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PLUTONIUM DECORPORATION BY MIXED LIGAND CHELATES

MASTER

Final Report
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ABSTRACT

The effects of mixed ligand chelates on the removal of plutonium and thorium from the tissues of experimental animals were investigated. The primary ligands, L_1, used to date were mainly EDTA and DTPA, while the secondary ligands, L_2, were of the bidentate type, as, for example, catechol, salicylic acid, and benzohydroxamic acid. A suitably chosen mixed ligand system binds Pu or Th at physiological pH's about 10^{11}-10^{15} times greater than EDTA or DTPA. An ultrafiltration screening procedure was employed in which soluble Pu or Th salts were injected into mice, the livers were removed several days later and homogenized in the presence of different concentrations of the single and mixed chelants. Similarly, in vivo studies were carried out in which mice were treated 4 hours post Pu (monomeric) injection (I.P.) with a single injection (I.P.) of the chelant systems. Results to date show that the mixed chelant systems are roughly 10 times more effective than EDTA or DTPA alone. Thus, under conditions where DTPA alone (5×10^{-5}M) removed 1.1% of Pu or Th, DTPA + equimolar catechol removed 13.5% and 15% respectively. Catechol alone had no significant effect. In an in vivo decorporation experiment, a single injection of DTPA plus catechol reduced the liver burden from 45% to 2.3% and the skeleton from 22% to 5.5%.

Future research on decorporation of Pu consists in the screening and testing of several dozen promising mixed ligand chelants. The best of these undergo genetic and toxicological testing including determinations of endogenous trace metal removal.

I. INTRODUCTION

As described in the initiating proposal, we carried out ultrafiltration screening experiments using selected mixed chelants and ^{239}Pu and ^{234}Th radionuclides. We also ran some in vivo experiments. Some of the results obtained in the ultrafiltration and in vivo experiments are described below. It is gratifying to report that the results obtained thus far bear out the expectations expressed in the initiating proposal, namely, that the decorporation of Pu and Th by selected mixed ligand chelates is, indeed, far greater than that of EDTA or DTPA alone.

In view of my 6 months sabbatical leave, which began 2 months after the project was funded, the research did not progress as rapidly or completely as it would if I had been in residence. However, I kept close track of the investigations and received frequent writeups. In any event, most of the funds allotted to the project were unexpended, so that I am now able to resume personal supervision and operation of the investigation in collaboration with highly qualified faculty members at the Ph.D. level at Hope College.

II. MATERIALS AND METHODS

A. Ultrafiltration

Female Hilltop mice (25-30 g) were injected intraperitoneally with monomeric plutonium or thorium citrate. The following day the livers are removed and placed in the homogenizer tube containing 1.35 ml of chelant of a given composition and concentration. After homogenization, 50λ samples are taken for counting. Then 1.5 ml samples of homogenate are placed in prepared dialysis (Visking) bags. After centrifugation for 40 minutes at about 900g, 50λ samples of the clear ultrafiltrate are deposited directly on planchets, dried, flamed, and counted.

The ultrafilterability is defined as:

$$\% \text{ Ultrafilterability} = \frac{\text{cpm/vol (ultrafiltrate)}}{\text{cpm/vol (homogenate)}} \times 100$$

B. In Vivo

Mice were injected I.P. with 0.3 ml of monomeric ^{239}Pu citrate solution. Four hours later the mice were injected I.P. with single or mixed chelant. Three days later the mice were killed and the liver, kidneys, and femur were removed for Pu assay. The tissues were wet ashed in fuming

nitric acid, and then made to volume. 5 to 50λ volumes were taken for counting by direct deposit on planchets.

C. Comments

The work should proceed at a far more rapid rate at Hope College because: (1) More qualified research associates; (2) Direct participation in the experiments by the principal investigator; and (3) The use of a liquid scintillation counter which includes the ashing of the tissue samples directly in the counting vials.

III. RESULTS AND DISCUSSION

A. Ultrafiltration

Some of the results are summarized in Figures 1 and 2. Note the large improvement obtained with the mixed chelants, especially catechol -- even though catechol itself had little or no effect. For example, DTPA alone at 5×10^{-5}M caused about 1.1% of ^{234}Th to be removed from the liver while DTPA + Catechol removed 13.5%! Similarly, DTPA + Catechol removed more than 15% of ^{239}Pu compared to 1.1% for DTPA.

The efficiency of removal, that is, ultrafilterability, increased if the molarity of the secondary ligand, e.g., catechol or benzohydroxamic acid was increased relative to EDTA or DTPA.

When the concentration of the primary ligand concentration was increased, the synergistic effect of the mixed ligand chelate system was still apparent (Fig. 2).

Several dozen combinations can be expected to be as good or better than those described here. We have found synergistic effects involving HEDTA + NTA and other systems, as suggested in the combinations mentioned (Table IV, page 30 in proposal).

B. In Vivo Decorporation

The DTPA + Catechol system was markedly more effective than DTPA alone for the decorporation of ^{239}Pu (Fig. 3). This was manifested in the liver, skeleton, and kidneys. The mixed chelant system reduced the Pu in the liver, for example, from 45% to 2.3% in a single injection. Similarly, the skeleton burden was reduced from 22% to 5.5%!

IV. FUTURE RESEARCH

We have a systematic series of investigations to carry out as described in the proposal. However, now that we have direct experimental evidence for the marked synergistic effects of mixed ligand chelates, we can now sharpen and refine our research plan.

The approach we propose to follow in the immediate future is as follows:

1. Ultrafilterability screening using EDTA and DTPA, in general, as the primary ligands, and numerous bidentate and tridentate chelants as the secondary ligands. Molar ratios of 1:1 and 1:2 will be employed.

2. Acute toxicity (full dose-response) in vivo of various mixed chelant systems in preparation for decorporation of ^{239}Pu and ^{234}Th. (The latter will be run simultaneously in the liquid scintillation counter in order to increase our productivity.)

3. Earlier emphasis on in vivo decorporation experiments will be given than previously scheduled. In other words, the in vivo experiments will be carried out while the ultrafiltration screening experiments are in progress.

4. The most promising mixed chelant systems will be subjected to detailed studies, e.g. (1) Chronic toxicity including cytogenetic effects; (2) Effects on monomeric and polymeric Pu will be tested; (3) Effects of mixed chelant systems when administered at different times following Pu administration.

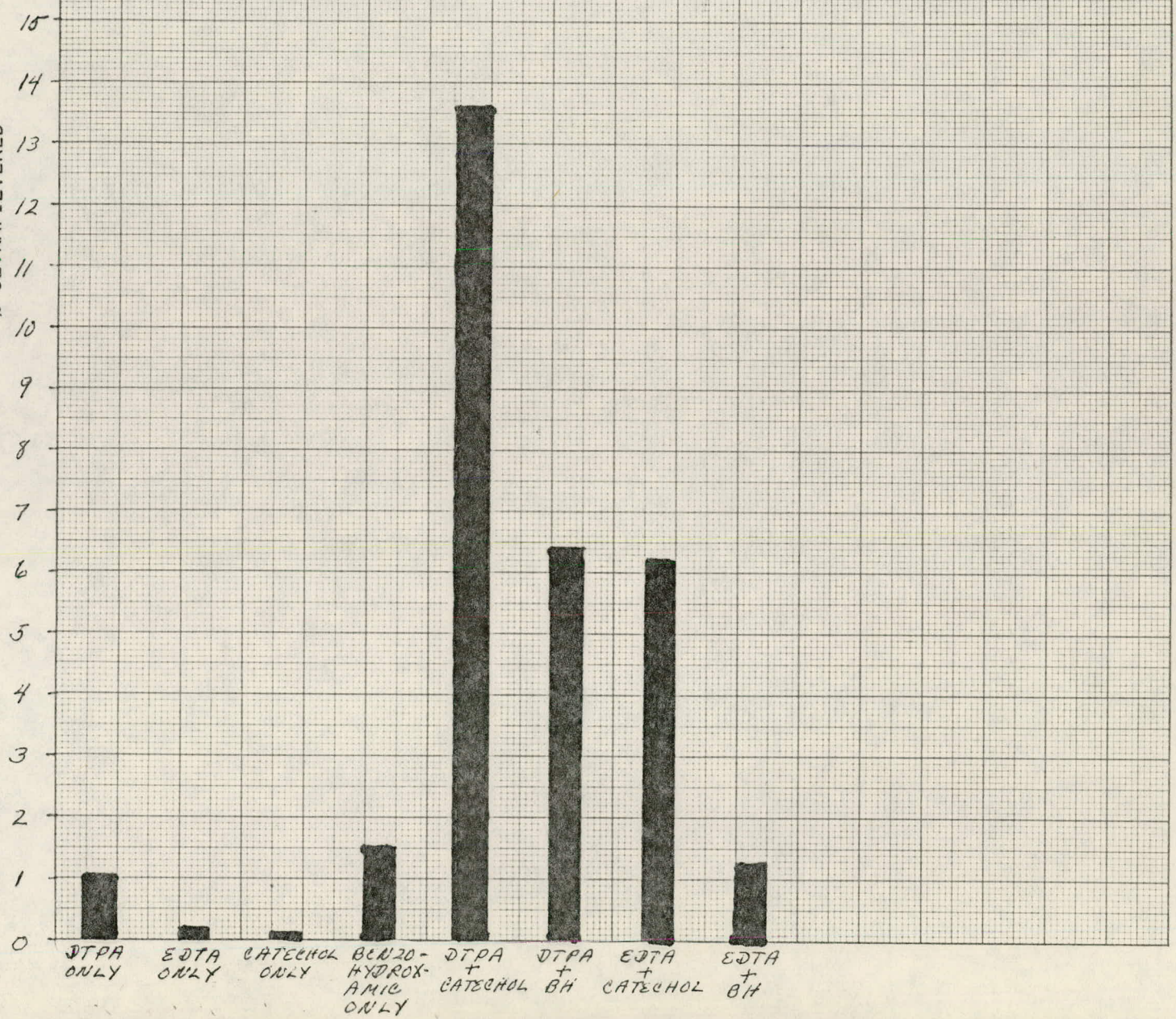
5. The schedule of experiments and the details to which a given mixed chelant system will be investigated will depend on the effectiveness relative to the single chelant system.

V. COMPLIANCE, EFFORT EXPENDED, ETC.

The principal investigator devoted a minimum of 40% of his time to the research project during the months of June, July, and August, 1976. In early September, as he so informed ERDA, he went to the Netherlands on sabbatical leave and returned to the University of Pittsburgh on March 2, 1977. During his leave he devoted about 10% of his time to the project by frequent communication with his research assistant. During March 1977 and continuing into April he has devoted about 65% of his time to the project. However, as he informed ERDA, he is transferring his research activities to Hope College, Holland, Michigan 49423 as of April 15. Arrangements for the transfer of the Contract to Hope College are in progress.

Since the principal investigator was on leave for part of the program, and did not fully staff the project, most of the funds for the program were unexpended and he has requested that these unexpended funds be used to support the Contract for one year beginning April 15, 1977 without additional funding so that the research can be resumed as rapidly as possible. In view of the very promising and unprecedented findings, the principal investigator is looking forward to early completion of the screening stages of the research program in collaboration with especially well qualified collaborators as described in the modified proposal submitted last month to the Office of University Programs in ERDA.

FIGURE 1: Ultrafilterability of monomeric ^{234}Th as affected by mixed chelants.
Primary chelants = DTPA or EDTA
$5x10^{-5}M$ and $1x10^{-4}$
Secondary chelants = $5x10^{-5}M$



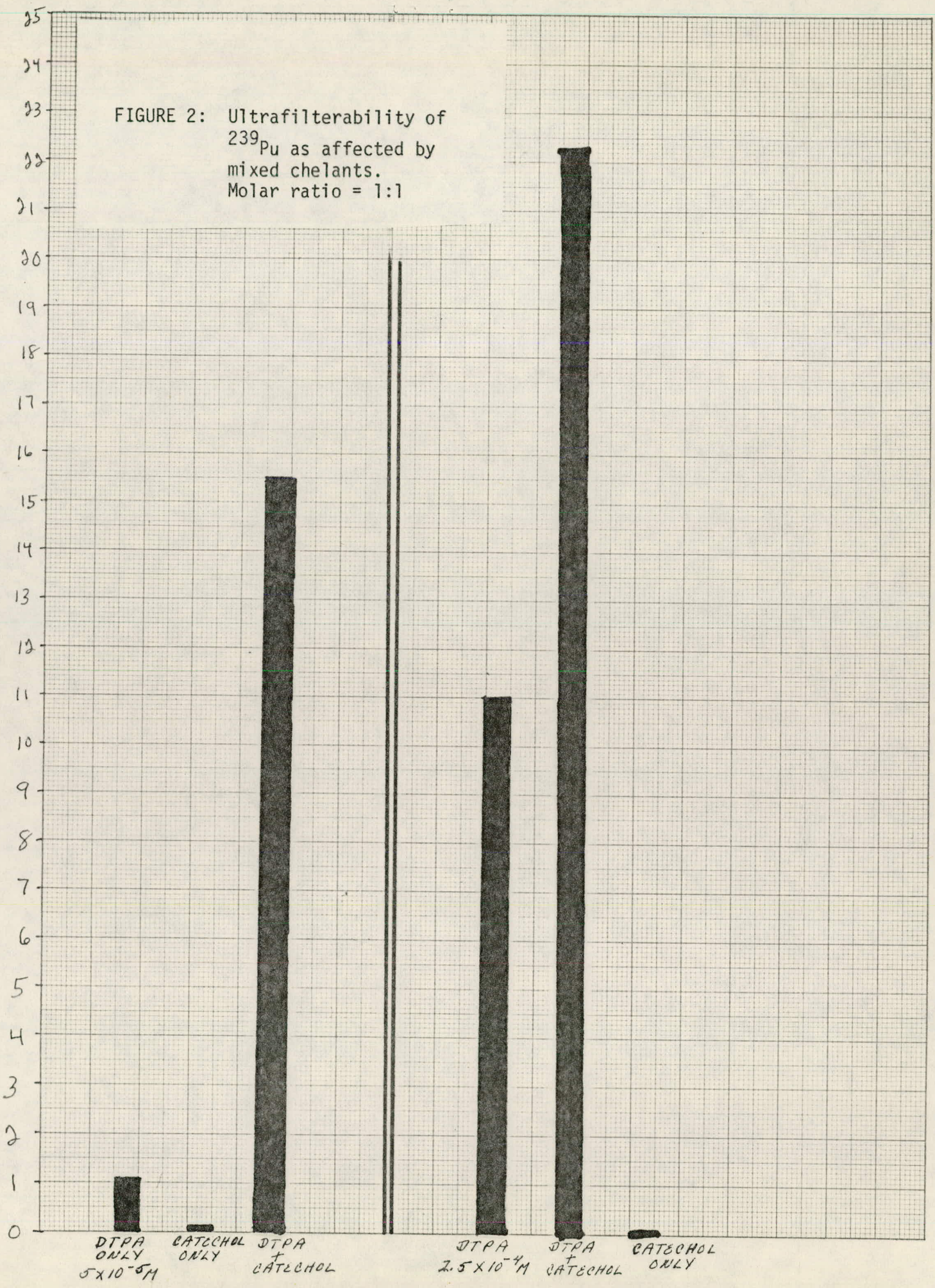


FIGURE 2: Ultrafilterability of ^{239}Pu as affected by mixed chelants. Molar ratio = 1:1

FIGURE 3:* In vivo decorporation of monomeric ^{239}Pu from mice given Pu I.P., then 4 hours later 0.34 μmoles/kg of DTPA or DTPA + catechol. The mice were killed 4 days later.