faculty Appointee

(4) Transferred to Cellular and Molecular Section

(5) Leave of absence

PATHOLOGY SECTION

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Patricia L. Hackett, Ph.D. (2)
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Glenda S. Vogt, B.A. (4)

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V. T. Faubert (4)
Daphne S. Harrah (5)
Marilyn J. Bottorff Hooper (1)
Darlene H. Hunter
Martha C. Perkins
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R. L. Van Citters, M.D.
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Seattle, Washington

(1) Transferred to Inhalation Toxicology Section

(2) Terminated

(3) Leave of Absence

(4) Transferred to Cytology Section

(5) Retired

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E. L. Hunt, B.S. (3)
M. Kashima, Ph.D. (3)
D. D. Mahlum, Ph.D.
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M. R. Sikov, Ph.D.
V. H. Smith, Ph.D.
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-
- (1) *Terminated*
(2) *Faculty Appointee*
(3) *National Institute of Radiological Sciences, Chiba City, Japan, Fellow of Japan AEC*
(4) *Transferred to Inhalation Toxicology Section*
(5) *Youth Opportunity Program (YOP)*

ATTACHED PERSONNEL

R. J. Olson, M.S. - Applied Mathematics Department
J. M. Thomas, Ph.D. - Applied Mathematics Department

GENERAL SERVICES
FACILITIES OPERATION DEPARTMENT

Janitors - W. L. Roquemore (Foreman)
Ruby Maupin
Alma Moore
G. Salazar
Mildred Straws

MAINTENANCE
CRAFT SERVICES DEPARTMENT

K. E. Harding - Foreman	Material Coordinator - W. D. Elliott
Carpenters - E. C. Bolin	Millwright - F. W. Reynolds
R. W. Garretson, Jr.	Pipefitter - L. R. Nelms
Electrical - J. T. Caraway	
Instruments - R. N. Martin	
S. A. Wilson	

RADIATION MONITORING
RADIATION PROTECTION DEPARTMENT

Specialist - G. M. Rolph⁽¹⁾
 M. W. Leale

Radiation Monitors - H. W. Dains
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(1) Transferred to WADCO

PUBLICATIONS AND PRESENTATIONSOPEN LITERATURE PUBLICATIONS

BAIR, W. J. "Experimental Animal Studies of Inhaled Plutonium," Health Physics, vol. 19, pp. 75-76. 1970. (Abstract)

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COOPER, P. H. and W. J. CLARKE. "Marrow Hematopoiesis in Pitman-Moore Swine Following ⁹⁰Sr Ingestion," pp. 71-83. In: W. J. Clarke, E. B. Howard and P. L. Hackett (eds.), Myeloproliferative Disorders of Animals and Man. CONF-680529. CFSTI, Springfield, Virginia. 1970.

CRAIG, D. K. "Some Factors Involved in the Interpretation of Rotameter Air Flow Readings," Health Physics, vol. 19, p. 347. 1970. (Abstract)

CRAIG, D. K., H. W. W. ADRIAN, and D. J. J. C. BOUWER. "The Effect of Iodine Concentration on the Efficiency of Activated Charcoal Adsorbers," Health Physics, vol. 19, pp. 223-224. 1970.

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PHILLIPS, R. D. "Mechanisms of Injury for the Radiation-Induced Decrease in Cold Tolerance," J. Appl. Physiol., vol. 28, pp. 821-825. 1970.

PHILLIPS, R. D. "The Effect of Dose and Partial-Body X-Irradiation on the Lethality Response of Rats to Low Temperature," Radiation Res., vol. 42, pp. 175-180. 1970.

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SANDERS, C. L. "Maintenance of Phagocytic Function Following $^{239}\text{PuO}_2$ Particle Administration," Health Physics, vol. 18, pp. 82-85. 1970.

SANDERS, C. L. and R. R. ADEE. "Ultrastructural Localization of Inhaled $^{239}\text{PuO}_2$ Particles in Alveolar Epithelium and Macrophages," Health Physics, vol. 18, pp. 293-295. 1970.

SANDERS, C. L. and W. J. BAIR. "The Effect of DTPA and Calcium on the Translocation of Intraperitoneal Administered $^{239}\text{PuO}_2$ Particles," Health Physics, vol. 18, pp. 169-173. 1970.

SANDERS, C. L., R. C. THOMPSON, and W. J. BAIR. "Lung Cancer: Dose Response Studies with Radionuclides," pp. 285-303. In: M. G. Hanna, Jr., P. Nettesheim, and J. R. Gilbert (eds.) Inhalation Carcinogenesis. CONF-691001. CFSTI, Springfield, Virginia. 1970.

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SIKOV, M. R., D. D. MAHLUM, and E. B. HOWARD. "Localization and Effect of ^{239}Pu in the Prenatal Rat and the Placenta," Radiation Res. vol. 43, p. 250. 1970. (Abstract)

SIKOV, M. R. and J. M. THOMAS. "Prenatal Growth of the Rat," Growth, vol. 34, pp. 1-14. 1970.

SMITH, V. H., R. L. AMSTER, and J. M. THOMAS. "Biological Disposition of Promethium after Intravenous Intramuscular and Subcutaneous Administration to Swine." Radiation Res., vol. 43, p. 249. 1970. (Abstract)

STUART, B. O., P. J. DIONNE, and W. J. BAIR. "A Dynamic Simulation of the Retention and Translocation of Inhaled Plutonium Oxide in Beagle Dogs," vol. 2, pp. 721-737. In: M. W. First and J. M. Morgan, Jr. (eds.), Proceedings of the Eleventh AEC Air Cleaning Conference. CONF-700816. CFSTI, Springfield, Virginia. 1970.

STUART, B. O., D. H. WILLARD, and E. B. HOWARD. "Uranium Mine Air Contaminants in Dogs and Hamsters," pp. 413-427. In: M. G. Hanna, Jr., P. Nettesheim, and J. R. Gilbert (eds.), Inhalation Carcinogenesis. CONF-691001. CFSTI, Springfield, Virginia. 1970.

THOMPSON, R. C. "A Review of the Radiosensitivity of the Tissues in Bone, ICRP Publication 11," Health Physics, vol. 18, p. 299. 1970. (Book Review)

TOMBROPOULOS, E. G. and J. M. THOMAS. "Effect of 800 R. Thoracic X-Irradiation on Lung Tissue Biochemistry," Radiation Res., vol. 44, pp. 76-86. 1970.

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PRESENTATIONS AT SOCIETY MEETINGS AND SYMPOSIA

BAIR, W. J. "Long Term Inhalation Toxicity Studies at BNW," Radiation Research Society, Dallas, Texas, March 5, 1970.

BAIR, W. J. "Experimental Animal Studies of Inhaled Plutonium," Plutonium Symposium at the International Radiation Protection Association Congress, Brighton, England, May 3-8, 1970.

BAIR, W. J. "Acceptance Remarks" at the Ernest O. Lawrence Memorial Award Ceremony in Washington, D.C., May 28, 1970.

BAIR, W. J. "Toxicology of Inhaled Plutonium," Eleventh AEC Air Cleaning Conference, Richland, Washington, August 31 - September 3, 1970.

BAIR, W. J. "Toxicology of Inhaled Plutonium - Experimental Animal Studies," Presented at Seminar on Radiation Protection Problems Relating to Transuranium Elements -- Sponsored by Euratom and European Nuclear Energy Agency of the Organization for Economic Cooperation and Development, Karlsruhe, Germany, September 21-25, 1970.

BAIR, W. J. "Plutonium Inhalation Studies," Seminar presented at the Atomic Energy Commission Headquarters in Rome, Italy, September 30, 1970.

BALLOU, J. E. "The Translocation of Thorium Decay Products from ThO_2 in the Dog Lung," Annual Meeting of Health Physics Society, Chicago, Illinois, June 7-11, 1970.

CLARKE, W. J., R. F. PALMER, and J. M. THOMAS. "Effects of Strontium-90 on Reproduction, Growth and Subsequent Neoplasia Development in Miniature Swine," Radiation Research Society, Dallas, Texas, March 5, 1970.

CRAIG, D. K. "Some Aerosol Physics Factors to be Considered in Conducting Animal Inhalation Exposures," Informal Seminar at the Lovelace Foundation, Albuquerque, New Mexico, March 26, 1970.

CRAIG, D. K. "Some Factors Involved in the Interpretation of Rotameter Air Flow Readings," Annual Meeting of Health Physics Society, Chicago, Illinois, June 20 - July 2, 1970.

DILLEY, J. V. "Deposition, Retention and Excretion of Inhaled $^{239}\text{PuF}_4$ In Beagle Dogs Treated with Diethylentriaminepentaacetic Acid (DTPA)," Radiation Research Society, Dallas, Texas, March 1-5, 1970.

DRUCKER, H. and S. BORCHERS. "Thermolysin: Calcium and Temperature Induced Autodigestion," Presented to the Northwest Branch of the ASM, Newport, Oregon, September 11-12, 1970.

DRUCKER, H., S. BORCHERS, and J. T. YANG. "The Effect of Calcium on Thermolysin," Presented to the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 12-17, 1970.

DRUCKER, H. "Kinetic Properties of Thermolysin: The Role of Ca^{++} ," Presented to the American Society for Microbiology, Boston, Massachusetts, May, 1970.

ERDMAN, H. E., D. D. MAHLUM, and M. R. SIKOV. "Age-Related Differences in Binding of ^{65}Zn in the Liver and Muscle of Rats," International Radiation Research Congress, Evian, France, June, 1970

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