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A STUDY OF THE TRANSLOCATION OF PLUTONIUM
AND AMERICIUM FROM WOUNDS

FOR THE PERIOD: December 16, 1968 to December 15, 1969

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INTRODUCTION

The rationale for this research project has been explained in detail in the First Technical Progress Report and in a special report. (1, 2) In essence, the research was begun to determine if the ratio of reactor produced plutonium isotopes to the inherently present ^{241}Am would be altered during the translocation of the material from contaminated wound sites to major points of deposition. This was deemed important because the 59.5 keV gamma photon of ^{241}Am was used as a tracer for plutonium in the in vivo or external whole-body counting estimation of body and lung burdens. The assumption implicit in this method was that the plutonium isotopes and ^{241}Am remained in fixed ratio in the body. However, urine bioassay data had indicated that ^{241}Am was excreted more rapidly than plutonium which raised doubts about the accuracy of the external counting method. The use of the therapeutic removal agent DTPA (diethylene-triaminepentacetate) also cast doubt on the constancy of the ratio.

The approach to this experimental study has been to simulate contaminated puncture wounds in beagle dogs with plutonium chemical forms which are typical of facilities such as the Rocky Flats Division, Dow Chemical Company. The first experiment employed air oxidized plutonium, a relatively insoluble material, while the experiment now underway uses plutonium nitrate a soluble form, at least, in

acid solution. Where possible the industrial health physics and medical handling procedures are adopted.

Half the animals received intravenous infusion of DTPA and the other half are untreated. Wound site excision takes place only when there is evidence that the injected radionuclide is leaking from the wound. During the time of the experiment, samples of urine, feces and blood are collected routinely and as the dogs are serially sacrificed blood and tissue samples are taken.

Chemical assays of excreta, blood and tissue samples are performed at the Rocky Flats Plant. In vivo measurements with scintillation and diode detectors, analysis and correlation of data are being made at Colorado State University.

The appearance of one case of generalized lymphosarcoma in forty dogs of the first experiment and hyperplasia of some lymph nodes in three other dogs has prompted a histopathological study of tissues from selected dogs at time of sacrifice.

This report is the second of its kind and summarizes project activities and information gathered between December 16, 1968 and December 15, 1969. Most of the effort has been spent in completing the plutonium oxide implants; collecting and assaying excreta, blood and necropsy samples; and systematically collecting in vivo counting data with NaI (Tl) detectors at the wound site and at the cervical lymph node area. Because of delays in the completion of plutonium and

americium assays, due to the fire at Rocky Flats, the second experiment with the plutonium nitrate implants was not begun until September 15, but 16 dogs comprising the 12 month and 1/2 month groups have been implanted since then.

Included in this report are:

1. A brief description of the experimental implant procedure and analytical techniques which are used.
2. A summary of the findings through December 15, 1969.
3. A list of publications and papers arising from this research. Special Report COO-1787-6 entitled "Relative Translocation and Distribution of Pu and Am from Experimental PuO_2 Subcutaneous Implants in Beagles", which describes our first experiment in detail, accompanies this report.

Materials and Methods

1. Experimental Design

The second experiment in this research project is to study the movement of Pu and Am from $\text{Pu}(\text{NO}_3)_4$ subcutaneous injection. The experiment will use 48 adult male beagles from the specific pathogen free (S P F) beagle colony of the Collaborative Health Laboratory at Colorado State University. These animals were randomly divided into six study groups, eight to a group, with half of each group randomly selected to receive DTPA treatment. Animals of the same

group were housed one or two to a kennel and were given the animal care described below. The groups were defined by the length of study time that each was to receive. This change in experimental design was made to improve the statistical evaluation for DTPA treatment and for temporal measurements.

2. Animal Care

The animals were maintained in accordance with provisions of the Laboratory Animal Welfare Act.⁽³⁾ The kennels are located on the Foothills Research Campus of Colorado State University (see Figure 1). The kennel design⁽⁴⁾ is as follows: 15 x 15 feet in size, 1 foot gravel base, 4 foot high, 2 inch mesh cyclone fence, and concrete curbs extending 12 inches above the gravel level. Shelter is provided from sunlight by panels of galvanized sheet metal on the fence and from storm and cold by concrete houses with wooden floors.

The dogs were fed a guaranteed nutritionally adequate dry ration of Purina dog meal. Automatic watering devices insure a constant source of fresh, clean water in the summer while daily filling of watering pans was necessary in winter. Cleaning and sanitation of feed and water containers were carried out as per required standards⁽³⁾.

Regular medical diagnosis and treatment of conditions arising in the experimental animals were carried out. An immunization

program was followed for each dog. The veterinary medical personnel and facilities within the College of Veterinary Medicine and Biomedical Sciences of Colorado State University provide a broad capability for the needs associated with experimental animal research.

3. Implanting Procedures

The plutonium oxide (oxidized under ambient conditions) used for the implants in the first experiment was prepared and provided by Rocky Flats and has been described previously⁽¹⁾. The plutonium nitrate which is used in the second experiment is in a 2.0 N HNO_3 and the activity concentration is about 15 $\mu\text{Ci/ml}$ with an ^{241}Am concentration of 750 parts per million parts of plutonium. The plutonium is contained in vials (rubber diaphragm stoppered) which contain 5 ml of solution. Solution calibration is performed each time a group of dogs is injected.

Laboratory space for this study is located in the Radiation and Environmental Stresses Building on the Foothills Campus. The laboratory space was prepared for handling radioactive materials by protecting walls, floors and equipment with wax and plastic covering. The usual personnel protective equipment including respirators was used. All plutonium implants were made under the surveillance of a radiation monitor from Rocky Flats. Air samples and contamination

surveys were taken to assure adequate containment and control during and after the injections.

Hypodermic needles and syringes are used for making the injections. The standard site for the puncture wound contamination is the subcutaneous tissue of the left dorsal metacarpal area.

An ultrashort acting anesthetic (sodium thiamylal for injection N.F. 4% solution) is used to prepare the animals for the implant. A hypodermic needle is positioned subcutaneously in the desired spot on the left dorsal metacarpus of the dog. Proper positioning is determined by palpation and by observing that there is no back-flow of blood. This latter observation indicates that intravenous injection had not occurred. By use of a tuberculin syringe and needle, an aliquot of the plutonium nitrate solution (about 0.10 ml) is drawn into the syringe. The syringe is joined to the needle previously positioned in the dog's foot and the injection is made. Upon withdrawal of the needle, an alpha survey meter is used to determine the extent, if any, of surface contamination. The dog's leg is decontaminated at least once and then repeated until no activity is detected.

4. DTPA Therapy

Following the implant, the dog is placed in a metabolism cage for quantitative excreta collection. About two hours after the simulated contamination has been performed, the animals designated to

receive therapeutic treatment are given DTPA injections. Those dogs are given 0.25 grams of DTPA per injection diluted in 10 ml of physiological saline solution. The usual level of DTPA administration for industrial workers is 1 gram per infusion. The 0.25 grams per injection for the dog was selected by comparing the standard man blood volume⁽⁵⁾ to that of the dog (9.5% of body weight)⁽⁶⁾. The dogs in this study average 14 kilograms in weight. Blood volume determinations based on a radio-iodinated serum albumin method⁽⁷⁾ have been taken on a few of the dogs. The data verified the above cited number and that the average beagle blood volume is 25% of the standard man volume.

5. Sample Collection

Each dog is maintained in a metabolism cage on a standard schedule where 24 hour urine and feces samples are collected. Initial samples are collected for the first seven days, then on the 9th, 11th and 14th days. (Blood samples are obtained by jugular venepuncture and started two hours after administration of Pu with samples taken every two hours for eight hours and then one sample daily.) The dogs are returned to the outside kennels for two weeks and then placed back in the metabolism unit for three days for sample collections. Each dog is then scheduled for two to three days in a metabolism cage at two weeks post injection and for each month thereafter to

procure samples and to administer DTPA, where applicable, until the specified time of sacrifice.

The dogs are sacrificed by anesthetic of pentobarbital sodium and exsanguination. The tissue samples collected at necropsy are as follows: left eye, left testis, left kidney, left adrenal gland, trachea (small piece to represent body cartilage), lung (tip of one lobe), spleen, liver (about a fourth of the organ was analyzed), sternum, left 10th rib, two lumbar vertebrae, piece of skull, left femur, left axillary lymph node, right cervical lymph node, left cervical lymph node, thyroid and the implant site (left front paw sectioned at the carpometacarpal joint), and bile.

6. Chemical Analysis

All samples collected are assayed for plutonium and americium activity in Rocky Flats Laboratories. The general chemical procedures for each sample type has remained essentially the same as previously reported. (1)

7. In vivo Counting Equipment and Procedures

In vivo counts on each dog were made on days 1 through 7, 9, 11, 14, 28, 29, and 30 and then monthly following the plutonium implants. Measurements were made with both a lithium drifted germanium detector and a NaI (Tl) wound counter.

The scintillation counter was used to measure the activity at the wound site and in the left supra-scapular or cervical lymph node. This lymph node is the first major node draining the area in the dog's foot and leg which contains the plutonium. (8) The count rate as a function of time was recorded.

The diode detector is a top mounted, right angle looking, planar model with an active entrance area of 10 cm^2 and a lithium drifted depth of 7.5 mm. It is housed in an evacuated chamber 12 mm behind a 0.25 mm beryllium window and maintained at "liquid-nitrogen" temperature. The resolution of the detector is about 2.0 keV for full width at half maximum for the 59.6 keV ^{241}Am gamma ray.

The germanium detector was used to measure the 51.6 keV gamma emission of ^{239}Pu and the 59.6 gamma emission of ^{241}Am . Counts were taken on the wound site, the left cervical lymph node, and the liver area of each dog. These data will be correlated with chemical assay data to investigate the feasibility of determining the relative translocation of these two nuclides by in situ counting techniques.

Experimental protocol is shown on the next page.

Summary of Findings to December 15, 1969

Forty dogs have been implanted with air oxidized plutonium oxide. Their excreta and selected tissues have been analyzed for plutonium and ^{241}Am content over the period from May 2, 1968 to May 1, 1969. The details of the experiment, the various correlations which were studied and the conclusions derived are discussed in detail in an accompanying Special Report (COO-1787-6 - Relative Translocation and Distribution of Pu and Am from Experimental PuO_2 Subcutaneous Implants in Beagles).⁽²⁾ In summary, the following observations and conclusions were obtained:

The translocation of Pu and Am from PuO_2 implants in the paw of the dog indicates the relative metabolism of these two elements. Two mechanisms of translocation, lymphatic transport and blood distribution, were important in the disposition of the subcutaneously injected material.

The amount of Pu at the implant site and in the liver was estimated by the method of external ^{241}Am 59.6 keV gamma ray analysis. This method assumed no differential metabolism of Pu and Am. In vivo estimation of the liver Pu content prior to necropsy by this method was compared to that determined by chemical assay techniques. The ^{241}Am tracer method overestimated the Pu liver burden by an average of 4.5 fold for untreated dogs and 3.6 fold for DTPA treated dogs.

Calculations of the Pu/Am alpha activity ratio in blood, urine, feces, and tissue samples were used as indexes for the relative metabolism of these elements in the dog. This ratio was divided by the calculated Pu/Am alpha activity ratio of the implant material at the sample assay time to correct for the buildup of ^{241}Am activity over the course of the experiment. These values, called multiples or corrected ratios, provided a basis for comparison of the relative metabolism of Pu and Am with in vivo counting as the frame of reference. Multiples greater than 1.0 indicated relative enhancement of Pu concentration and multiples less than 1.0 indicated Am enhancement.

Regression analyses of the multiples or corrected ratios of all sample types as a function of post-implant time were completed. These analyses did not show a significant time relationship at the 95 percent confidence level for any sample type.

Mean multiples for each sample type from both untreated and DTPA treated dogs were computed to measure treatment effect and to compare sample types (Tables 1, 2, 3, 4, 5). A significant DTPA treatment effect was observed in urine, liver, and proximal femur tissue samples. Enhancement of the relative Pu content was observed in these three sample types following DTPA treatment. Samples from DTPA treated dogs showed mean multiple increases of 2, 3,

Table 1. Mean Multiple of Pu/Am Ratio for Major Sites of Pu and Am Deposition in Soft Tissues

Sample	Treatment	Mean Multiple	95% Confidence Interval
Liver	None	0.28	0.01 - 0.68
	DTPA	1.49	0.68 - 2.31
Spleen	None	1.03	0.61 - 1.45
	DTPA	1.09	0.67 - 1.51
Kidney	None	0.28	0.11 - 0.45
	DTPA	0.29	0.01 - 1.14
Left cervical lymph node	None	1.40	0.63 - 2.17
	DTPA	1.26	0.78 - 1.74

Table 2. Mean Multiple of Pu/Am Ratio for Various Types of Soft Tissue

Sample	Treatment	Mean Multiple	95% Confidence Interval
Left axillary lymph node	None	1.24	0.50 - 1.98
	DTPA	1.30	0.66 - 1.94
Right cervical lymph node	None	0.59	0.15 - 1.03
	DTPA	0.56	0.01 - 1.22
Lung	None	0.60	0.33 - 0.87
	DTPA	0.68	0.47 - 0.89
Adrenal gland	None	1.98	0.67 - 3.29
	DTPA	0.95	0.42 - 1.48
Thyroid gland	None	0.071	0.049 - 0.093
	DTPA	0.091	0.051 - 0.131
Eye	None	0.39	0.22 - 0.56
	DTPA	0.46	0.23 - 0.69
Trachea	None	0.78	0.01 - 1.69
	DTPA	0.45	0.13 - 0.77
Testis	None	0.34	0.06 - 0.62
	DTPA	0.58	0.18 - 0.98

Table 3. Mean Multiple of Pu/Am Ratio for Minor Sites of Pu and Am Deposition in Skeletal Tissue

Sample	Treatment	Mean Multiple	95% Confidence Interval
Rib	None	3.11	1.34 - 4.88
	DTPA	2.30	1.50 - 3.10
Skull	None	1.31	0.63 - 1.99
	DTPA	1.04	0.68 - 1.40
Femur shaft	None	1.56	0.71 - 2.41
	DTPA	1.99	1.19 - 2.79

Table 4. Mean Multiple of Pu/Am Ratio for Major Sites of Pu and Am Deposition in Skeletal Tissue

Sample	Treatment	Mean Multiple	95% Confidence Interval
Sternum	None	3.41	1.60 - 5.22
	DTPA	5.62	3.54 - 7.70
Vertebrae	None	5.74	2.83 - 8.65
	DTPA	5.96	1.86 - 10.06
Proximal femur	None	1.93	1.13 - 2.73
	DTPA	6.02	3.60 - 8.44
Distal femur	None	1.48	0.70 - 2.26
	DTPA	2.92	1.68 - 4.16

Table 5. Relative Pu and Am Distribution in Blood, Urine, and Feces

Sample	Treatment	Mean Multiple of Pu/Am Ratio	95% Confidence Interval
Blood	None	0.95	0.80 - 1.09
	DTPA	0.80	0.68 - 0.93
Urine	None	0.79	0.71 - 0.87
	DTPA	1.47	1.28 - 1.65
Feces	None	2.11	0.88 - 3.34
	DTPA	2.24	0.89 - 3.59

and 5 fold for urine, proximal femur tissue, and liver tissue, respectively. Most of the other tissue samples showed a trend toward Pu enhancement for DTPA treated dogs but the enhancement was not significant at the 95 percent confidence level.

The magnitude of the multiple differed for sample type. Enhancement of Am collection averaged about 15 in the thyroid tissue while Pu enhancement in the vertebrae averaged about 6. These two tissues represented the two multiple or corrected ratio extremes in the data obtained. The general result was Am enhancement in soft tissues and Pu enhancement in skeletal tissue. In the case of skeletal tissue, there seemed to be a direct relationship between the hemopoietic activity of bone and its Pu collection enhancement.

These data suggested the need to consider the relative metabolism of Pu and Am in interpreting in vivo measurement of Pu burdens via Am measurement. Differential translocation and excretion of these two elements were indicated. The administration of DTPA therapy was shown to have an additional effect on the relative collection of Pu and Am in some body tissues and fluids.

As previously reported, rapid movement of the material from the implant site to the first major lymph node draining the site was observed by in vivo counting of the lymph node.⁽¹⁾ Data on the dynamics of Pu and Am localization in the lymphatic system were obtained (Figures 1 and 2). The kinetics of the lymph node

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concentration of the implanted material were best described by power function equations. The equation fitting the data for untreated dogs was:

$$A = 0.255 T^{0.343} - 0.191$$

and for DTPA treated dogs was:

$$A = 1.65 T^{0.101} - 1.729$$

where A is the activity accumulated at the lymph node and T is the post-implant time in days. Because of the dispersion of the data these two equations were not significantly different at the 95 percent confidence level. About 10 percent of the node burden was accumulated in the first two days. [This finding supports the current industrial practice of immediately excising any wound in which measurable amounts of Pu or Am are detected.] The movement of the subcutaneously implanted material to the superficial cervical lymph node ranged from an average of 3.3 percent of the estimated initial amount implanted after 1/2 month of implant time to 13.3 percent after 12 months of implant time.

The International Commission on Radiological Protection's model showing the principle metabolic pathways of radionuclide intake and absorption in the body did not show a wound site to lymph

node pathway.⁽⁹⁾ The present experimental data strongly indicated the existence of this pathway.

The left superficial cervical lymph node of the dogs received radiation doses estimated to range from 363 rads for a two week dog to greater than 31,000 rads for a long-term dog. The probable induction of lymphosarcoma in one experimental animal was observed and is reported in more detail in a later section. The potential for induction of lymphosarcoma as a result of a plutonium contaminated wound was indicated by this experimental observation.

The use of a lithium drifted germanium detector for simultaneous in vivo measurement of ^{241}Am and ^{239}Pu gamma rays was investigated. This method of external measurement was in need of further optimization by shielding out background radiation and by detector collimation.

Occurrence of Lymphosarcoma

Four months after being administered 5.8 μCi of PuO_2 one dog showed a generalized lymphadenopathy. The condition of the animal deteriorated rapidly thereafter. A radiographic examination was done and showed enlargement of the lymph nodes in the anterior mediastinum and sublumbar region. A radiographic diagnosis of generalized lymphosarcoma was given and the animal was put to sleep and necropsied. This animal had not been treated with DTPA.

Gross findings at necropsy confirmed the radiographic diagnosis. The anterior mediastinum lymph nodes and the mesenteric and sub-lumbar lymph nodes were markedly enlarged. Histologic examination of the cervical and prescapular nodes revealed an absence of normal lymph node architecture. The nodes were diffusely infiltrated with lymphoreticular cells varying in appearance from that of mature lymphocytes to lymphoblasts and reticulum cells. Neoplastic lymphocytes were observed infiltrating adjacent adipose and collagenous tissues. The histopathologic diagnosis was lymphosarcoma.

Alpha radioautography of sections of kidneys, liver, spleen, and prescapular lymph nodes revealed various concentrations of Pu within these tissues. The left prescapular lymph node had the heaviest concentration of radioactive material followed by the liver. The estimated dose to the prescapular lymph node was 7052 rads. The amount of Pu concentrated at this lymph node relative to the total amount injected was estimated to be 5.4 percent of the initial dose.

The authors recognize that a single case of lymphosarcoma following implantation of PuO_2 into the subcutaneous tissues of forty dogs is not proof that the condition was induced by Pu contamination. However, in the colony from which these dogs were obtained, only one other case of lymphosarcoma, in an 11 year-old dog, has been diagnosed in the last seven years out of a total population numbering

in excess of 8000 dogs. The fact that this animal was only 1 1/2 years old and the heavy concentration of Pu detected by alpha radioautography in the prescapular and cervical lymph nodes must be considered.

Several other animals have had marked destruction of the germinal centers in the prescapular lymph nodes. In several dogs other lymph nodes were enlarged. Microscopically, the enlargement was accounted for by hyperplasia rather than neoplasia. It is quite possible that the dose given to some of these animals may have been too large and have induced destruction of the lymphoid tissue rather than inducing a neoplastic process.

More detailed studies of the processes involved in the translocation of Pu from subcutaneous deposition sites and its exact location and effect on target organs are clearly necessary.

List of Publications and Papers

- COO-1787-1 Watters, R. L., J. L. Lebel, L. J. Johnson and E. H. Bull, "A Study of the Translocation of Plutonium and Americium from Wounds", First Technical Progress Report (1968).
- COO-1787-2 Watters, R. L. and L. J. Johnson, "The Movement of Plutonium and Americium from Wound Sites", Presented at the Midyear Topical Symposium of the Health Physics Society, Los Angeles, California January 31, 1969.
- COO-1787-3 Johnson, L. J., R. L. Watters, C. R. Lagerquist and S. E. Hammond, Relative Tissue Distribution of Pu and Am from Experimental PuO₂ Contaminated Puncture Wounds, (Abstract) Health Physics, 17 p. 383 (1969).
- COO-1787-4 Johnson, L. J., E. H. Bull, J. L. Lebel and R. L. Watters, "Kinetics of Lymph Node Activity Accumulation From Subcutaneous PuO₂ Implants", Health Physics (in press).
- COO-1787-5 Lebel, J. L., E. H. Bull, L. J. Johnson and R. L. Watters, "Lymphosarcoma Associated with Nodal Concentration of Plutonium in Dogs: A Preliminary Report", Submitted to J. of Veterinary Science.
- COO-1787-6 Johnson, L. J., Relative Translocation and Distribution of Pu and Am from Experimental PuO₂ Subcutaneous Implants in Beagles, Special Report to U. S. Atomic Energy Commission (Dissertation).
- COO-1787-7 Johnson, L. J., R. L. Watters, C. R. Lagerquist and S. E. Hammond, "Relative Distribution of Plutonium and Americium Following Experimental PuO₂ Implants", Submitted to Health Physics.

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