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Distribution and excretion of lanthanides: comparison between europium salts and complexes

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Europium (^{152,154}Eu) was intravenously injected into rats as: (i) the chloride salt at pH 7.4, (ii) the chloride salt at pH 3, (iii) the albumin complex and (iv) the DTPA complex, and tissue uptake was determined 24 h later. For the chlorides, the target organ for uptake was liver (about 60% of dose) whilst europium complexes were rapidly excreted in urine and were predominantly taken up into the kidney (about 0.5% of dose) and bone. Liver uptake of $EuCl_3$, pH 7.4, corresponded to that of a colloidal material with most ^{152}Eu present in the non-hepatocyte population; however, $EuCl_3$, pH 3, was handled in a different manner, with significant uptake by hepatocytes. The differing tissue distributions of $EuCl_3$ and Eu–albumin suggest that plasma albumin does not readily bind injected $EuCl_3$. Renal uptake of europium, although a relatively low proportion of the injected dose, was associated with many subcellular fractions, including lysosomes, suggesting significant intracellular uptake and thus possible retention.

Keywords: albumin, distribution, DTPA, europium, lanthanide

Introduction

Previous studies with salts of lanthanide metals and weak lanthanide complexes have identified liver and spleen as the main organs for sequestration of light and intermediate lanthanides (Durbin *et al.* 1956, Evans 1990), and also long-term retention of lanthanides in bone (Durbin *et al.* 1956, Berke 1968) and kidney (Berke 1968). Target organ toxicity in the liver has been reported with specific effects on the function of hepatocytes (Arvela 1979) and Kupffer cells (Koudstaal *et al.* 1991). Berry *et al.* (1989) have noted that lanthanides cause lesions to the proximal convoluted tubules in kidney. In addition to these target organ toxicities, lanthanide salts exhibit potent physiological activities by acting as calcium analogues (Evans 1990). Judged by their intravenous LD_{50} values (5–50 mg Ln kg^{-1}; Venugopal & Luckey 1978) lanthanide salts would be classed as 'highly toxic'. Stable lanthanide complexes, such as the gadolinium–diethylenetriaminepentaacetic acid (Gd–DTPA) ligand used as a contrast agent in magnetic resonance imaging (MRI), are rapidly excreted in the urine (Lauffer 1987), have minimal tissue uptake and retention, and are classed as relatively non-toxic (i.v. LD_{50} 1.5 g Gd kg^{-1} for Gd–DTPA; Lauffer 1987). The handling and biological activity of lanthanides are of interest and concern because of increasing human exposure through industrial and clinical applications of these metals.

The chemistry of lanthanide ions in blood is very complex because lanthanides form very insoluble phosphates, bicarbonates and hydroxides, but may also interact with macromolecules such as albumin (Schomäcker *et al.* 1988) and transferrin (Zak & Aisen 1988), and low molecular weight components e.g. citrate and lactate, to form soluble complexes. The partitioning amongst endogenous ligands in plasma and subsequent tissue distribution is also altered by the pH at which lanthanide salts are administered because lanthanides readily form stable colloids at neutral pH (Aeberhardt *et al.* 1962). Thus distribution and toxicity data from experiments in which lanthanide salts are parenterally administered are difficult to assess because the availability of Ln^{3+} under physiological conditions is unknown.

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This study compares the distribution and excretion of several different chemical forms of europium. Europium was administered i.v. into rats as (i) the chloride salt at pH 7.4, (ii) the chloride salt at pH 3, (iii) Eu–albumin complex (log stability constant, log K_s, of 6.4; Schomäcker *et al.* 1988) and (iv) the stable DTPA chelate (log K_s of 22.4; Martell & Smith 1974). The distribution of europium amongst hepatocytes and liver sinusoidal cells is investigated because there is uncertainty as to the relative importance of each cell type to liver lanthanide uptake. The subcellular distribution of lanthanides in the kidney is studied to determine if soluble lanthanides handled by the kidney are retained intracellularly.

Materials and methods

Preparation of europium compounds

$EuCl_3 \cdot 6H_2O$ (99.99%) was purchased from Aldrich (Gillingham, Dorset, UK) and $^{152}EuCl_3$ (1.0 mCi ml^{-1}, 0.078 mg Eu ml^{-1}) from Amersham (Amersham, Bucks, UK). Rat albumin (globulin-free) was supplied by Sigma (Poole, Dorset, UK) and DTPA (free acid, 98% purity) by Aldrich.

Solutions for i.v. administration (Table 1) were made by mixing $^{152}EuCl_3$, $EuCl_3$ and ligand (if present) in 0.15 M NaCl, pH about 4. The pH was adjusted to the required value with NaOH/HCl. The Eu–albumin complexes (containing 35 mg albumin ml^{-1}) were prepared at one-fifth of the europium concentration of the chlorides so that albumin could be injected at close to physiological concentrations (about 32 mg albumin ml^{-1} in serum; Baker *et al.* 1979).

Labeling of albumin with ^{125}I

Rat albumin was labeled with ^{125}I by the method of Fraker & Speck (1978). Albumin (125 mg in about 2 ml of NaCl, pH 5) and then 0.5 mCi of $Na^{125}I$ (Amersham) were added to an iodogen-coated tube. Unreacted ^{125}I and iodogen were separated from albumin with the aid of a PD10 desalting column (Pharmacia, Milton Keynes, Bucks, UK) that had been equilibrated with 0.15 M NaCl, pH 5. Approximately 20% of added ^{125}I bound to the albumin. The final albumin concentration of the [^{125}I]albumin was determined by the BioRad protein assay (BioRad, Hemel Hempstead, Herts, UK) with bovine serum albumin as standard.

Acid precipitables were prepared by adding an equal volume of ice-cold 10% (w/v) trichloroacetic acid to aliquots of plasma and urine. Precipitated protein was sedimented by centrifuging at 3000 r.p.m. for 10 min and the supernatant decanted off. ^{125}I activity was counted in the supernatant and pellet, reflecting free and albumin-bound ^{125}I, respectively.

Dosing the tissue collection

The compounds (Table 1) were injected into the ventral tail vein of male Wistar albino rats (200–250 g). The animals were placed in metabolism cages for collection of urine and feces.

After 24 h, the animals were killed by overdose of Sagatal (Rhone-Poulenc, Dagenham, Essex, UK). An aliquot (0.5 ml) of blood was placed into a heparinized tube and another aliquot was allowed to clot. Tissues (see Results, Table 2) were weighed and an appropriate aliquot of each was assayed for ^{152}Eu or ^{125}I. The remainder of the liver was placed in phosphate buffered saline for fractionation of cell types (see below). One kidney was placed in ice-cold 0.25 M sucrose, 5 mM Tris–HCl, pH 7.4, for subcellular fractionation (see below). Appropriate aliquots of urine and feces were also taken for counting.

^{152}Eu was counted in the pre-set ^{125}I channel of a Searle (1185R) gamma counter with an average efficiency of 30.7 ± 0.7% (mean ± SD of six observations).

Isolation of liver cells

Cells were isolated from the liver by the method of Lawrence & Benford (1991). Non-sedimenting material (at 50 × *g*) was kept as the non-parenchymal cell fraction and sedimenting material as the hepatocytes. Hepatocyte viability was assessed by Trypan blue dye exclusion. The cell fractions, solubilized in 0.1 M NaOH, were assayed for protein by the BioRad protein assay; bovine gamma globulin standards were also diluted in 0.1 M NaOH.

Table 1. Formulation of europium compounds for injection

Group	Compound	Eu:ligand molar ratio	Dose (mg Eu kg^{-1})	Solution (mg Eu ml^{-1})	Activity (μCi ml^{-1})
A	$^{152}EuCl_3$, pH 7.4	–	0.1	0.1	25
B	$^{152}EuCl_3$, pH 3.0	–	0.1	0.1	25
C	^{152}Eu–albumin, pH 7.4	1:4	0.1	0.02	5
D	^{152}Eu–DTPA, pH 7.4	1:3	0.1	0.1	25
E	Eu–[^{125}I]albumin, pH 7.4	1:4	0.1	0.02	340

All compounds were prepared in 0.15 M NaCl.

Subfractionation of the kidney

The kidney was fractionated into classical subcellular fractions by differential pelleting exactly as described by Andersen *et al.* (1987). The distribution of ^{152}Eu and ^{125}I amongst the lysosomal subfractions was examined by further subfractionating the mitochondrial/lysosomal (ML) fraction by rate sedimentation (Andersen *et al.* 1987), adapting the method to a swing-out motor.

The subfractions were analyzed for protein by the same method used for liver cells above. Aliquots (1 ml) of the subcellular fractions and the subfractions were assayed for ^{152}Eu or ^{125}I activity.

Results

Tissue distribution and excretion of europium

The dose of europium (0.1 mg Eu kg^{-1}; Table 1) was chosen to minimize toxicity (rat i.v. LD_{50} of about 20 mg Ln kg^{-1}; Venugopal & Luckey 1978) such as hepatic effects which occur after i.v. injection of above 1 mg Ln kg^{-1} as the chlorides (Lazar 1973, Arvela 1979).

The tissue deposition and excretion of ^{152}Eu and ^{125}I, depicted in Figures 1 and 2 as percentages of the administered dose, show that liver, spleen, kidney and lung were the most important soft tissues in the sequestration of europium. The relative concentrations of ^{152}Eu and ^{125}I in tissues, expressed as percentage of dose per unit weight or unit volume of tissue, are tabulated in Table 2. $EuCl_3$ at physiological pH (Group A) distributed in a pattern consistent with the particulate nature of the europium (27% of this 'solution' was retained on 0.22 μm filters). It was sequestered mainly in the liver, and also in the spleen and lung. Although the recovery of europium in the kidney was low, this was higher than other soft tissues. The liver retention of $EuCl_3$ injected at pH 3 (Group B) was slightly lower than at pH 7.4 but acidification of $EuCl_3$ essentially prevented uptake by spleen and lung. Urinary and fecal excretion of $EuCl_3$, pH 3, were marginally higher than in Group A but the excretion was much lower than the complexes (Figure 2).

Europium, from Eu–albumin (ratio, 1:4; Group C), was cleared from the blood and mainly excreted in the urine over the 24 h collection period. Only a small fraction of the europium was sequestered by the sampled soft tissues. The recoveries, expressed as a percentage of the dose (mean ± SD), of ^{152}Eu–albumin, $^{152}EuCl_3$, pH 3, and $^{152}EuCl_3$, pH 7.4, in sampled tissues were 41.2 ± 4.2, 60.6 ± 13.3 and 79.9 ± 25.6, respectively, suggesting that upto 60, 40 and 20% of the respective europium compounds were in unsampled tissues, probably bone

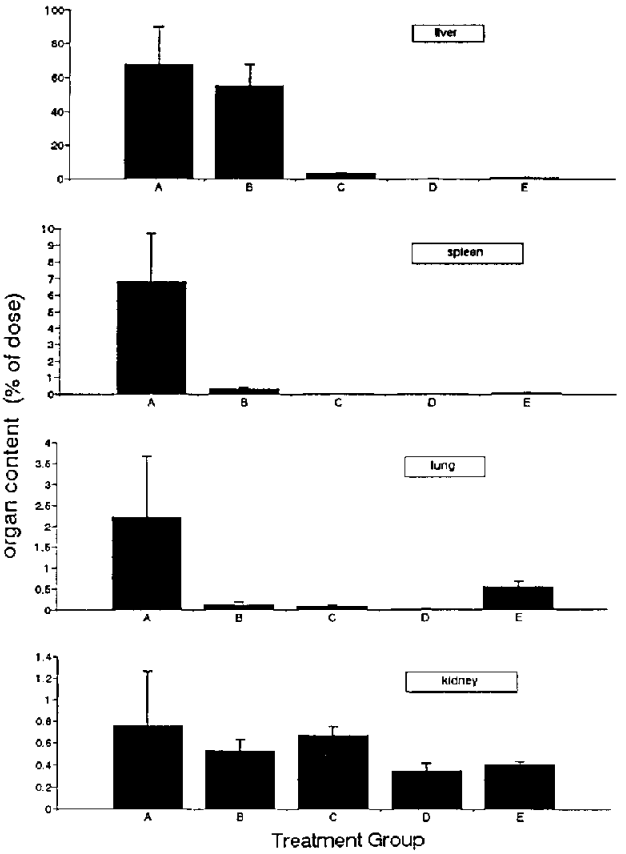

Figure 1. Comparison of the organ distribution of ^{152}Eu (Groups A–D) and ^{125}I (Group E). Group A was administered $^{152}EuCl_3$, pH 7.4; Group B $^{152}EuCl_3$, pH 3; Group C ^{152}Eu–albumin; Group D ^{152}Eu–DTPA; and Group E Eu–[^{125}I]albumin (see Table 1 for further details), 24 h prior to autopsy. Results are shown as amount of dose in organ (mean + SD from three to four animals).

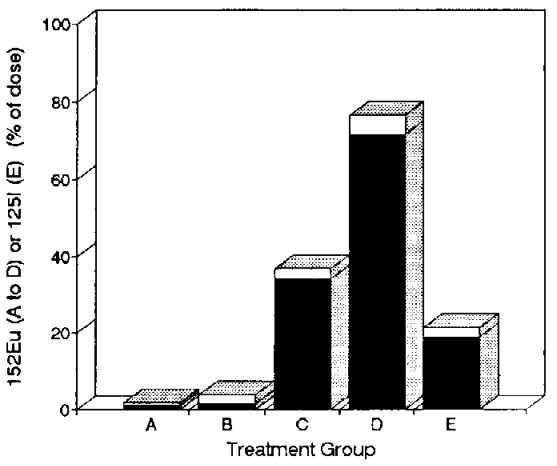

Figure 2. Cumulative urinary and fecal excretion of ^{152}Eu and ^{125}I, 24 h after dosing. Groups A–E are described in Table 1 and the legend to Figure 1. □, feces; ■, urine.

Table 2. Comparison of tissue uptake of ^{152}Eu and ^{125}I, 24 h after i.v. administration of $^{152}EuCl_3$, ^{152}Eu–albumin and Eu–[^{125}I]albumin

Location	Percentage of dose per gram or per milliliter of tissue (SD in parentheses)				
	$^{152}EuCl_3$, pH 7.4	$^{152}EuCl_3$, pH 3	^{152}Eu–albumin	^{152}Eu–DTPA	Eu–[^{125}I]albumin
Liver	6.52 (2.52)	5.06 (0.67)	0.35 (0.06)	< 0.01	0.08 (0.01)
Spleen	12.10 (5.62)	0.51 (0.05)	0.05 (0.01)	< 0.01	0.12 (0.02)
Kidney	0.42 (0.27)	0.28 (0.05)	0.41 (0.04)	0.20 (0.03)	0.22 (0.02)
Lung	2.02 (1.33)	0.11 (0.05)	0.08 (0.02)	< 0.01	0.41 (0.11)
Sternum	0.24 (0.05)	0.13 (0.01)	0.39 (0.02)	< 0.01	0.10 (< 0.01)
Heart	0.14 (0.03)	0.04 (0.01)	0.04 (< 0.01)	< 0.01	0.29 (0.03)
Testes	< 0.01	0.01 (< 0.01)	< 0.01	< 0.01	0.12 (0.03)
Small intestine	0.06 (0.02)	0.13 (0.03)	0.03 (< 0.01)	< 0.01	0.17 (0.03)
Pancreas	0.07 (0.05)	0.01 (< 0.01)	0.02 (< 0.01)	< 0.01	0.21 (0.03)
Plasma	< 0.01	< 0.01	< 0.01	< 0.01	1.27 (0.15)
Serum	< 0.01	< 0.01	< 0.01	< 0.01	1.30 (0.14)
Red blood cells	< 0.01	< 0.01	< 0.01	< 0.01	0.38 (0.09)

Results are the mean percent of the dose per gram or per milliliter of tissue from three or four animals.

(Berke 1968). Europium was almost totally excreted in the urine when it had been administered as the stable Eu–DTPA (ratio 1:3) complex. The kidney was the only organ that contained, intracellularly and/or in the filtrate, appreciable amounts of ^{152}Eu at 24 h after administration of the DTPA complex. Indeed, the kidney contained very similar recoveries of europium irrespective of which form of europium was administered.

In contrast with the ^{152}Eu moiety (Group C) of Eu–albumin, a substantial proportion of the [^{125}I] albumin dose (Group E) was retained in blood (Tables 2 and 3). Almost all (93%) of the plasma ^{125}I was protein associated, judged by acid precipitation. Generally, concentrations of ^{125}I label associated with soft tissues were greater than ^{152}Eu from Eu–albumin; however, kidney and, particularly, liver concentrations of ^{125}I label were lower. At autopsy, 8.5% of the ^{125}I had been sequestered by the thyroid and 19% excreted in the urine (not associated with protein) suggesting that a fraction of the [^{125}I]albumin had been metabolized.

Table 3. Blood retention of ^{152}Eu and [^{125}I]albumin 24 h following i.v. injection of Eu–albumin (ratio, 1:4)

	Proportion of the dose in (% of dose)		
	serum	plasma	red blood cell
^{152}Eu Group C (n = 3)	0.012	0.016	0.001
^{125}I Group E (n = 4)	11.42	11.15	1.93

Plasma, serum and volumes were calculated on the basis of 4.0 ml plasma or serum and 2.4 ml RBC per 100 g of rat (Baker *et al.* 1979).

Distribution of europium and albumin in the cells of the liver

The cells of the liver were separated into parenchymal (hepatocytes) and non-parenchymal cells (Kupffer cells, endothelial cells, Ito cells and bile duct cells). The average viability of hepatocyte preparations was 83%, judged by exclusion of Trypan blue dye.

^{152}Eu from $EuCl_3$, pH 7.4, was predominantly located in the non-parenchymal fractions that contained phagocytic Kupffer cells (Figure 3) but was also present with hepatocytes. Administering europium in the more soluble forms of $EuCl_3$, pH 3, and Eu–albumin increased the proportion of europium associated with the hepatocytes. The livers from Eu–DTPA-treated rats were not used because only negligible amounts of ^{152}Eu radioactivity were detected at the time of autopsy (Table 2). In order to isolate the cells the livers were flushed with an EDTA (2 mM) buffer to loosen cell junctions by removing Ca^{2+}; this would probably also remove any weakly bound extracellular lanthanide and so these results measure either strongly bound extracellular europium or intracellular europium.

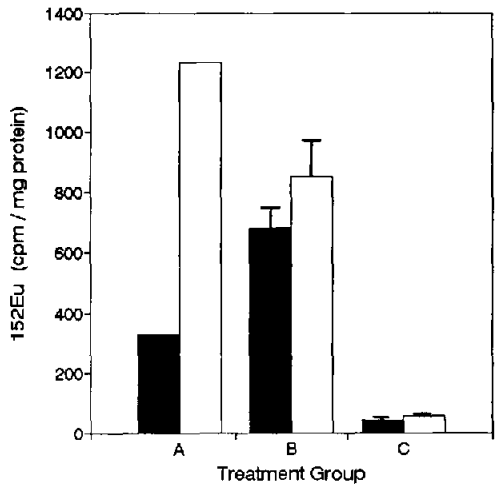

Figure 3. Partitioning of ^{152}Eu between hepatocytes and other cells of the liver. Group A received $^{152}EuCl_3$, pH 7.4; Group B $^{152}EuCl_3$, pH 3; and Group C ^{152}Eu–albumin, 24 h prior to separation of liver cell types. Results are the averages from two, three and four animals for Groups A, B and C, respectively (SD is shown for Groups B and C). ■, hepatocytes; □, other cells.

The very small amounts of ^{125}I label present in the liver were associated at 1.6-fold greater concentration with non-parenchymal cells than with hepatocytes.

Uptake and distribution of europium in the kidney

The recovery of ^{152}Eu in the various subcellular fractions of the kidney is presented in Table 4. The presence of ^{152}Eu in the particulate fractions indicates significant intracellular uptake. The supernatant (soluble) fraction is composed of all unsedimentable material from the cellular cytosol, vascular spaces and the tubular filtrate. Vascular ^{125}I (Table 2) is responsible for one-third of the soluble ^{125}I activity in the soluble fraction, based on a kidney vascular volume of 13% (Pfaller & Rittinger 1980); if vascular ^{125}I is sutracted from renal uptake, then 70.5% of ^{125}I is present in the soluble fraction, and 14.5, 7.5 and 7.5% in the nuclear, microsomal and ML fractions, respectively.

A difference of note between europium derived from $EuCl_3$ and Eu–albumin was the higher concentrations obtained from the latter in the ML fraction. ^{152}Eu which is associated with lysosomes probably represents uptake via the endocytic pathway, and for this reason it was decided to further fractionate the ML fraction in order to resolve various populations of lysosomes and establish possible association of ^{152}Eu with them (Figure 4). ^{152}Eu from $EuCl_3$, pH 7.4, was associated with unsedimentable (soluble and very small particulates) material and mitochondria or small lysosomes. ^{152}Eu and [^{125}I]albumin from Eu–albumin were also associated with small lysosomes and, especially, large lysosomes. Whilst with the ^{125}I label this is the expected fate of a protein reabsorbed from the tubular filtrate (Anderson *et al.* 1987), the presence of ^{152}Eu in these lysosomal populations strongly suggests that europium bound to albumin was also delivered intracellularly.

Discussion

When injected at pH 7.4, $EuCl_3$ has a similar tissue distribution to systemically administered colloidal cerium (Aeberhardt *et al.* 1962) and other particulate materials. At acid pH, however, this more soluble form of europium is not sequestered by liver and lung and uptake by Kupffer cells is reduced. Acidic europium, like gadolinium, may also inhibit Kupffer cell function and kill Kupffer cells (Koudstaal *et al.* 1991), and thus reduce Kupffer cell uptake. The hepatic association of $EuCl_3$, from 'free' and even colloidal europium, with hepatocytes confirms observations (Aeberhardt *et al.* 1962, Berry *et al.* 1989) that hepatic uptake of lanthanides is not solely attributable to Kupffer cells. Berry *et al.* (1989), by the use of electron microprobe analysis, detected cerium with phosphate in the lysosomes of hepatocytes and Kupffer cells, suggesting that lanthanides are indeed internalized via the endocytic

Table 4. Localization of ^{152}Eu and ^{125}I in subcellular fractions of kidney cortex after i.v. administration of $^{152}EuCl_3$, pH 7.4, ^{152}Eu–albumin and Eu–[^{125}I]albumin, expressed as the percentage of the homogenate recovered in each fraction

Subcellular fraction	Amount of radioisotope in the fraction as a percentage of the homogenate (%)		
	$^{152}EuCl_3$, pH 7.4	^{152}Eu–albumin	Eu–[^{125}I]albumin
Nuclear	35.0	27.5	9.0
ML	11.1	21.0	4.8
Microsomal	19.2	12.8	4.8
Soluble	34.7	38.7	81.4

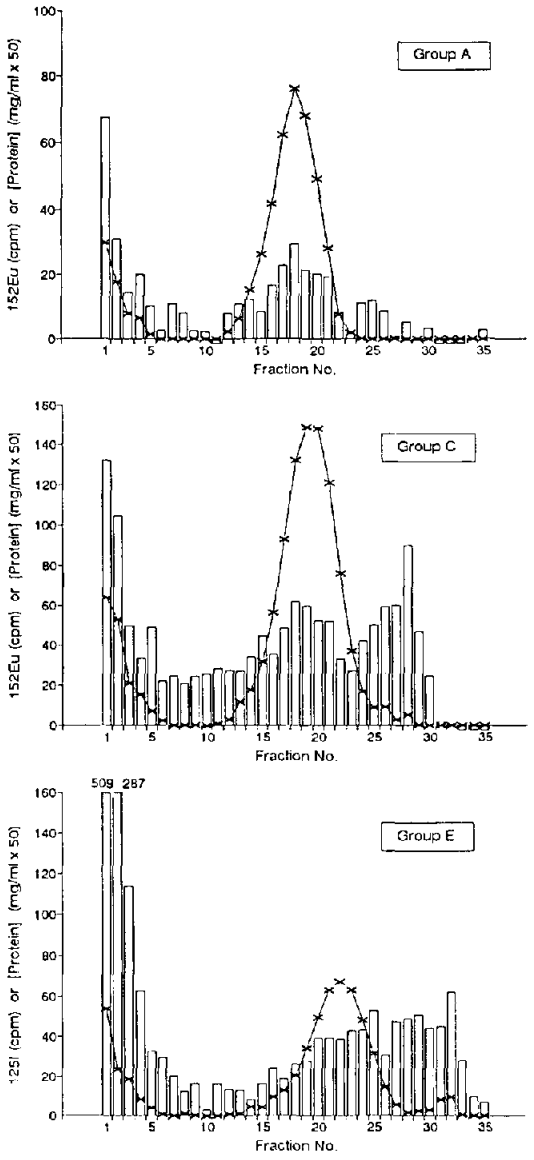

Figure 4. Distribution of ^{152}Eu and ^{125}I in mitochondrial and lysosomal subfractions of kidney cortex. Group A was dosed with $^{152}EuCl_3$, pH 7.4; Group C with ^{152}Eu–albumin (□); and Group E with Eu–[^{125}I]albumin (□), 24 h prior to kidney subfractionation. The protein peak (—×—) in early fractions (nos 1–7) corresponds to unsedimentable material, the central peak (fraction nos 11–27) corresponds to mitochondria, brush-border membranes and small lysosomes, and a further small peak (fraction nos 27–33) corresponds to large lysosomes (protein droplets).

pathway into hepatocytes and phagocytosed into Kupffer cells; the metals are then deposited as insoluble phosphate in lysosomes. The negligible hepatic uptake of albumin (Group E) suggests that the Eu–albumin is not endocytosed by hepatocytes whilst the weak Ln–transferrin complex may not be formed in blood (Zak & Aisen 1988) and thus not contribute to uptake. Possibly europium uptake by hepatocytes may be a consequence of internalization of europium-coated plasma membrane during endocytosis.

The differing handling of $EuCl_3$ and Eu–albumin by the rat suggests that albumin is not a major plasma ligand for europium injected as the chloride. Europium appears to form stable complexes with albumin because only small amounts of europium were recovered in spleen, liver and lung. This is consistent with observations that albumin prevents europium precipitation in phosphate-containing media (Kanapilly 1980, Schomäcker *et al.* 1988, Bingham & Dobrota 1992). The relative abundance of albumin in plasma and the stability of the Eu–albumin complex (log K_s of 6.4; Schomäcker *et al.* 1988) make the possibility of redistribution to other plasma ligands very remote. Europium, from Eu–albumin, appears to distribute mainly to bone, although our results cannot confirm this because the sternum is not representative of bone skeleton. Both mineral and osteoid components of bone may be involved in uptake of lanthanides (Evans 1990).

Albumin is normally filtered at the glomerulus in small amounts (Bowman's space:plasma ratio of albumin is less than 0.01; Dworkin & Brenner 1985) but this can be increased by reduction in the net negative charge of the molecule or by neutralization of the glomerular basement membrane charge (Dworkin & Brenner 1985). In the filtrate, Eu–albumin may dissociate under the slightly acidic conditions (pH 6–7; Koeppen *et al.* 1985) and/or following partial degradation of albumin by ecto-proteases on the luminal face of the brush-border membrane. The presence of europium from Eu–albumin in lysosomes in kidney (Group C) suggests that intact Eu–albumin complex is reabsorbed by proximal tubular cells. Small amounts of Eu–albumin complexes formed in blood from the chlorides or DTPA may be partially responsible for the relatively consistent uptake of europium by kidney. Berry *et al.* (1989) have observed cerium deposited on the glomerular basement membrane following administration of cerium chloride.

The water soluble Eu–DTPA complex is rapidly excreted in urine, as has been found with the Gd–DTPA complex (Lauffer 1987). Although there appears to be no data available in the literature on how much lanthanide remains chelated to DTPA in urine, its very high stability constant (log K_s for Eu–DTPA is 22.4; Martell & Smith 1974) and well characterized stability in blood, as shown by lack of tissue binding and *in vitro* studies (Tweedle *et al.* 1988) suggest that the complex remains intact. A

small but significant proportion of the i.v. administered complex was excreted in the feces, suggesting some biliary or intestinal excretion of europium. Direct sampling of bile is necessary to confirm biliary excretion. The kidney retention of Ln–DTPA is of interest because of the clinical applications of Gd–DTPA in MRI imaging. Whilst the proportion of the dose (0.35%) retained is low, it is not clear if this residual amount may represent a significant risk. In patients receiving Gd–DTPA at the routinely used dose of 0.1 mmol Gd–DTPA kg^{-1} (15.7 mg Gd kg^{-1}), it is estimated that 0.055 mg kg^{-1} of europium would be retained. This is about 1/300th of LD_{50} for i.v. administration of lanthanides, as the salt, in male rats (Venugopal & Luckey 1978). However, lanthanides clearly exhibit very potent biological activity on some systems *in vitro*, e.g. membrane Ca^{2+} transport systems (Evans 1990). Furthermore, administration of cerium chloride to rats has been reported to cause lesions to cells of the proximal convoluted tubule (Berry *et al.* 1989). On the other hand, the form of europium retained may be relatively inert, e.g. precipitated as the phosphate in lysosomes. More definitive data of the form, amount and long-term effects of the retained lanthanides in the kidney are required.

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