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# The bioinorganic chemistry of Np, Pu and Am in mammalian liver

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**Abstract**

Investigations of the sub-cellular distribution patterns of $^{239}$Np and $^{237}$Np in rat liver have shown that the distribution of neptunium is time and mass dependent. One hour after administration, $^{237}$Np deposits predominantly in the cytosol, whereas at later times after exposure (24 h to 40 days) the nuclei and lysosomes are the main binding sites. In contrast, 24 h after exposure, the $^{239}$Np was found mainly in the cytosol. Investigations in baboons with $^{237}$Np, $^{239}$Pu and $^{241}$Am have shown that the cytosol was the main binding site 24 h after exposure, and that nuclei and lysosomes were involved at later times after exposure (10 days). In cytosol, $^{239}$Pu and $^{241}$Am were bound mainly to ferritin irrespective of the time of sacrifice. For $^{237}$Np, it was shown, both in rats and baboons, that the radionuclide binds to two proteins soon after exposure (1–24 h) with molecular weights of 450 and 200 kDa, respectively. The former was identified as ferritin, but the latter remains unidentified. At later times (1–40 days), Np was found to be bound mainly by ferritin and by high-molecular weight compounds. 

*Keywords:* Actinides; Biochemistry; Rat; Primates

## 1. Introduction

The local effects of incorporated radionuclides in target cells are difficult to predict because of the lack of knowledge concerning the intracellular distribution of the elements. After incorporation, radionuclides can be deposited in various cellular compartments, depending on the element, the chemical form and the mass administered. The internal deposition of plutonium, americium and curium in the liver cells has been extensively studied during the last 20 years [1–6]. These studies showed that these elements were associated mainly with lysosomal structures and that different mechanisms of lysosomal uptake are involved for actinides as polymeric and monomeric forms [7]. All these studies were of prime interest but, unfortunately, limited to rodents. Moreover, only few of them were focused on neptunium, whereas the sparse data obtained to date indicated that this element had a distinctly different behaviour from the other actinides [8,9]. The purpose of this paper is to present new data obtained with neptunium in rats and some other studies undertaken with plutonium, americium and neptunium in baboons. The preliminary data obtained with baboons confirm most of the results obtained in rodents, and showed that neptunium behaves differently from plutonium and americium.

## 2. Material and methods

The methods used to study subcellular distribution and the chromatographic procedures used to study protein–metal interactions have been described elsewhere [8,9].

## 3. Results

Fig. 1 illustrates the quantitative association of $^{237}$Np with the different structures of rat liver cells, after administration as nitrate. This figure shows that, 1 h after administration, Np was deposited predominantly in the cytosol (69%), whereas at later times after exposure (1–40 days) the radionuclide was bound to the heaviest fractions of the cells (30–55% in the nuclei and 9–20% in mitochondria/lysosomes, respectively). Separation of lysosomes from mitochondria showed that neptunium was bound mainly by the secondary and tertiary lysosomes, and that mitochondria were not involved in the binding of the

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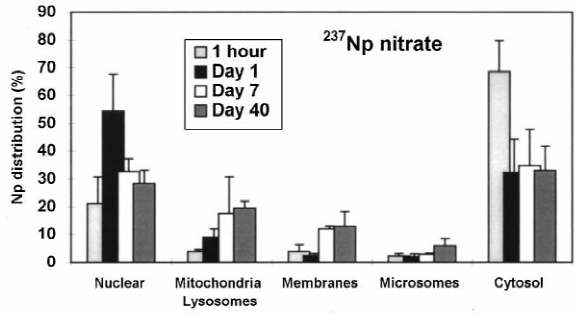


Fig. 1. The subcellular distribution of neptunium in rat liver 1 h to 40 days after injection of $^{237}$Np nitrate.

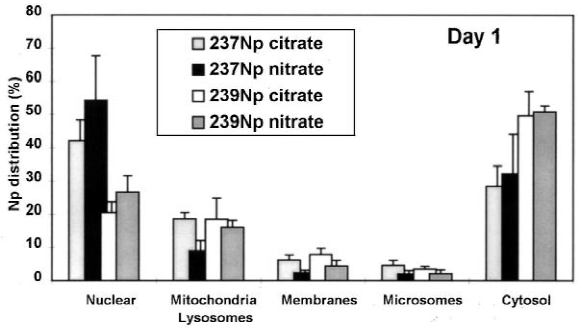


Fig. 2. The subcellular distribution of neptunium in rat liver 1 day after injection of $^{239}$Np or $^{237}$Np.

element (results not shown). This intracellular distribution depends, however, on the mass of the radionuclide injected. Fig. 2 shows that the nuclei were the main target organelles 1 day after contamination when high masses of Np were injected (1.2 mg $^{237}$Np kg$^{-1}$). By contrast, when lower masses of Np were injected (17 pg $^{239}$Np kg$^{-1}$), a large fraction (50%) remained in the cytosolic fraction. In this study, the initial chemical form of the radionuclide (nitrate or citrate) had no effect on its intracellular distribution.

The distribution of $^{237}$Np amongst the proteins of liver cytosol 1 h to 40 days after injection of $^{237}$Np nitrate is shown in Fig. 3. This figure shows that the distribution of the radionuclide is time dependent. In the cytosol of rats sacrificed early after contamination (1 h to 1 day), neptunium eluted mainly in two peaks (Fig. 3). The first coincided with the elution of ferritin, whereas the second is associated with an unidentified protein of MW 200 kDa. When animals were sacrificed either 7 or 40 days after contamination, the major part of the radionuclide was associated with the ferritin while the rest co-eluted with macromolecules of MW>1500 kDa. In all cases, the fraction eluted with the low-molecular weight compounds represented less than 5% of the total cytosolic radionuclide.

The results of the experiments with two baboons injected with a mixture of $^{237}$Np, $^{239}$Pu and $^{241}$Am as citrate (29.2 μg kg$^{-1}$ $^{237}$Np, 1.3 μg kg$^{-1}$ $^{239}$Pu and 25 ng kg$^{-1}$ $^{241}$Am, respectively) are summarised in Figs. 4 and

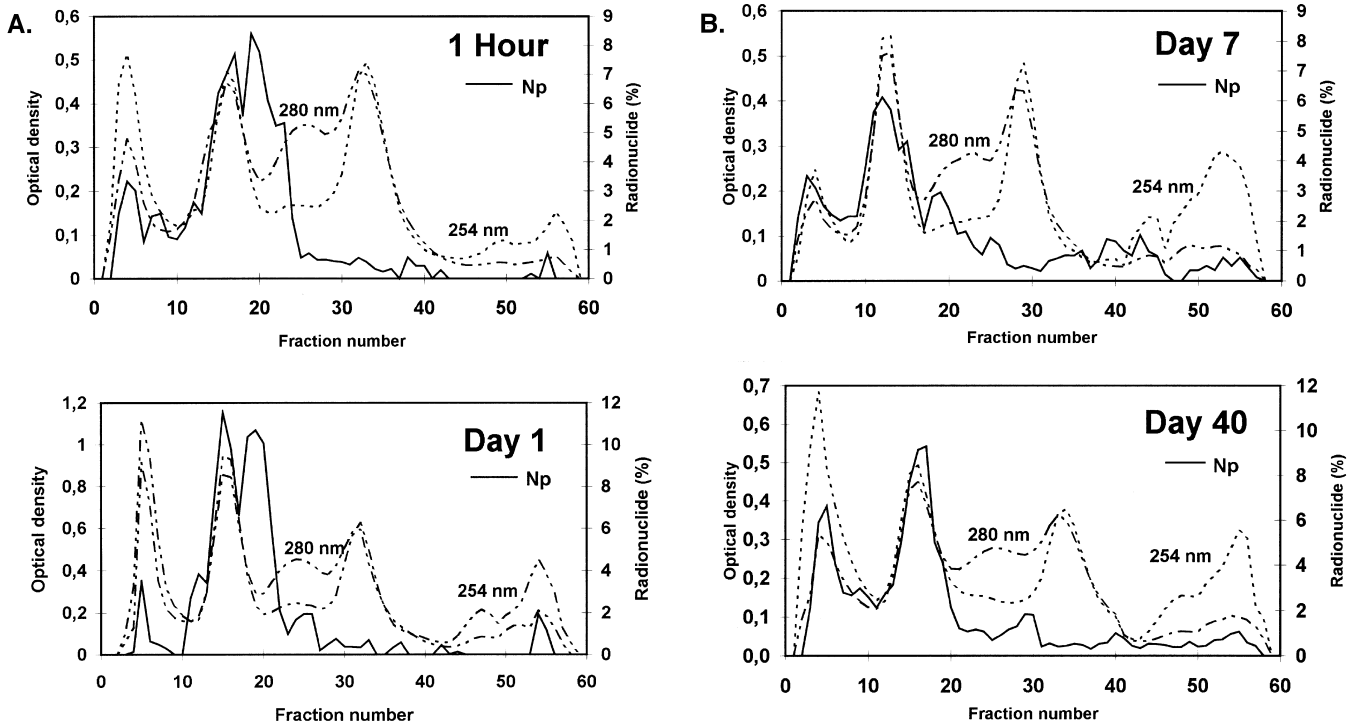


Fig. 3. The elution profile of $^{237}$Np nitrate after chromatography on Sephacryl HS300 of cytosol of rat liver cells.

5. Fig. 4 shows the association of the three radionuclides with the subcellular structures of the liver cells. One day after injection, the three radionuclides were located predominantly in the cytosol, while the nuclear and mitochondria/lysosomes fractions accounted at most for 15–18% of the total radioactivity. At later times after exposure (day 10), the radionuclide distributed mainly in the nuclear structures (38–42%), whereas the mitochondria/lysosomes contained the highest relative specific activity (RSA= Act%/Proteins%) (results not shown). Once again, separation of the lysosomes from mitochondria on a metrizamide gradient showed that secondary and tertiary lysosomes were the only structures involved in the binding of the three elements (results not shown).

The neptunium distribution in the cytosolic fraction of the baboon livers was similar to that observed in rats. One day after injection, neptunium was bound to ferritin and to an unidentified compound of MW 200 kDa. At later times after exposure (day 10), neptunium was bound mainly to ferritin and to macromolecules of MW>1500 kDa (Fig. 5). This distribution is definitely different from that observed here with americium and plutonium. These two radionuclides seemed to be associated mainly with ferritin whatever the time elapsed between the injection and the sacrifice.

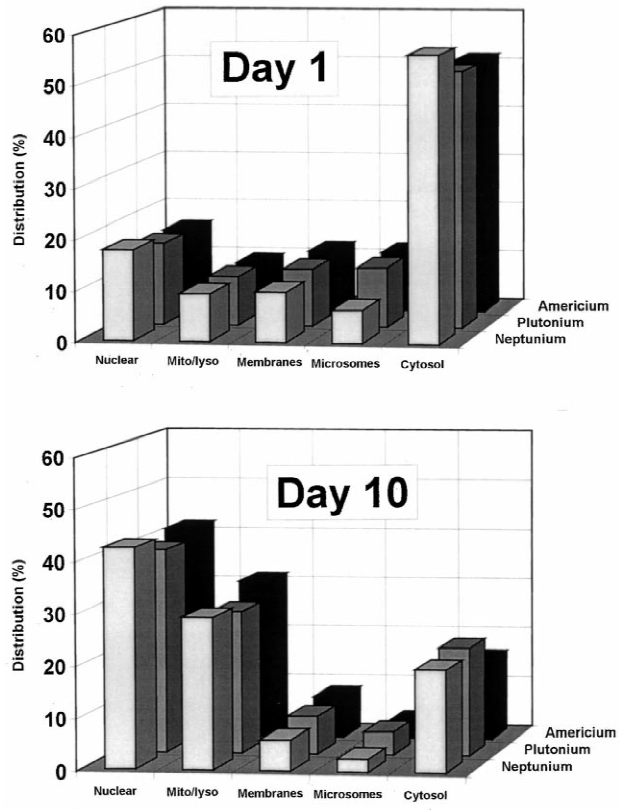


Fig. 4. The subcellular distribution of americium, plutonium and neptunium in baboon liver cells.

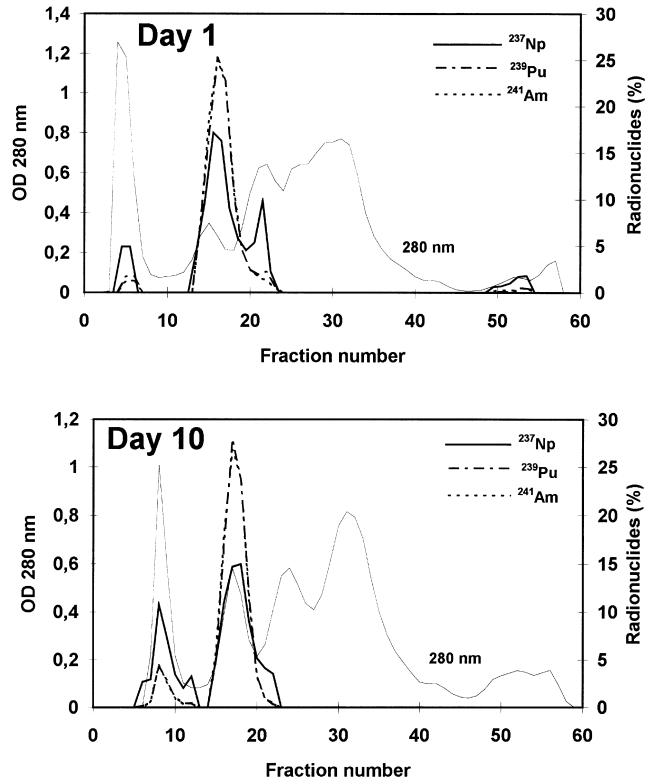


Fig. 5. The elution profile of $^{241}$Am, $^{239}$Pu and $^{237}$Np after chromatography on Sephacryl HS300 of cytosol of rat liver cells.

## 4. Discussion

The main results obtained on the bioinorganic chemistry of neptunium, plutonium and americium in the liver cells can be summarised as follows.

After their entry into the blood, the three actinides are distributed amongst the different target organs in the form of both transferrin and low-molecular weight complexes [10]. These three radionuclides are then transferred into the liver cells by an unknown mechanism. Other studies with iron showed that the $Fe^{3+}$–Tf complex combines with a specific receptor on the hepatocyte plasma membrane, and that the metal is translocated alone into the cell interior, before being transferred to apoferritin [11]. For plutonium, americium and neptunium, we can reasonably assume that a similar mechanism is involved since these three radionuclides have the same molecular carriers as iron. After passage through the cell membrane, neptunium appears to be bound first by a cytosolic protein of MW 200 kDa. This protein has not yet be identified but some evidence suggests that it could be calmodulin [12]. We cannot say at this stage that plutonium and americium are, or are not, bound by this protein just after their translocation into the cells; if they are, as supposed elsewhere for plutonium [13], this phenomenon should be very transient since 24 h after injection this protein was free from contamination

with both Pu and Am. However, the three radionuclides seem to be progressively transferred to ferritin and to high-molecular weight (HMW) compounds. This is particularly true for neptunium for which, 10 days after contamination, 30% of the cytosolic radionuclide was associated with these HMW compounds. Previous studies have shown that plutonium and americium were bound to lipofuscin in the canine liver [2,5], and it is not impossible that our HMW compound was lipofuscin. Nevertheless further analyses are needed to confirm this speculation. Another possibility is that these HMW compounds correspond to some material resulting from lysosomal rupture. The measurement of the acid phosphatase activity in the cytosol shows that, in the experiments with baboons, less than 15% of the total marker enzyme was present in this fraction. This indicates that this phenomenon cannot be excluded but is probably very restricted.

After passage through the cytosol the radionuclides are distributed amongst the various organelles, with preferential accumulation in lysosomes and nuclei. Previous studies have shown that neptunium in rat liver cells was bound by some proteins of the nuclear matrix, which have low turn-over and a very strong affinity for the radionuclide [9]. Such data are for the moment unavailable for plutonium and americium, but other studies showed clearly that a large fraction of these two radionuclides are also bound to the nuclear structures [5,14]. This translocation to the nuclear structures could be mass dependent; when low masses were injected, the radionuclide seems to deposit predominantly in the lysosomes and cytosol, whereas high masses cause saturation of the sites and complexation elsewhere. This have been demonstrated in vitro for $^{239}$Pu and $^{238}$Pu [14] and in vivo for $^{237}$Np and $^{239}$Np [8]. When accumulated by lysosomes, the radionuclides are probably bound as ferritin complexes [13]. A search for other ligands involved in the retention of the radionuclide inside the lysosomal structures has been made but, for the moment, without success.

The biochemical behaviour of americium, plutonium and neptunium proposed here is not radically different from what was known previously about the bioinorganic chemistry of these actinides. Nevertheless, it emphasises the particular behaviour of neptunium when compared with americium and plutonium. We cannot say for the moment if this behaviour is due to the relatively lower specific activity of $^{237}$Np, and hence higher mass. Nevertheless, this point has to be taken into consideration for the calculation of the doses resulting from the incorporation of the three radionuclides.

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