3. MODIFICATION OF RADIATION EFFECTS

SUMMARY

Arthur Lindenbaum, Group Leader

In previous Annual Reports, the work described in this section was included, largely for administrative purposes, among the diverse activities being carried out by the Biochemistry Group of the Division of Biological and Medical Research. This year, however, in recognition of the importance and increased scope of the program on plutonium metabolism and therapy, the submissions are treated as a separate entity. Likewise, much diversity will be noted in the kinds of activities being carried out under this program. The unifying objective underlying the varied research activities reported here continues to be the decorporation of dangerous polyvalent radionuclides. While all radionuclides are to varying degrees hazardous when deposited in living tissue, the metals of the actinide series--and in particular, plutonium-239--represent, by any measure, one of our most serious radiological problems. Our therapeutic objectives, therefore, are mainly directed toward decorporation of plutonium. To verify that therapeutic procedures developed in a variety of small animal species will ultimately be effective in humans, a larger species, the dog, is being increasingly used as an experimental animal.

In the design of appropriate experiments, a balance has been sought among the various approaches possible. Thus, in whole animal work the mouse is the species of choice because of the relatively low cost of obtaining data. Results of particular importance are then verified in the dog, using fewer numbers of animals for economy. The results obtained in metabolic experiments have provided the basic information needed for the development of new therapeutic concepts, procedures, and drugs. Frequently, metabolic and autoradiographic studies have pinpointed specific organs, cells, or subcellular tissue components that are particularly involved in plutonium deposition and retention. Ultimately, to make full use of the information gained from the above studies, selected results must be reinvestigated and interpreted at the molecular level. Over the past year the biochemical and molecular aspects of radionuclide metabolism and therapy have received increased attention, as they will in the future.

The present report covers new results in all of the areas described above. In duration-of-life experiments with mice injected with different levels of monomeric and highly polymeric plutonium, new data have been obtained on pathological changes in various tissues and on the life-shortening

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effects of these doses. These results supplement previously reported information on differences in tissue deposition and retention following administration of these two forms of plutonium.

In dogs injected with monomeric plutonium we have demonstrated (as previously shown in mice) the value of a regimen of early and prolonged treatment with DTPA (diethylenetriaminepentaacetic acid) for minimizing the plutonium burden in the soft tissues and skeleton. These results have immediate implication for DTPA treatment in man.

New studies in mice have verified the action of pyran copolymer antiviral agents in enhancing the effectiveness of DTPA for removal of polymeric plutonium from the liver. The net effect is much like that of glucan, but with the possible advantage that a water-soluble, rather than particulate, substance is injected. Time, dosage, and toxicity studies are now being carried out in preparation for therapeutic testing of the most effective of these compounds in dogs.

Continuing studies of the effectiveness of liposome-encapsulated DTPA in removing polymeric plutonium deposited in the mouse liver and skeleton are described in an abstract.

Recent application of autoradiographic procedures for quantitatively comparing short- and long-term localization of monomeric and polymeric plutonium in dog liver has shown that there is no net translocation of monomeric plutonium within the liver between 6 and 90 days following injection. In a cooperative clinical study of the possible use of a lanthanide radionuclide, ^{153}Sm, for tumor localization, the autoradiographic techniques developed for plutonium have been used to measure the differential deposition of radio-samarium in tumors and in normal tissues.

One of the molecular studies presently underway aims at synthesis of a variety of DTPA esters. The diethyl ester has already been prepared and tested for toxicity in mice. These compounds are designed to bring DTPA into contact with plutonium deposits unavailable to the action of ionic DTPA. Other synthetic work is directed toward the preparation of ^{14}C-labeled DTPA of high specific activity, to be used in elucidating the delayed action of this chelating agent and for other purposes.

Significant changes in personnel have occurred over the past year. Dr. M. W. Rosenthal has taken up other duties in the Laboratory and two new PhD-level staff members, M. H. Bhattacharyya and R. A. Guilmette, have joined our group. Their backgrounds, training, and scientific viewpoints will inevitably contribute new insights, approaches, and perhaps new directions to our program.

MODIFICATION OF RADIATION EFFECTS STAFF

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Bhattacharyya, Maryka H. (Assistant Biochemist)
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*Terminated in 1974.

†Now Division Editor.

METABOLIC AND THERAPEUTIC STUDIES WITH PLUTONIUM AND AMERICIUM

*Arthur Lindenbaum, Marcia W. Rosenthal, Maryka H. Bhattacharyya, Raymond A. Guilmette, John E. Parks, G. Steve Kalesperis, Elizabeth S. Moretti, David P. Peterson, John J. Russell, and Nancy Doan**

PURPOSE AND METHODS

The broad objective of this program is to develop new approaches to the therapy of poisoning by radioactive and nonradioactive metals. Plutonium-239 and americium-241 have received major attention in recent years because of the potentially great radiological health hazard represented by alpha-emitting nuclides used for nuclear power production and allied technology. Compounds of the actinide series (as well as those of other polyvalent heavy metals) exhibit highly variable tendencies to hydrolyze and polymerize, to aggregate, and to bind to proteins and other biological components, both in solution and *in vivo*. This range in physical-chemical character has been shown to influence the deposition, retention, effectiveness of therapy, and delayed pathological effects of these nuclides. Thus, information obtained with plutonium and americium aids in understanding the behavior of other nuclides of the actinide, lanthanide, and rare earth series in living tissues. Metabolic studies of actinide compounds also provide useful information on the translocation and deposition of other colloids and macromolecules in tissues.

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*Fall 1974 participant in the Undergraduate Honors Research Participation Program, St. Olaf College.

Previous work has demonstrated the effectiveness of chelating agents, such as diethylenetriaminepentaacetic acid (DTPA), for removal of plutonium and related elements from blood, bone, and soft tissues. Attention is now being directed toward (a) other therapeutic approaches aimed at removal of that portion of the plutonium not readily removed by DTPA, and (b) improving our understanding of the molecular interactions occurring among actinide compounds, tissue constituents, and therapeutic substances.

In past years we have used the mouse and rat to develop a variety of therapeutic procedures for removal of plutonium and to explore the mechanisms of actinide deposition and removal. Promising results with these species are now being extended to the dog. In this larger, longer-lived species there is slower bone turnover; also, the retention of plutonium in the liver is believed to resemble more closely that of man.

PROGRESS REPORT

Deposition of Polymeric Plutonium in Beagle Dogs

Although much is known about the short- and long-term pattern of deposition of monomeric plutonium in the tissues of the dog, most of our information on the deposition kinetics of polymeric plutonium has been obtained in the mouse. Accordingly, studies with polymeric plutonium are now being carried out in the dog. A group of three dogs previously injected with 0.1 μCi/kg of highly polymeric plutonium were sacrificed after 1 year to allow comparison with tissue deposition values obtained 6 and 90 days after injection of similar preparations of polymeric plutonium. The data obtained are entirely consistent with expectations based on previous results with mice and dogs: the plutonium burdens in the liver, spleen, and lymph node sample one year after injection were 73%, 5%, and 0.03% of the amount injected, respectively, approximately the same as in these tissues at 6 and 90 days. Comparison of plutonium burdens in all skeletal elements analyzed (femur, third lumbar vertebra, mandible + teeth) showed that the plutonium concentrations at one year were greater than at 90 days, which in turn were greater than at 6 days. These successive increases are presumably due to gradual translocation of plutonium from soft tissue deposition sites. Another group of dogs in this study will be sacrificed 2 years post-injection to follow these trends further and to carry out a continuing autoradiographic investigation of changes, over a 2-year period, in the microdistribution of polymeric plutonium in the liver.

Therapeutic Studies in Beagle Dogs

In comparative studies of plutonium decorporation in dogs, the effects of DTPA therapy delayed for 3 months after injection of 0.3 μCi/kg of monomeric plutonium have been compared with results of the same therapeutic regime (70 mg/kg, twice weekly, for 12 weeks) begun at 6 days. At the end of the delayed therapy about 7% of the injected plutonium remained in the liver, compared to 1.2% at the end of therapy begun at 6 days (1). The difference between these values may reflect the processes of tissue binding, intracellular incorporation, etc., that gradually sequester the plutonium and inhibit chelation by DTPA. Delayed DTPA treatment resulted in only small differences in the plutonium content of the skeletal samples from

levels found in controls or in dogs receiving early treatment. In a second comparison, DTPA treatment was begun at 6 days but continued for only 4 weeks before sacrifice. In this case, the livers contained about 9% of the injected plutonium and less removal of plutonium from the femurs and third lumbar vertebra was found than after 12 weeks of treatment. These results reinforce other data indicating the value of early, and prolonged, treatment with DTPA.

Because the skeleton undergoes a continuous process of remodeling, deposits of any bone-seeking substance gradually tend to become buried in cortical areas impenetrable by chelating agents like DTPA. In the case of plutonium, one of our therapeutic goals is to promote bone resorption, thereby exposing buried plutonium. To this end two procedures have been tested in dogs. In one, DTPA was injected intravenously as the pentasodium salt slowly (to avoid tetany) rather than as $CaNa_3DTPA$. The rationale was that plasma calcium lost in the formation of $CaNa_3DTPA$ would be replaced by skeletal calcium, in the course of which plutonium might also be removed or exposed to be chelated by the DTPA. This treatment was a part of the study, described above, in which 12 weeks of DTPA treatment was begun 3 months after monomeric plutonium. In the second approach, a corticoid drug, methylprednisolone acetate, known to increase osteoclastic activity in the skeleton with minimal effects on water balance, was administered intramuscularly with and without concomitant DTPA therapy. Injections of corticoid were given twice per week for 4 weeks, beginning 6 days after injection of monomeric plutonium (as part of a study described above). In brief, no significant additional plutonium removal from the skeleton was achieved by either approach over the removal obtained with $CaNa_3DTPA$ alone. In both experiments it is possible that treatment with either of these substances begun less than 6 days after plutonium administration, or with a different dose regimen, might have shown some effectiveness. Because of the importance of achieving maximal skeletal depletion of any of the alpha-emitting actinides, this problem will continue to be an experimental challenge; other approaches to skeletal removal are being considered.

Therapeutic Studies in Mice

The manipulation of normal physiological processes to promote additional removal of actinides from tissues beyond the amounts removed by DTPA alone is an approach to therapy that has shown promise in our hands. In recent work with mice (2-6), several new adjunct compounds or preparations have been shown to amplify the effect of DTPA in removing plutonium from the liver. One of the most promising of these adjunct compounds is designated by the supplier, Hercules, Inc., as pyran copolymer XA-124-177 (peak MW $\sim$ 36,000). It is a water-soluble compound, unlike glucan, the first such adjunct substance tested, whose particulate properties may present difficulties in intravenous injection. The pyran copolymers have been shown by others to have antiviral properties; whether these properties are related to their action in promoting plutonium removal has not yet been determined. Recent mouse studies have verified the adjunct action of pyran copolymer XA-124-177 in removing hepatic plutonium. We are now determining the optimal dosage level and treatment schedule for this drug in the mouse. (Testing in dogs would be an obvious next step.)

Although oral administration of pyran copolymer XA-124-177 was ineffective, a single intravenous injection given with concomitant DTPA therapy increased the amount of mid-range polymeric plutonium removed from the mouse liver by a factor of 2 as compared to treatment with DTPA alone [14.4% of the injected dose (ID) vs. 7.5% ID net removal due to therapy]. The effect of this same pyran compound in treating contamination by highly polymeric plutonium was even more pronounced, i.e., ∿ 5 times as much plutonium was removed from the liver compared to DTPA treatment alone (27.7% ID vs. 5.7% ID net removal due to therapy). Preliminary results suggest that pyran copolymers used as adjuncts to DTPA therapy are at least as effective in removing polymeric plutonium from the liver as liposome encapsulation of DTPA. The toxicities of several other antiviral drugs are being determined in anticipation of testing those that show promise for plutonium decorporation.

Effects of Physical-Chemical State of Plutonium on Lifetime Pathological Changes in Mice

The high carcinogenicity in bone of ^{239}Pu is believed due to the high local energy of its alpha radiation and its selective deposition near the cells lining the endosteal surfaces of the skeleton. Evidence indicating that these are the cells at risk in the induction of bone tumors by irradiation is based on our earlier demonstration (7,8) that monomeric plutonium, which is deposited almost entirely on bone surfaces, was at least twice as carcinogenic in bone as an equal bone deposition of a mid-range polymeric plutonium, which is located in the marrow as well as on the bone surfaces. Several years ago we initiated an experiment to extend these studies to a highly polymeric form of plutonium and to compare, under identical experimental and environmental conditions, the induction of bone tumors by monomeric plutonium and by a highly polymeric plutonium, using more exactly characterized plutonium preparations in mice of the same age from a closed, carefully controlled colony (9). This highly polymeric plutonium had a much higher deposition in liver and spleen, and a much lower deposition in bone, and on bone surfaces, than the mid-range polymeric plutonium preparation used previously. At 6 days after injection of the monomeric plutonium, the total skeletal burden was 43.2% of the injected amount (femurs x 13), and the total marrow burden was calculated from a standard sample to be 0.95%. After injection of highly polymeric plutonium, the bone burden was 3.3%, and the marrow 2.0%. Subsequent annual reports have described (a) the distribution and long-term retention of the two forms of plutonium; (b) the translocation of polymeric plutonium from liver to bone surfaces with time; (c) the development of anemia and dose-related hematopoietic deaths after polymeric plutonium; (d) reduction of survival by the highest level of monomeric plutonium; and (e) progressive shortening of the mean survival time by the three higher levels of polymeric plutonium (2, 10-12).

Analysis of morbidity data, primarily from observations at autopsy, shows that tumors of the lymphatic system (including lymphocytic leukemia, myelogenous leukemia, and reticulum cell tumors) were the primary cause of death of almost 30% of the control mice, not injected with plutonium; lesions in a number of different tissues were responsible for the other deaths. In mice given polymeric plutonium, anemia and tumors of bone and lymph were the major causes of death. Some deaths were caused by hemorrhages in the spleen, apparently related to radiation damage from the high

concentrations of the polymeric plutonium. In mice with monomeric plutonium, no large hemorrhages were observed, and either lymphatic or bone tumors were the cause of most deaths.

The total occurrence of lymphatic tumors was high in all groups of mice. These tumors occurred in 72% of the control mice. Their incidence was increased over this control level in all groups of mice receiving monomeric plutonium except the group given the highest level, but was decreased in the groups in which life-shortening was also observed.

A detailed histopathological study of all skeletal areas diagnosed as abnormal in terminal radiograms has been completed in a cooperative study with Dr. T. E. Fritz (in consultation with Dr. L. S. Lombard). Two of the 58 duration-of-life control mice (3.4%) had malignant tumors in the bone. After injection of monomeric plutonium at 0.07, 0.14, 0.27, 0.47, and 0.96 μCi/kg, the incidence of mice with malignant bone tumors increased with dose: 5.3%, 11.6%, 35.3%, 55.9%, and 61.5%, respectively. After injection of polymeric plutonium, doses of 1.1, 2.2, and 3.8 μCi/kg, which resulted in the same initial bone burdens as the three lowest levels of the monomeric form, produced a bone tumor incidence of 42%, 50%, and 25%, respectively. With both forms of plutonium, increasing dose resulted in a shortening of the latent period, and an increased age-specific death rate with bone tumors.

Most of the tumors were osteosarcomas of the osteogenic type, and consisted of well-differentiated, rapidly proliferating, bone-forming osteoblastic cells. In some tumors, however, other forms of morphologic differentiation sometimes predominated over the obvious osteoid-producing cells. No apparent relationship existed between the histologic variability in tumor type and either the form or the dose level of plutonium injected. Most of the osteosarcomas appeared to originate from the endosteal bone. Non-neoplastic osteoporotic and osteosclerotic changes were also distributed throughout the animals of the several groups, including the controls.

Interpretation of the comparative effectiveness of monomeric plutonium and highly polymeric plutonium in the induction of bone tumors in this experiment must take into consideration a number of complex and interrelated factors, including: (a) the initial microlocalization of the monomeric plutonium on bone surfaces and of two-thirds of the polymeric plutonium in the marrow; (b) the different long-term retention patterns of the two forms, particularly as regards the increasing bone levels and the continuing lay-down of plutonium on bone surfaces after administration of the polymeric form; (c) the effect of polymeric plutonium on life-shortening; and (d) radiation damage to the hematopoietic system, liver, and spleen by polymeric plutonium.

Enzymatic Dissociation of Liver Parenchymal Cells from Plutonium-Treated Mice

New biochemical studies of the association of different physical-chemical forms of ^{239}Pu with non-cellular, cellular, and subcellular components of the liver have been initiated in the mouse. It is anticipated that such studies, when extended to other species such as the dog and hamster, will aid in understanding the mechanisms responsible for the grossly longer hepatic retention half times of monomeric plutonium in these animals (and

presumably man) as compared to those in mice and rats. These studies are also expected to contribute to our knowledge of the fundamental mechanisms involved in plutonium uptake in liver and other tissues, and to aid in devising better detoxification procedures.

Data are presently being obtained on the kinetics of association of plutonium with mouse liver parenchymal cells following intravenous injection of 0.1 μCi/kg of monomeric plutonium. To carry out this work, a method has been developed for the isolation of parenchymal cells from dog and mouse livers. The technique involves incubation of a tissue mince with buffered lysozyme solution, followed by dispersion of the incubated tissue mince with gentle strokes in a Potter-Elvehjem homogenizer (clearance, 1.0 mm) to release individual parenchymal cells. The cells are then isolated by differential centrifugation. By this means, the plutonium associated with liver parenchymal cells can be isolated free from plutonium associated with Kupffer cells or contained in extracellular spaces of the liver. The role of the parenchymal cells and their subcellular components in handling the liver plutonium burden can then be investigated. Subcellular components prepared from the isolated cells can be analyzed for plutonium content without the problems presented by heterogeneous mixtures of liver cell types or by the mixing of cell-associated and non-cell-associated plutonium during the tissue homogenization step.

In preliminary tests of this procedure using dog livers, cell yields averaged 6.4×10^7 parenchymal cells isolated per gram of fresh liver. Cell yields from mice were much lower, averaging 2.3×10^6 parenchymal cells isolated per gram of fresh liver. In the case of both dogs and mice, cell yields obtained so far have been consistently lower in plutonium-treated than control animals, possibly indicating increased cell fragility in plutonium-irradiated livers.

Autoradiographic Studies

Quantitative autoradiographic measurements of the microdistribution of monomeric plutonium in the dog liver 90 days following injection, reported last year as preliminary results (3), are now complete. Both track density and the fractions of tracks associated with parenchymal or littoral cells were found to be unchanged from values obtained at 6 days. Thus, no translocation of plutonium in the liver between 6 and 90 days following injection of monomeric plutonium could be detected by autoradiographic techniques.

Autoradiographic estimation of plutonium localization in different regions of a canine liver lobe 6 days following injection of polymeric plutonium has also been completed. As reported earlier (13), particulate plutonium is phagocytized primarily by reticuloendothelial elements of the liver, namely the Kupffer cells. In the present work only a slightly higher concentration of plutonium was found in the central region of the liver lobe, as compared to the peripheral region. This is in contrast to autoradiographic results with polymeric plutonium in the dog liver reported earlier (13). This difference may be due to the use of a slightly more polymeric solution in the earlier experiment, possibly resulting in physical entrapment of some of the larger particles in addition to uptake by phagocytosis. To extend the information obtained at 6 days, the quantitative

microdistribution in liver of highly polymeric plutonium at 3 months and 1 year after injection is currently being determined.

In addition to liver studies, bone specimens from dogs sacrificed at 6 and 90 days after injection of monomeric plutonium, and at 6, 90, and 365 days after injection of highly polymeric plutonium are now being prepared for autoradiographic and histological evaluation.

In cooperation with several members of the Chemistry and Physics Divisions of ANL and some of the staff of Rush University Medical Center we are participating in the evaluation of intravenously injected ^{153}Sm citrate as a tumor-localizing agent, using ICR white mice bearing sarcoma-180 as experimental animals. Autoradiographic examination of tumor tissue sections removed 24 hours after injection show a moderately high ratio ($\sim$ 5:1) of ^{153}Sm activity associated with cells in the peripheral regions of the tumor (no activity in the necrotic central regions), as compared to activity levels in adjacent areas of normal tissue. It has been shown (14) by direct radiochemical measurement of mouse tissues that between 24 and 48 hours after ^{153}Sm injection the activity ratios of tumor/normal tissues are increased, indicating a faster rate of removal of ^{153}Sm from non-tumor tissue than from the tumors.

Use of Electron Microscopy and X-Ray Microanalysis to Detect Subcellular Localization of Plutonium

The availability of a scanning electron microscope equipped with an Ortec energy dispersive X-ray analyzer has raised the possibility of combining the visualization of tissue sections at high magnifications with high resolution detection of plutonium located in subcellular deposition sites (15). In preliminary tests of the apparatus, X-ray peaks corresponding to plutonium have been obtained from electron-dense areas located on grids to which a solution of highly polymeric plutonium had been applied. However, the detection system as used at Argonne was not sensitive enough to produce X-ray peaks corresponding to plutonium from areas of liver sections where plutonium had already been located by EM autoradiography. More sensitive X-ray detector systems are available, however, and the feasibility of using these for our purposes is presently being investigated. This technique, when developed, could locate plutonium more quickly and accurately than can α-track recording by EM autoradiography. Also, development of such a sensitive detection method would permit estimations of the cellular and subcellular deposition of other heavy metal environmental pollutants.

Molecular Studies

Synthesis of ^{14}C-DTPA. Methods for the synthesis of DTPA labeled with ^{14}C in the 2-carbon have been successfully adapted to a micro-scale (6 millimoles) preparation, with a procedure which will allow maximum label incorporation. Problems encountered in the purification have been overcome by a combination of selective precipitation and ion-exchange chromatography. These procedures are also expected to be of use in the recovery of the pure ^{14}C-labeled DTPA from excreta of experimental animals. In the course of this work, a new technique for the rapid colorimetric microanalytical determination of DTPA has been developed. A preparation of low specific

activity DTPA is in progress to determine the percent label incorporation and the radiochemical purity of the synthetic DTPA.

Synthesis of Lipid-Soluble DTPA Esters. Previous work (16) has established that esterification of DTPA can enhance its ability to promote the removal of plutonium internally deposited in the liver, presumably by facilitating the penetration of the DTPA through cellular membranes. Intracellular hydrolysis of these esters then presumably liberates the free chelating agent. Unfortunately, the toxicity of the pentaethyl ester precludes its therapeutic use. To determine whether other, possibly less toxic, DTPA esters might be of interest we have developed a procedure for the synthesis of DTPA esters in which both the number and nature of the ester groups can be varied. The key intermediates in these syntheses are acid anhydride derivatives of DTPA. The diethyl ester of DTPA has been prepared in pure form and completely characterized by chemical analysis, and by infra-red and nuclear magnetic resonance spectroscopy. Preliminary experiments have established that the toxicity of this compound is not appreciably different from that of DTPA. Small quantities of the monoethyl ester of DTPA have been isolated and a compound believed to be the triethyl ester has been obtained; modification of the syntheses to provide macroscopic quantities of these derivatives is in progress.

Vitamins A_1 and A_2 are selectively taken up by the liver. Therefore, esterification of these vitamins to DTPA may provide a liver-specific vehicle for delivery of this chelating agent. Preliminary experiments with octadecyl alcohol have indicated that the esterification of DTPA with long-chain lipid-soluble alcohols is possible; chromatographic procedures for the isolation of pure DTPA-lipid-soluble esters are being developed.

Mössbauer Spectroscopy of ^{237}Np. Mössbauer spectroscopy offers the possibility of providing chemical information about the binding of certain radioelements in intact tissue samples. In order to assess the feasibility of this approach, spectra of $^{237}Np^{4+}$ and $^{237}NpO_2^+$ as perchlorates were obtained in frozen aqueous solution, at concentration levels approximating those that might occur in biological specimens. Satisfactory spectra were obtained in both cases, thus demonstrating the potential utility of this technique in characterizing the binding sites for internally deposited actinide ions.

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REMOVAL OF POLYMERIC PLUTONIUM FROM DOGS WITH DTPA AND GLUCAN*

Marcia W. Rosenthal, Arthur Lindenbaum, David W. Baxter, G. Steven Kalesperis, Elizabeth S. Moretti, and John J. Russell

A highly polymeric preparation of plutonium-239 was injected intravenously into five groups of beagle dogs. An untreated control group was killed at six days after injection. The four treated groups, killed at 90 days, received one of the following by intravenous injection: a) 15 mg/kg of glucan on days 6, 34, and 62; b) 100 mg/kg of $CaNa_3DTPA$, twice-weekly for 12 weeks, beginning on day 6; c) both of these treatments; or d) saline. Between days 6 and 90 the level of plutonium in the liver decreased from 92.2% to 85.6% of the injected dose (ID) after saline, to 81.6% after glucan, to 77.8% after DTPA, and to 71.0% after both glucan and DTPA. The decrease to 71.0%, an approximately additive effect, was statistically significant. In dogs treated with either saline or glucan, the bone, and soft tissues other than the liver, had increased plutonium levels that did not occur in animals treated with DTPA. The 90-day excretion of plutonium in feces was low in all groups (3.3% ID, or less), while urinary plutonium was 3.5% ID after saline, 4.0% after glucan, 13.4% after DTPA, and 21.1% after glucan plus DTPA. These results confirm in dogs, as previously determined in mice, the action of glucan as an adjunct to DTPA in removal of polymeric plutonium from the liver.

*Abstract of a paper to be published in Radiation Research.

REMOVAL OF POLYMERIC PLUTONIUM BY DTPA DIRECTED INTO CELLS BY LIPOSOME ENCAPSULATION*

Marcia W. Rosenthal, Yueh-Erh Rahman, Elizabeth S. Moretti, and Elizabeth A. Cerny

DTPA (diethylenetriaminepentaacetic acid) encapsulated within lipid spherules (liposomes) removes more plutonium from the liver and femurs of mice injected with polymeric plutonium than conventional nonencapsulated DTPA. A single intravenous injection, at 3 days after plutonium, of 100 mg/kg of $CaNa_3DTPA$ encapsulated in liposomes made with phosphatidylcholine and cholesterol (3:1) reduced the plutonium in liver to 43-51% of the control level at 10 days, compared to 60% after nonencapsulated DTPA. In the femurs, the plutonium was reduced to 60.4-62.5% of the control level compared to 83-113%. Liposomal DTPA was equally effective when given intraperitoneally, or when stored for 3 days before use. Liposomal DTPA at doses as low as 25 mg/kg was not significantly less effective than a higher dose of 100 mg/kg. Four once-weekly injections of liposomal DTPA continued to give improved removal of plutonium compared to conventional DTPA. When given 24 days after plutonium, liposomal DTPA had a greater advantage over nonencapsulated DTPA in the liver than at 3 days and removed 22% of the plutonium from the femurs, compared to no removal by the nonencapsulated form. To date, DTPA liposomes made with lipids other than phosphatidylcholine and cholesterol, or with different surface charges, offer no advantage for plutonium removal.

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*Abstract of a paper to be published in Radiation Research.