# Biochemical Studies of the Interactions of Plutonium, Neptunium and Protactinium with Blood and Liver Cell Proteins*

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A sound knowledge of the behaviour of actinide elements in animals and man is an essential prerequisite for the realistic assessment of the hazards which may result from actinide release into the environment from nuclear installations, reprocessing plants or waste repositories. The liver and skeleton are the principal deposition sites for Pu and other actinides in all the animal species examined, including man, and within the liver cells, the lysosomes form the major intracellular deposition sites for Pu(IV) and for Am(III), Cm(III) and Cf(III) [1, 2]. Recent evidence indicates that Np and Pa are also deposited in lysosomes after injection of Np(V) and Pa(V) [2, 3]. In blood serum, actinides are transported on the iron-carrier protein transferrin [2] and the transfer from the cell membrane to the lysosome appears to involve interactions with ferritin and other intracellular proteins.

This report discusses the interactions of $^{239}$Pu, $^{237}$Np and $^{233}$Pa (elements which are important in relation to the long-lived waste from the nuclear fuel cycle) with serum and other proteins.

## Experimental

The methods used to study subcellular distribution and the chromatographic procedures used to study protein–metal interactions have been described elsewhere [2–4].

## Results

Figure 1 illustrates the almost quantitative association of the actinides with the iron-transport protein transferrin in rat blood serum. This Figure shows the elution profiles obtained when the fractions containing the albumin plus transferrin peak obtained by chromatography of serum on Sephacryl S-300 are rechromatographed on DEAE–Sephadex. This peak contains all the radioactivity in the serum and the ion-exchange chromatography indicates that it is wholly associated with transferrin. Similar studies with Th [1] show that this actinide is also carried on transferrin in serum; less convincing evidence suggests that Am and Cm are also associated with this protein [1].

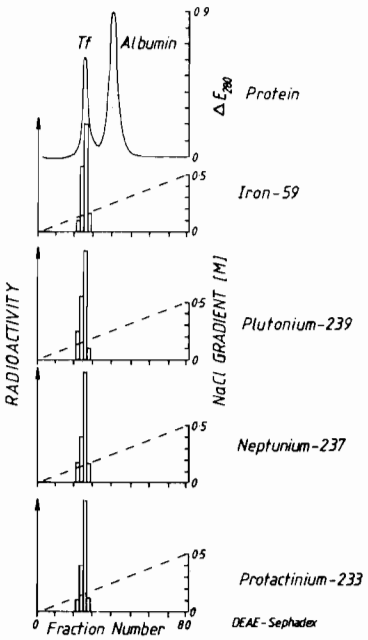


Fig. 1. The elution profiles of $^{59}$Fe, $^{239}$Pu, $^{237}$Np and $^{233}$Pa after chromatography on DEAE–Sephadex of the albumin–transferrin fraction obtained by gel-filtration of rat blood serum on Sephacryl S-200. The results show a clear association with transferrin.

Evidence obtained by carrier-free electrophoresis for the deposition of Pu, Np and Pa in the lysosomes of rat liver is shown in Fig. 2. This Figure shows the clear association of Pu, Np and Pa with the lysosomal marker enzyme, acid phosphatase, and the virtual absence of radioactivity in the peak of activity of the mitochondrial marker, glutamate dehydrogenase.

The distribution of $^{233}$Pa amongst the proteins of liver cytosol 0.5 and 12 h after injection of $^{233}$Pa citrate is shown in Fig. 3. At the earlier time, much of the protein-bound activity is associated with an unidentified protein of $M_r$ *ca.* 200 000, but by 12 h after injection the major part of the protein-bound activity is associated with the iron-storage protein ferritin. At both times some of the radioactivity is bound by low molecular weight ligands, of which citrate could be one component. Essentially similar results have been obtained with plutonium [5].

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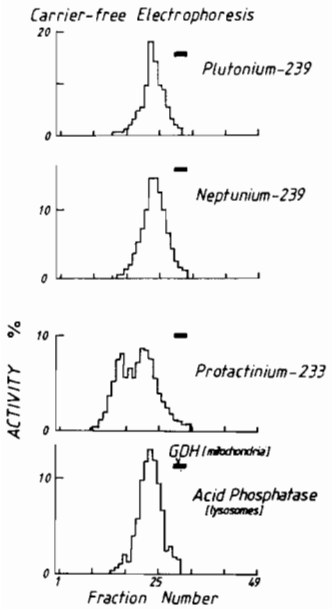


Fig. 2. The results of the subcellular fractionation of rat liver organelles by carrier-free electrophoresis. $^{239}$Pu, $^{237}$Np and $^{233}$Pa show similar mobility to the lysosomal marker enzyme, acid phosphatase. The horizontal bar corresponds to the three peak fractions of the mitochondrial marker, glutamate dehydrogenase (GDH).

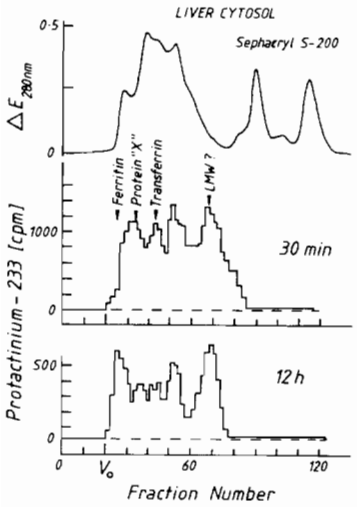


Fig. 3. The elution profile obtained by chromatography of the soluble fraction (cytosol) of liver obtained from rats injected with $^{233}$Pa citrate, 0.5 to 12 h previously. At 0.5 h the main protein bound species is the complex with 'Protein X', while after 12 h ferritin represents the major binding protein.

## Discussion

Figure 4 represents a diagrammatic representation of current knowledge about the biochemical interactions of actinides in the mammalian body. Following

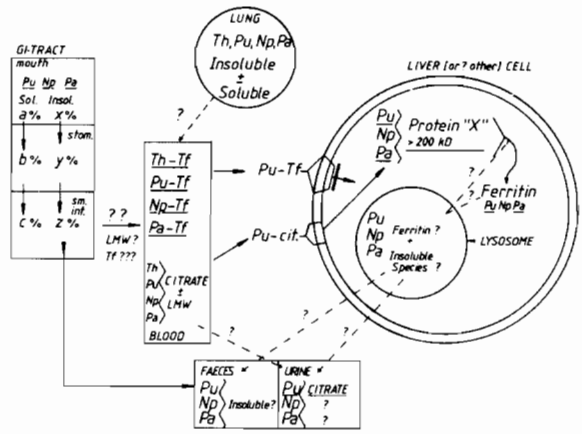


Fig. 4. Diagrammatic representation of the interactions of actinides with proteins and other ligands, the blood, cells and body compartments.

ingestion, the actinides undergo major changes in the fractions of soluble and insoluble material during the passage from the mouth to the absorptive surfaces in the duodenum [6, 7]. The mechanisms of transfer from gut or lung to the blood are not yet known, but they most probably involve low molecular weight complexes; in the blood transferrin represents the major binding species, but again a small fraction of low molecular weight complexes may be important in transfer from the blood through the cell membrane. Complexation with transferrin appears to block cellular uptake, at least by liver cells [8]. After passage through the cell membrane, the actinides appear to be bound first by a protein of $M_r$ *ca.* 200 000, so far unidentified, but after a few hours ferritin becomes the major binding protein. Ferritin or other iron-storage proteins may represent the major binding species within the lysosomes. The mechanisms by which actinides are lost from cells is not known. Although Fig. 4 indicates many areas of ignorance or uncertainty, the importance of complex formation with proteins and other biological ligands is clearly demonstrated. In the gastrointestinal tract and in the lungs, hydrolytic reactions also appear to play an important role in relation to all three actinides. The remarkable similarity in the interactions of Pu, Np and Pa with proteins and other ligands *in vivo* suggests that the latter two elements, which in simple aqueous solutions exist predominantly in the pentavalent state, probably undergo reduction to the tetravalent state under physiological conditions.

## Acknowledgement

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## References


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