EFFECT OF DTPA $CaNa_3$ ON THE DISTRIBUTION OF Ce-144 IN THE SOLUBLE CYTOPLASMATIC FRACTION OF ISOLATED HEPATOCYTES

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INTRODUCTION

Numerous animal experiments and therapeutics applications have demostrated the usefulness of DTPA $CaNa_3$ to increase excretion of actinides and lanthanides in cases of internal contamination.

Because of the high affinaty of these radionuclides for the liver, hepatocytes in primary culture was chosen as a simple model system.

The purpose of this work was to study the distribution of Ce-144 between compounds of high and low molecular weight of the soluble cytoplasmatic fraction obtained from isolated hepatocytes after they were incubated with and without DTPA $CaNa_3$, since the effectiveness of this chelating agent to remove contaminants is larger while they remain in this fraction.

MATERIALS AND METHODS

The methodology consisted in the intraperitoneal contamination of Wistar rats with Cl_3 Ce-144. Prior to sacrifice, 48 h later, the liver was perfused "in situ" with modified Hank's buffer supplemented with collagenase 0.05 % and then removed and digested in a volume of the same complete buffer.

The suspension was centrifuged and the cells resuspended in Waymouth's MB 752/1 medium supplemented with 10 % Fetal bovine serum and antibiotics.

Aliquots of the final suspension were incubated in a humidified 5 % CO_2 air incubator at 37°C during 6 h. The chelator DTPA $CaNa_3$ was added at a final concentration of 5×10^{-3}M, prior incubation. A corresponding control was processed.

The suspension was removed, centrifuged and the cells were resuspended in TRIS 5 mM - Sucrosa 0.25 M and ruptured in a Dounce homogenizator. The soluble cytoplasmatic fraction was obtained by centrifugation at 105,000 x g for 80 min. The supernatant was examined by gel permeation chromatography through ULTROGEL ACA-22 columns (1.6 x 80 cm) using 0.1M TRIS-ClH, pH 8.0, containing 0.1M ClNa and 0.02 % sodium azide as eluting buffer. The column was calibrated using horse spleen ferritin and human transferrin.

Fractions of 4 cm^3 were collected at a flow rate of 0.15 cm^3/min. The eluate was monitored spectrophotometrically at 254 nm and each fraction was counted for radioactivity by a multichannel, well-crystal, scintillation counter.

RESULTS

Two peaks with Ce-144 activity were observed with the hepatocytes with DTPA $CaNa_3$ (fig 1) and with the controls (fig 2). One of the peaks was coincident with the elution volume of ferritine (MW 700,000 - 800,000) and the other was located in the lower limit of exlusion of the gel (fractionation range 100,000 - 1,200,000). The chromatographic profile for a preparation of Ce^{144}-DTPA complex is shown in figure 3. The ratio between Ce-144 activity in high and low molecular weight fractions is 0.08 for the sample incubated with DTPA $CaNa_3$ and 3.60 for controls.

DISCUSSION

In the treatment of actinides and lanthanides contamination with DTPA, the amount of radionuclides which can be removed from the liver decrease with the length of time wich elapses between contamination and the start of treatment.

This decreasing efficiency of DTPA probably reflects the transfer of nuclide from the soluble fraction to the formed elements of the cell becoming less accesible for reaction with the chelating agent.

Our previous experiments showed that Ce-144 content in the rat liver reached a maximun at 2 days P.I. and that the fraction of Ce-144 in the cytosol was larger in hepatocytes incubated with DTPA.

The purpose of the present work was to investigate the distribution of Ce-144 amongst proteins of the liver cytosol with and without DTPA using gel chromatography on Ultrogel ACA-22.

At 48 hours after injection the major cerium-bearing peak was in the region of the ferritin elution volume. In the presence of DTPA the pattern of isotope destribution had changed. The proportion of cerium in the low molecular weight region had increased and in the ferritin region had declined.

According the hypothesis of Bhattacharyya et al, DTPA may be taken up by the parenchymal cells of the liver by pinocytosis of extracellular fluid. Fusion of pinocytotic vesicles with lysosome containing Pu-239 could result in direct chemical contact between the retained DTPA and hepatically deposited Pu. Excretion of Pu-DTPA complex into bile was demonstrated.

The observed differences in the distribution of cerium by chelate action might be explained by this hypothesis but we can not assert yet that the increase is due to the Ce-DTPA complex.

Clearly more experiments need to be done to elucidate the finer details of the mechanism for DTPA-induced removal of lanthanides from the liver rat.

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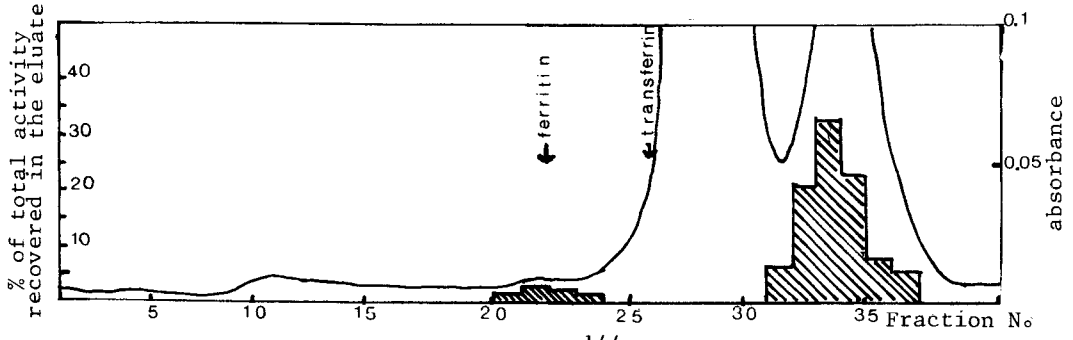

FIG. 1: UV absorbance at 254 nm and Ce^{144} γ - radioactivity profiles obtained by chromatography on ULTROGEL AcA 22 of the soluble fraction of hepatocytes incubated with DTPA Ca Na_3

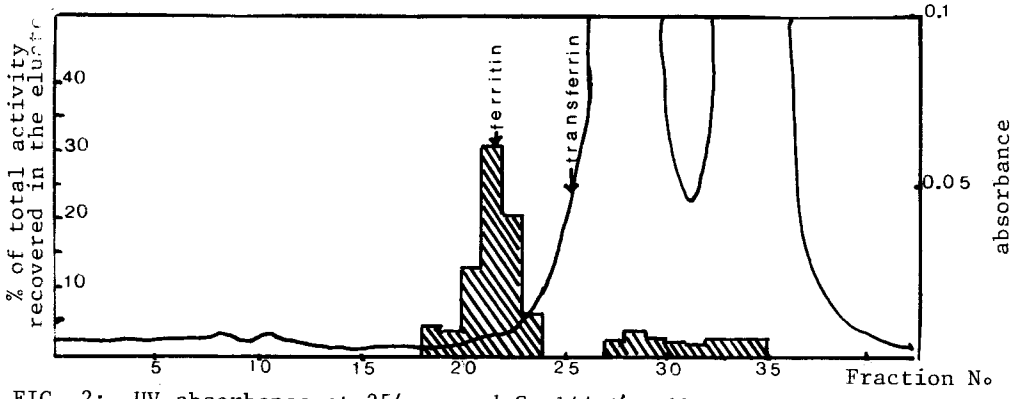

FIG. 2: UV absorbance at 254 nm and Ce-144 γ radioactivity profiles obtained by chromatography on ULTROGEL AcA 22 of the soluble fraction of hepatocytes

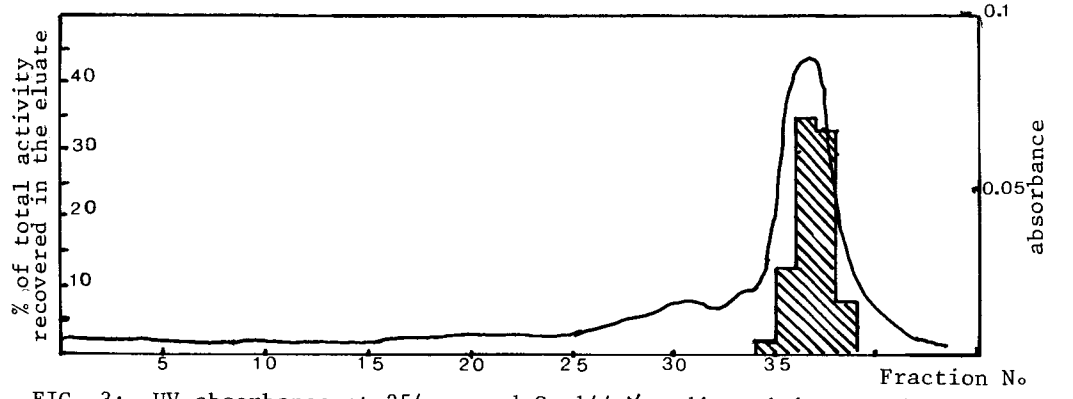

FIG. 3: UV absorbance at 254 nm and Ce-144 γ radioactivity profiles obtained by chromatography on ULTROGEL AcA 22 of the complex Ce^{144} - DTPA