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Retention of Plutonium in Mouse Tissues as Affected By Antiviral Compounds and Their Analogs

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RETENTION OF PLUTONIUM IN MOUSE TISSUES AS AFFECTED BY ANTIVIRAL COMPOUNDS AND THEIR ANALOGS*

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Abstract

RETENTION OF PLUTONIUM IN MOUSE TISSUES AS AFFECTED BY ANTIVIRAL COMPOUNDS AND THEIR ANALOGS

The chelating agent DTPA (diethylenetriaminepentaacetic acid) is an effective therapeutic substance for decorporation of extracellular monomeric plutonium in the mouse and dog, but is much less effective in removing intracellular polymeric plutonium (Pu-P). In the absence of effective therapy, this intracellular plutonium is long retained in the body, particularly in reticuloendothelial tissues like the liver. Our interest, therefore, turned to the development of adjunct substances capable of removing additional plutonium from the liver beyond that removable by DTPA alone. We showed that glucan, a yeast cell wall polysaccharide, is a useful adjunct to DTPA for removal of Pu-P from the mouse liver. Its toxicity, however, makes it a less than desirable drug for potential human use. Therefore, we initiated a search for more soluble (and presumably less hazardous) therapeutic agents similar to glucan, i.e., capable of adjunct action with DTPA. Of over 20 substances tested the most successful results were obtained with two antiviral, antitumor compounds, the pyran copolymers

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XA-124-177 and XA-146-85-2. These are condensation products of divinyl ether and maleic anhydride. Another analog, EMH-227, prepared by condensation of acrylic acid and itaconic acid, was similarly successful. Maximal removal of plutonium from mouse liver was obtained with a single intravenous (I.V.) injection of 10-90 mg/kg of pyran copolymer given 5 days after I.V. Pu-P administration. Although these doses increased splenic uptake of plutonium, a dose of 10 mg/kg produced a minimal increase in the splenic burden while producing maximal removal of hepatic plutonium.

INTRODUCTION

Use of the chelating agent diethylenetriaminepentaacetic acid (DTPA) has been widely accepted as the "treatment of choice" for removal of radionuclides of the actinide series from human patients. However, recent studies in this laboratory, carried out with polymeric preparations of plutonium (Pu-P), have shown that DTPA possesses a serious practical limitation for removal of plutonium from organs (e.g., liver) in which phagocytes represent a significant fraction of the cellular population. The high charge density of DTPA, which is usually administered as the calcium trisodium salt, results in membrane impermeability; as a result, DTPA is relatively ineffective against plutonium deposited intracellularly. This limitation was indirectly confirmed by the demonstrations that Pu-P becomes concentrated in liver cell lysosomes [1] and that a significantly more lipophilic form of DTPA, the pentaethyl ester, was additive with $CaNa_3DTPA$ in its removal of plutonium from the mouse liver [2].

These metabolic and therapeutic results encouraged us to examine the phagocytic behavior of liver cells toward colloidal or particulate forms of plutonium circulating in the blood stream, as well as to consider other therapeutic substances more capable than DTPA of penetrating cell walls and interacting with intracellular Pu deposits. In the design of subsequent experiments, we adopted the working hypothesis that the development of means to control phagocytic function in organs rich in reticuloendothelial elements might allow us to control the uptake and retention of plutonium (as well as of other easily hydrolyzed toxic metals) in these organs. It is ironic that some of the resulting experiments suggested by this hypothesis led to results quite contrary to expectations. For example, our initial success in applying this hypothesis came from the use of glucan, a substance whose use (at least prior to Pu administration) would have been expected to increase the hepatic burden rather than reduce it. Nevertheless, the fact that several interesting therapeutic results have been achieved speaks well for the use of a unifying principle to be tested by the design of appropriate experiments and the evaluation of results obtained therefrom.

To summarize briefly our results with glucan in the mouse and dog [3-6], we have demonstrated that this polysaccharide, which is extracted from cell walls of the yeast *S. cerevisiae*, removes Pu-P from the liver when used alone and, when used as an adjunct to DTPA, removes a significant additional fraction of Pu-P from the liver. Because of the clinical problems inherent in injecting a particulate substance, however,—glucan, unless carefully administered, was found to produce toxic symptoms in dogs (shock, tachycardia, vomiting, convulsions) [5]—it probably is not an ideal adjunct to DTPA for plutonium decorporation in man.

We therefore initiated a search for more soluble (and presumably less hazardous) substances capable of producing a therapeutic effect similar to that of glucan. In this paper, we describe results obtained with two classes of compounds: (1) polysaccharides of biological origin, analogous to glucan, and (2) several types of antiviral (interferon-inducing) compounds or their chemical analogs, some of which are known to induce the appearance of glucan-like inclusions within hepatic cells. In addition, early studies aimed at reducing Pu uptake in mouse liver by blockade or depression of the reticuloendothelial system (RES) are described.

MATERIALS AND METHODS

Plutonium injection solutions

Polymeric plutonium (Pu-P) was generally prepared from $Pu(NO_3)_4$ in concentrated HNO_3 by dilution with water and adjustment to pH 6-7 by drop-wise addition of 1 N NaOH. The degree to which the plutonium underwent hydrolysis and polymerization was determined by ultrafiltration through cellophane bags [7]. The fraction of ultrafilterable (% UF) plutonium in the preparations used in the studies presently described ranged between 8% and 45%. These so-called ungraded preparation of "mid-range" polymeric plutonium [8] were injected intravenously (I.V.) into either male or female CF 1 or female $B6CF_1$/Anl mice 70-100 days of age. The amount injected ranged between 0.7 μCi/kg and 3.0 μCi/kg. The mice received food and water *ad lib.*

Therapeutic test solutions

A list of adjunct test substances, giving the dose(s) and route(s) of administration, the supplier, and the basis for selection is given in Table I. Therapy with $CaNa_3DTPA$ (Geigy or Fluka AG), usually 100 mg/kg (0.25 mmoles/kg) injected intraperitoneally (I.P.), was begun 5 days after administration of plutonium and continued biweekly thereafter for a total of seven to fourteen injections.

Methyl palmitate was suspended in an aqueous vehicle consisting of 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate) and 5% dextrose; the suspension was homogenized for 10 minutes in a Virtis-45 homogenizer just prior to use. The suspension was injected one and two days prior to plutonium injection. Particle sizes ranged downward from six micrometers.

Colloidal carbon was prepared from Gunther-Wagner carbon suspension C 11/1431a according to a procedure described by Biozzi *et al.* [9]. The suspension was injected I.V. into mice five hours before plutonium injection, a time found by these investigators to be optimal for blockade of phagocytes.

The preparation of yeast glucan and its therapeutic application against plutonium in mice have been described previously [4]. Isosclerotan suspensions were prepared for injection in the same way as for glucan. The preparation of other glucan analogs was as follows: Blue Dextran 2000 was prepared as a 0.4% solution in isotonic saline and injected I.V. on days 5, 6, 7 after Pu-P injection [4]. The bacterial cell wall compounds 168-gal

and BR 290-gal were suspended in isotonic saline to provide a concentration of 6 mg/ml, stirred vigorously just before use, and injected I.V. on day 5 after injection of Pu-P.

Poly I:poly C, used as supplied by the manufacturer, was injected I.V. 5 days after injection of Pu-P. The tilorone compounds RMI 10008DA, RMI 10024DA, RMI 11645DA, and RMI 11877DA, as well as the interferon-inducing compound designated as BL-20803 (4(3-dimethlaminopropylamino)-1,3-dimethyl-1H-pyrazolo [3,4-b] quinoline dihydrochloride), were diluted in isotonic saline, adjusted to pH $\sim$ 4, and administered I.V. 5 days after Pu-P. (RMI 10008DA was also given by gavage, 200 mg/kg, 5 days after Pu-P). Quinacrine hydrochloride was dissolved in saline, filtered through an 8 μm Millipore membrane and given as a single dose by gavage.

The pyran copolymers (pyrans), copolymers of divinyl ether and maleic anhydride, as well as the compound EMH-138, a copolymer of acrylic acid and maleic anhydride, and EMH-227, a copolymer of acrylic acid and itaconic acid, were dissolved in double distilled water, adjusted to pH $\sim$ 7 with NaOH, and were usually administered in a single I.V. injection to some mice, and orally to others, 5 days after Pu-P.

Analysis of animal tissues

At the end of each experiment the mice were sacrificed under sodium pentobarbital anesthesia. The livers, spleens, and both femurs were removed and analyzed for plutonium by a liquid scintillation technique as previously described [10].

Optimization of dose level and of time of administration of a pyran copolymer

In order to achieve maximum effectiveness of pyran copolymer XA-124-177, used as an adjunct to DTPA for plutonium decorporation, experiments were performed to determine the optimum time of administration relative to Pu-P injection, and to determine the optimum I.V. dose for plutonium removal. In the first study, groups of five female $B6CF_1$/Anl mice were injected I.V. with 50 mg/kg of pyran XA-124-177 at times ranging from 5 days before, to 21 days after, injection of 1 μCi/kg of Pu-P (19% UF). In addition, except for one control group, the mice received biweekly I.P. injections of $CaNa_3$DTPA. The groups were sacrificed either at 33 or 54 days after injection of Pu-P, and the livers, spleens, and femurs were removed for assay of plutonium content.

In the second study, groups of five female $B6CF_1$/Anl mice that had been injected I.V. 5 days earlier with 1 μCi/kg Pu-P (19% UF) were injected I.V. with doses of pyran XA-124-177 ranging from 0.1 mg/kg to 90 mg/kg of pyran. The animals were also given DTPA biweekly as in the previous experiment. All groups of animals were sacrificed at 33 days after injection of Pu-P, and the livers, spleens, and femurs were removed and assayed for plutonium content.

RESULTS

The effects of all substances so far tested in mice are shown in Tables II and III, which have the following organization: Table II - RES

inhibitors (carbon black and methyl palmitate, also Tween 20, used as a vehicle for the latter); Table III, A. - RES stimulators and analogs (representative results with glucan and Blue Dextran 2000 as reported by Rosenthal *et al.* [4] are included along with isosclerotan and the *B. subtilis* cell wall extracts 168-gal and BR 290-gal); and Table III, B. - Interferon-inducers and analogs (the pyrans, tilorones, quinacrine hydrochloride, Poly I:poly C, BL-20803, EMH-138, EMH-227).

In comparison with saline-treated control mice, no significant effect on the hepatic uptake of Pu occurred when the RES was depressed prior to Pu injection by carbon black, or by methyl palmitate (Table II).

The basis for intercomparison of the various adjunct compounds is partially statistical, partially subjective, and complicated by the use of plutonium injection solutions differing significantly in ultrafilterability. Therefore, the degree of effectiveness of various adjuncts was compared within experimental groups rather than as a whole. For each experiment shown in Table III, adjunct treatment effectiveness, calculated as the difference in % ID between DTPA-treated controls and adjunct-treated mice expressed as a percent of the former (bracketed values), was evaluated as follows: Values of under 10% represented ineffectiveness; with one exception (experiment Pu-108) adjunct treatment yielding values of 30% or greater was considered effective. In comparison with removal of liver plutonium by DTPA alone, given after Pu injection (Table III), no significant adjunct effect was noted for poly I:poly C, Blue Dextran 2000, the tilorones, BL-20803, or orally administered quinacrine hydrochloride or pyran XA-124-177; some additional removal was obtained with 168-gal, BR 290-gal and EMH-138, but the magnitudes of the adjunct effect were so small as to be of little practical interest; significant additional hepatic removal was achieved with intravenously-administered glucan, isosclerotan, pyran XA-124-177 (identical to NSC-46015), pyran XA-146-85-2, and EMH-227. Thus, the only soluble substances inducing removal of plutonium from mouse liver comparable to that induced by glucan particulates were the pyrans XA-124-177 and XA-146-85-2, and EMH-227. Tilorone RMI 10008DA, which gave a positive effect in one experiment was ineffective upon retesting, as were three other tilorones tested.

A single I.V. injection of pyran, given 5 days after Pu-P was essentially as effective as three daily injections begun at 5 days (Table III, B.). Approximately maximal removal of hepatic plutonium was achieved with pyran doses ranging between 10 mg/kg and 90 mg/kg. Pyran doses higher than 25 mg/kg, however, resulted in an approximately two-fold higher splenic burden of Pu than produced by 10 mg/kg (Table IV). The pyrans, like glucan, were without effect in reducing the plutonium burden in the femurs.

Injection of pyran XA-124-177 five days before Pu-P administration resulted in liver burdens at 33 days after Pu that were not significantly different than those of saline- or DTPA-treated controls (Table V), but the splenic burdens were 4 to 6 times those of saline controls and almost twice those of mice posttreated with pyran. Interestingly, the plutonium burdens in the femurs of mice pretreated with pyran were measurably less than those of either saline or DTPA control or of mice posttreated with pyran. As shown by the data obtained at 54 days after Pu-P, the injection of this pyran at 12 days or at 21 days after Pu-P is as effective for removal of hepatic Pu as an injection at 5 days after Pu-P.

DISCUSSION

It is apparent from the data in Table II that neither RES depression induced by the use of methyl palmitate, nor RES "blockade" produced by injecting colloidal carbon of sufficient dosage, affected the uptake by the liver of polymeric plutonium injected at times corresponding to maximum RES depression. (This depression was verified in our mice by measurement of vascular clearance of a test injection of carbon in additional mice.) Several possible explanations for the lack of effect of prior RES depression may be offered: a) The rate of disappearance of plutonium from the blood, or its rate of uptake in the liver, may not be correlated with the final amount deposited in the liver. b) The phagocytic cells of the liver may not play as important a role in the initial uptake of colloidal Pu as originally proposed [11]; perhaps it is mainly the hepatic parenchymal cells that are actively involved in the initial uptake of Pu-P. c) Production of a "blockade" of Kupffer cell phagocytosis by injection of some particles, e.g., colloidal carbon, may not affect the uptake of different particles, in this case colloidal Pu. Saba and Di Luzio [12], using an *in vitro* perfused liver test system, showed that the phagocytic uptakes of different kinds of particulates (e.g. colloidal gold, or albumin aggregates) were independent. They proposed the possibility of a "multiple opsonin system," with each opsonin, or serum factor, being specific for a set of physical and chemical particulate characteristics, e.g., charge, size, or surface receptor configuration.

The ultimate use of yeast cell wall glucan as a practical therapeutic agent for the decorporation of plutonium in man will probably be limited due to toxicological considerations. Nevertheless, it is interesting to note that yeast glucan, when given either alone or with DTPA, was effective in promoting increased removal of plutonium from the liver of both mouse [4] and dog [5,6]. Another glucan, isosclerotan, a polysaccharide isolated from the cell wall of the fungus *Sclerotinia libertiana* was as effective as glucan in increasing removal of Pu from the mouse liver (Table III, A.). On the other hand, similar bacterial cell wall polysaccharide preparations derived from *Bacillus subtilis* were not as effective as glucan or isosclerotan. Since isosclerotan, a polysaccharide structurally distinguishable from glucan, was found to be equally effective *vis-a-vis* glucan, it seems reasonable to suggest that the effectiveness of glucan in removing hepatic plutonium is not specific to the yeast glucan molecule. Clearly, the physical, chemical and biological factors involved in the mechanism of action in these two polysaccharides in inducing plutonium decorporation have yet to be elucidated.

Consideration of the possible relationship between the molecular structure of the substances tested in this work and their ability to decorporate plutonium might well focus on the chemical structure and reactive groups of DTPA, as compared to those of some of the adjunct compounds tested (see Figure 1). One of the first points to be noted in such a comparison is that DTPA has an arrangement of nitrogen and carboxyl groups which is nearly ideal for high binding affinity for a polyvalent metal like plutonium, whereas none of the other compounds shown has a molecular structure likely to exhibit a comparably high binding affinity. A second point is the similarity in the ring structures forming some of the repeating units of the pyrans and the glucans. Thirdly, the structure of the

tilorone shown (which is generally representative of the other tilorones, as well as compounds like quinacrine hydrochloride and BL-20803) is sufficiently different from those of glucan or pyran to suggest that further testing of substances similar to the tilorones might well be unprofitable, in view of their failure to remove hepatic Pu. Fourth, some of the molecular features of the compounds successfully used for plutonium removal are not shared; thus, the pyrans are rich in carboxyl groups, while the glucans possess none.

Chain length (or extent of polymerization) of the test substance may be important for plutonium decorporation. Yet glucan, with a molecular weight of about 6500, was an effective adjunct whereas a low molecular weight pyran, X18503-35-1 [13], was not.

It should be pointed out that the chemical makeup of some of these test substances does not necessarily relate to their physical state as injected into the body. Thus, glucan, with a molecular weight of ~ 6500 [14] is particulate in nature (the particles are basically the yeast cell wall "ghosts"). As injected, the glucan particles retain the approximate shape and dimensions of the yeast cell [4] and are rapidly phagocytized by liver cells. In comparison, all the pyran and EMH copolymers, though large in "peak height" molecular weights, were all soluble as injected.

To further complicate the picture, all the tilorones and pyrans tested are interferon-inducers, but only the higher molecular weight pyrans (and their analogs, the EMH copolymers) were effective in removal of hepatic plutonium.

In view of the variety of substances tested for plutonium decorporation, and the variability in the results obtained, it is important to reemphasize that the main object of this work was to obtain significant removal of otherwise long-retained plutonium with soluble adjunct substances of low toxicity. By this criterion, our interest clearly focuses on the two pyrans XA-124-177 and XA-146-85-2 and on the analogous copolymer, EMH-227. The approximate "peak height" molecular weights of these three substances are 32,000, 22,500 and 44,000 respectively [15]. It is, of course, possible that the similarity in magnitude of these values is coincidental, but it is also possible that this similarity provides a basis for selection of other analogs for therapeutic testing.

If polymers of relatively large molecular size and high negative charge are most efficacious as adjuncts to DTPA in reducing the plutonium burden in the liver, then the mechanism by which they remove plutonium may be quite different from the mechanism by which they induce increased interferon production in living tissues. Although the lower molecular weight pyrans are clinically desirable in that they induce antiviral effects with lower toxicity to the patient, it may well be that pyrans of larger molecular size will be required for plutonium decorporation. Further work with pyrans and their analogs, relating molecular size, shape, and charge to effectiveness in plutonium decorporation, is clearly needed.

In planning further therapeutic testing of successful adjunct compounds in another species, namely the dog, it is important that their

toxic side effects be minimized. The data shown in Table V indicate that effectiveness of intravenously administered pyran XA-124-177, for adjunct activity in removal of plutonium from the liver was achieved at doses of 10-90 mg/kg. For therapeutic use, the most desirable pyran dose (assuming this compound to be representative of the others) would be at the lower end of this range, perhaps 10-20 mg/kg. These low doses, which are in the range already used in human subjects for anti-tumor treatment, would minimize the increased Pu concentrations noted in the spleen after injection of 50 mg/kg or over, and would raise the therapeutic index--an important consideration in human applications.

On the basis of the present mouse data, it appears also that some latitude is allowable in choosing the optimum time of administration of a single dose of pyran XA-124-177 with respect to the injection of the polymeric plutonium (Table V). Treatment with pyran at 2 hours or 1 or 2 days after Pu, although of some value, was judged inferior to later administration of the drug. Treatment with pyran at 5, 12, or 21 days after Pu injection, it will be noted, were equally efficacious in increasing removal of Pu from the liver.

In summary, several types of compounds were tested as adjuncts to the chelating agent DTPA for effectiveness in removing additional amounts of polymeric plutonium from mouse liver beyond the amounts removable by DTPA alone. The three most successful adjunct compounds were the antiviral pyran copolymers XA-124-177 and XA-146-85-2 (condensation products of divinyl ether and maleic anhydride) and the analogous polyanionic compound EMH-227 (condensation product of acrylic acid and itaconic acid). Five days after I.V. injection of Pu-P, the mice, in addition to receiving a regimen of biweekly DTPA treatments, received a single I.V. injection of adjunct compound. Injection of the pyran copolymer IX-124-177 as long as 21 days after Pu-P also resulted in effective removal of hepatic Pu. In preparation for further therapeutic testing in plutonium-injected dogs, it was determined in the mouse that optimal pyran therapy consists of 10-20 mg/kg given as a single I.V. injection at 5 to 21 days after plutonium administration.

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REFERENCES

[1] RAHMAN, Y. E., LINDENBAUM, A., Lysosome particles and subcellular distributions of polymeric tetravalent plutonium-239, Radiat. Res. 21 4 (1964) 575.

[2] MARKLEY, J. F., Removal of polymeric plutonium from mice by combined therapy with the calcium chelate and penta-ethyl ester of DTPA, Int. J. Radiat. Biol. 7 4 (1963) 405.

[3] ROSENTHAL, M. W., SMOLER, M., LINDENBAUM, A., "Combined reticuloendothelial stimulation and long-term, intermittent DTPA therapy in poisoning by polymeric plutonium," Part IV, p. 403, Diagnosis and Treatment of Deposited Radionuclides (KORNBERG, H. A., NORWOOD, W. D., Eds) Excerpta Medical Foundation, Amsterdam (1968).

[4] ROSENTHAL, M. W., BROWN, H., CHLADEK, D. L., MORETTI, E. S., RUSSELL, J. J., LINDENBAUM, A., Removal of plutonium from mouse liver by glucan and DTPA, Radiat. Res. 53 1 (1973) 102.

[5] BAXTER, D. W., ROSENTHAL, M. W., LINDENBAUM, A., Decorporation of monomeric plutonium from the dog by glucan and/or DTPA, Radiat. Res. 55 1 (1973) 144.

[6] ROSENTHAL, M. W., LINDENBAUM, A., BAXTER, D. W., KALESPERIS, G. S., MORETTI, E. S., RUSSELL, J. J., Removal of polymeric plutonium from dogs with DTPA and glucan, Radiat. Res. 63 2 (1975) 253.

[7] LINDENBAUM, A., WESTFALL, W., Colloidal properties of plutonium in dilute aqueous solution, Int. J. Appl. Radiat. Isotop. 16 (1965) 545.

[8] LINDENBAUM, A., LUND, C., SMOLER, M., ROSENTHAL, M. W., "Preparation characterization and distribution in mouse tissues of graded polymeric and monomeric plutonium. Radiochemical and autoradiographic studies," Part II, p. 56, Diagnosis and Treatment of Deposited Radionuclides (KORNBERG, H. A., NORWOOD, W. D., Eds) Excerpta Medica Foundation, Amsterdam (1968).

[9] BIOZZI, G., BENACERRAF, B., HALPERN, B. N., Quantitative study of the granulopectic activity of the reticulo-endothelial system. II: A study of the kinetics of the granulopectic activity of the R.E.S. in relation to the dose of carbon injected. Relationship between the weight of the organs and their activity, Brit. J. Exp. Pathol. 34 (1953) 441.

[10] LINDENBAUM, A., SMYTH, M. A., "Determination of ^{239}Pu and ^{241}Am in animal tissues by liquid scintillation spectrometry," Section III, p. 951, Organic Scintallators and Liquid Scintillation Counting (HORROCKS, D. L., PENG, C.-T., Eds), Academic Press, Inc., New York (1971).

[11] SCHUBERT, J., FRIED, J. F., ROSENTHAL, M. W., LINDENBAUM, A., Tissue distribution of monomeric and polymeric plutonium as modified by a chelating agent, Radiat. Res. 15 2 (1961) 220.

[12] SABA, T. M., DI LUZIO, N. R., Kupffer cell phagocytosis and metabolism of a variety of particles as a function of opsonization, RES, J. Reticuloendothel. Soc. 2 (1966) 437.

[13] GUILMETTE, R. A., LINDENBAUM, A., Progress in the use of pyran copolymers for decorporation of polymeric plutonium, (to be published in Proc. Workshop on the Biological Effects and Toxicity of Pu-239 and Ra-226, Sun Valley, Idaho, (1975)).

[14] BELL, D. J., NORTHCOTE, D. H., The structure of a cell-wall polysaccharide of baker's yeast, J. Chem. Soc. (1950, Part 2) 1944.

[15] BRESLOW, D. S., EDWARDS, E. I., NEWBURG, N. R., Divinyl ether-maleic anhydride (pyran) copolymer used to demonstrate the effect of molecular weight on biological activity, Nature 246 16 (1973) 160.

[16] ROSENTHAL, M. W., MARSHALL, J. H., LINDENBAUM, A., "Autoradiographic and radiochemical studies of the effect of colloidal state of intravenously injected plutonium on its distribution in bone and marrow," Part II, p. 73, Diagnosis and Treatment of Deposited Radionuclides (KORNBERG, H. A., NORWOOD, W. D., Eds) Excerpta Medica Foundation, Amsterdam (1968).

TABLE I

SUBSTANCES TESTED FOR ADJUVANT EFFECT, WITH DTPA, ON REMOVAL OF PLUTONIUM FROM MOUSE TISSUES

Compound	Dose/Injection (mg/kg)	Route of Administration	Supplier	Basis for Selection
Methyl palmitate	1,100	I.V.	Eastman Organic Chemicals	RES depressant
Tween 20	110	I.V.	Sigma Chemical Co.	Vehicle for methyl palmitate
Colloidal carbon (Pelikan C11/1431a)	160	I.V.	Gunther Wagner, Germany	RES Blockade
Glucan	40 or 60	I.V.	Fleischmann Labs.	RES stimulant/depressant
Blue Dextran 2000	40	I.V.	Pharmacia Fine Chemicals, Inc., Sweden	Smaller sized polysaccharide similar to glucan
Isosclerotan	60	I.V.	Prof. Y. Satomura, Osaka City University, Japan	Sclerotinia cell wall polysaccharide similar to glucan
168-gal	60	I.V.	Dr. C. Birdsell, School of Dentistry, Loyola University (Chicago)	B. subtilis cell wall polysaccharides similar to glucan, but smaller particles
BR 290-gal	60	I.V.		
Poly I:poly C	5	I.V.	Microbiological Associates	Soluble polynucleotide; antiviral compound
Tilorones (RMI 10008DA, RMI 10024DA, RMI 11645DA, RMI 11877DA)	25 200	I.V. oral	Dr. R. F. Krueger, Merrill National Labs.	Soluble antiviral compound
BL-20803 (Lot #11997-42B)	25	I.V.	Dr. P. Siminoff, Bristol Labs.	Soluble antiviral compound
Quinacrine HCl	500	oral	Winthrop Labs.	Structure similar to tilorones; possible antiviral activity
Pyran copolymers (XA-124-177, XA-146-85-2, X18503-35-1, X18503-35-3)	50 or 70 400	I.V. oral	Dr. D. S. Breslow, Hercules, Inc.	Soluble antiviral compounds
Pyran copolymer NSC-46015	50	I.V.	Prof. A. E. Munson, Med. College of Va.	Soluble antiviral compound
EMH-138	50	I.V.	Prof. E. M. Hodnett, Oklahoma State University	Polyanionic copolymers analogous to pyrans
EMH-227	25	I.V.		

TABLE II

EFFECT OF RES INHIBITION PRIOR TO INJECTION OF POLYMERIC PLUTONIUM ON THE DISTRIBUTION OF Pu IN MOUSE TISSUES

		Percent of injected dose			
Experiment	Treatment	Liver	Spleen	Femurs	Marrow Sample*
CF 1 ♂ mice; sacrificed 5 days after injection of 30% UF Pu-P (Expt. Pu-47)	Saline	53.2	1.34	1.65	0.0799
	Colloidal carbon	49.4	1.84	2.01	0.0956
	Methyl palmitate	49.6	0.95	2.12	0.0641
	Tween 20	54.8	1.12	1.62	0.0713

*Procedure for obtaining mouse marrow samples is described in [14].

TABLE III

EFFECT OF VARIOUS SUBSTANCES GIVEN AS ADJUNCTS TO DTPA, 5 DAYS AFTER INJECTION OF POLYMERIC PLUTONIUM ON THE DISTRIBUTION OF Pu IN MOUSE TISSUES

A. EFFECT OF GLUCAN AND OTHER POLYSACCHARIDES OF BIOLOGICAL ORIGIN

Experiment	Treatment*	Percent of injected dose		
		Liver	Spleen	Femurs
CF 1, ♀ mice; sacrificed 29 days after injection of 14% UF Pu-P (Expt. Pu-69 [4])	Saline + Saline	28.4	1.57	2.11
	Saline + DTPA (7)	19.9	1.12	1.23
	Glucan (40 mg/kg on days 5, 6 and 7) + DTPA (7)	10.3 [48.2]†	0.645	1.18
	Blue Dextran 2000 (40 mg/kg on days 5, 6 and 7) + DTPA (7)	19.2 [3.5]	1.17	1.22
CF 1, ♀ mice; sacrificed 49 days after injection of 8% UF Pu-P (Expt. Pu-88)	Saline + Saline	33.8	1.06	1.47
	Saline + DTPA (11)	31.1	1.17	0.831
	Glucan (60 mg/kg) + DTPA (11)	20.2 [35.0]	1.22	N.A.
	Isosclerotan (60 mg/kg) + DTPA (11)	19.6 [37.0]	1.28	N.A.
	168-gal (60 mg/kg) + DTPA (11)	25.2 [19.0]	1.29	N.A.
	BR 290-gal (60 mg/kg) + DTPA (11)	25.1 [19.3]	1.36	N.A.

TABLE III (cont'd)

B. EFFECT OF ANTIVIRAL COMPOUNDS AND ANALOGS

Experiment	Treatment*	Percent of injected dose		
		Liver	Spleen	Femurs
CF 1, ♀ mice; sacrificed 49 days after injection of 8% UF Pu-P (Expt. Pu-88)	Saline + Saline	33.8	1.06	1.47
	Saline + DTPA (11)	31.1	1.17	0.831
	Pyran NSC-46015 (70 mg/kg on days 5, 6 and 7) + DTPA (11)	15.8 [42.1]	2.59	N.A.
	Tilorone RMI 10008DA (25 mg/kg) + DTPA (11)	22.8 [26.7]	1.09	N.A.
	Poly I:Poly C (5 mg/kg) + DTPA (11)	29.5 [5.1]	1.03	N.A.
CF 1, ♀ mice; sacrificed 32 days after injection of 45% UF Pu-P (Expt. Pu-100)	Saline + Saline	21.0	0.661	2.90
	Saline + DTPA (8)	14.9	0.681	1.70
	Pyran XA-124-177 (50 mg/kg) + DTPA (8)	7.49 [58.5]	1.80	1.47
	Pyran XA-124-177 (400 mg/kg, oral) + DTPA (8)	13.9 [6.7]	0.602	2.10
	Pyran XA-146-85-2 (50 mg/kg) + DTPA (8)	8.95 [39.9]	1.45	1.89
	Tilorone RMI 10008DA (25 mg/kg) + DTPA (8)	16.1 [-8.1]	0.632	1.61
	Tilorone RMI 10024DA (25 mg/kg) + DTPA (8)	14.3 [4.0]	0.537	1.33

TABLE III B. (cont'd)

Experiment	Treatment*	Percent of injected dose Liver	Spleen	Femurs
	Tilorone RMI 11645DA (50 mg/kg) + DTPA (8)	14.0 [6.0]	0.579	1.43
	Tilorone RMI 11877DA (50 mg/kg) + DTPA (8)	13.5 [9.4]	0.613	1.46
	BL-20803 (25 mg/kg) + DTPA (8)	16.0 [-7.4]	0.644	1.69
	Quinacrine HCl (500 mg/kg, oral) + DTPA (8)	16.0 [-7.4]	0.646	1.47
$B6CF_1$/Anl ♀ mice; sacrificed 33 days after injection of 19% UF Pu-P (Expt. Pu-108)	Saline + Saline	26.5	1.62	2.21
	Saline + DTPA (8)	23.5	1.59	1.52
	Pyran XA-124-177 (50 mg/kg) + DTPA (8)	18.9 [19.6]	5.12	1.71
	Pyran XA-124-177 (50 mg/kg) + Saline	24.8 [-5.5]	5.32	2.30
	EMH-138 (50 mg/kg) + DTPA (8)	20.3 [13.6]	3.56	1.68
	EMH-227 (25 mg/kg) + DTPA (8)	17.9 [23.8]	4.95	1.90

*A single adjunct treatment was given I.V. 5 days after Pu injection except where indicated. DTPA treatments were also initiated 5 days after Pu and continued biweekly (total number in parentheses).

$$\dagger [\ ] \equiv \frac{\%\ ID_{DTPA\ alone} - \%\ ID_{Adjunct}}{\%\ ID_{DTPA\ alone}} \times 100$$

N.A. = not analyzed

TABLE IV

EFFECT OF DOSE OF A PYRAN ADMINISTERED AS AN ADJUNCT TO DTPA ON THE RETENTION OF PLUTONIUM IN MOUSE TISSUES*

Pyran (mg/kg)	Percent of injected dose		
	Liver	Spleen	Femurs
0.0 (no DTPA)	26.5	1.62	2.21
0.0	23.5	1.59	1.52
0.1	25.0	1.60	1.51
1.0	24.8	1.62	1.65
10	20.8	2.48	1.58
25	19.1	3.79	1.77
50	18.9	5.12	1.71
75	20.7	5.36	1.71
90	20.6	4.91	1.65

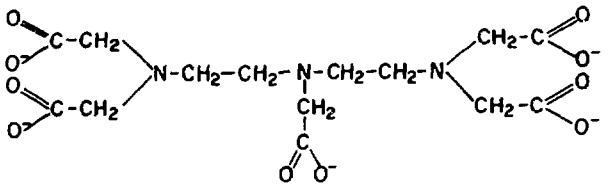
*Pyran XA-124-177 was administered I.V. 5 days after injection of polymeric Pu. DTPA (100 mg/kg or 0.25 mmoles/kg) was injected I.P. biweekly, beginning 5 days after Pu. Mice were sacrificed 33 days after Pu.

TABLE V

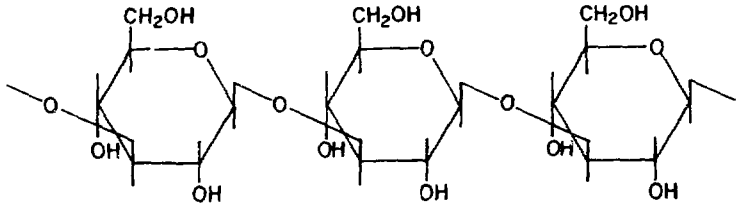
INFLUENCE OF TIME OF ADMINISTRATION OF PYRAN XA-124-177 ON THE RETENTION OF Pu IN MOUSE TISSUES*

Adjunct Treatment			Days post	Percent of injected dose		
Substance	Day injected relative to Pu	DTPA injections	Pu at sacrifice	Liver	Spleen	Femurs
Saline	5	0	33	27.2	1.56	2.01
Saline	5	8	33	22.7	1.40	1.64
Pyran	-5	0	33	28.1	8.99	1.81
Pyran	-5	8	33	22.7	8.21	1.08
Pyran	-1	8	33	23.1	7.30	1.28
Pyran	0.08	8	33	19.3	4.98	1.51
Pyran	1	8	33	21.7	4.39	1.95
Pyran	2	8	33	20.3	4.33	1.85
Pyran	5	8	33	16.5	4.40	1.70
Saline	5	0	54	22.6	1.44	2.15
Saline	5	14	54	21.3	1.43	1.54
Pyran	5	14	54	14.7	4.57	1.44
Pyran	12	14	54	14.9	4.91	1.58
Pyran	21	14	54	13.9	3.99	1.56

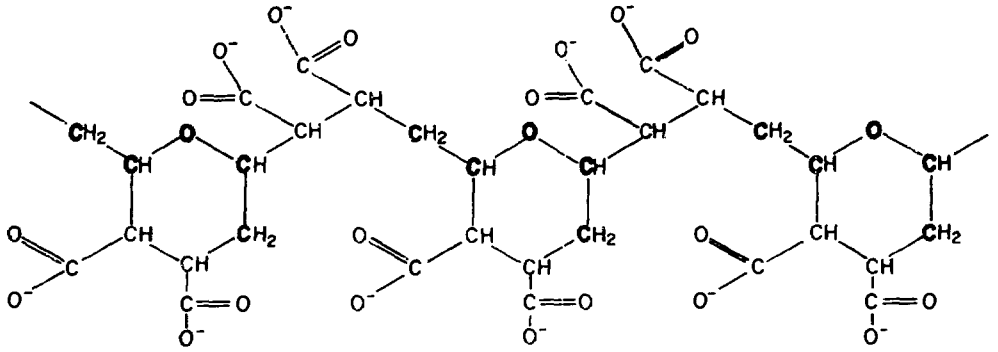
*Pyran was administered I.V. at times ranging from 5 days before, to 21 days after, injection of Pu-P. DTPA (100 mg/kg or 0.25 mmoles/kg) was injected I.P. biweekly, beginning 5 days after Pu.



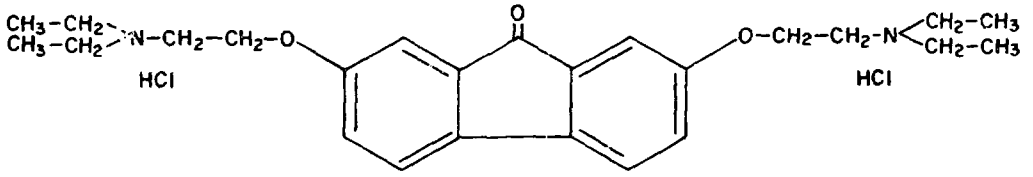
DIETHYLENETRIAMINEPENTAACETIC ACID
(DTPA)



β-1,3-GLUCAN, M.W.~6500



DIVINYL ETHER MALEIC ANHYDRIDE COPOLYMER
(PYRAN COPOLYMER, M.W. 5,200-32,000)



2,7-BIS[2-(DIETHYLAMINO)ETHOXY]FLUOREN-9-ONE DIHYDROCHLORIDE
(TILORONE)

Fig. 1. Chemical structures of some organic compounds used to promote the removal of plutonium from mouse tissues.