ESRF	**Experiment title:** Study on the localisation and kinetics of lanthanum incorporation into and efflux out of bone in chronic renal failure rats after oral dosing	**Experiment number**: MD 53
Beamline: ID21	**Date of experiment**: from: 19-11-03 to: 28-11-03	**Date of report**: 25-02-04
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ESRF – ID21

Background:

In patients with chronic renal failure (CRF), serum phosphorus levels increase due to insufficient renal excretion. To control serum phosphorus levels, phosphate binding agents are given orally, in order to reduce gastrointestinal uptake of phosphate. Severe side effects limit the use of the most commonly used drugs (aluminium hydroxide and calcium carbonate). Lanthanum carbonate has been proposed as a possible alternative. Despite its very low gastrointestinal absorption, some accumulation of La in bone was observed (up to 6 µg/g wet weight) in CRF rats dosed for 12 weeks with 2 g/kg/day. We have previously shown a mineralisation defect may develop in these animals, most likely secondary to a severe lanthanum carbonate induced phosphate depletion, rather than a direct effect of La on the bone. In a previous experiment at the ESRF (LS-1709), we were able to localise La at the outer edge of the mineralised bone. However, due to limitations in sample preparation and positioning, no clear information regarding the localisation relative to the unmineralised bone (osteoid) and the mineralisation front, was obtained. With the improved sample positioning now available at ID21, thanks to the installation of a new videomicroscope allowing in-situ observation of the samples, and by using adjacent sections stained for light microscopy, more accurate sample positioning and localisation of La is possible.

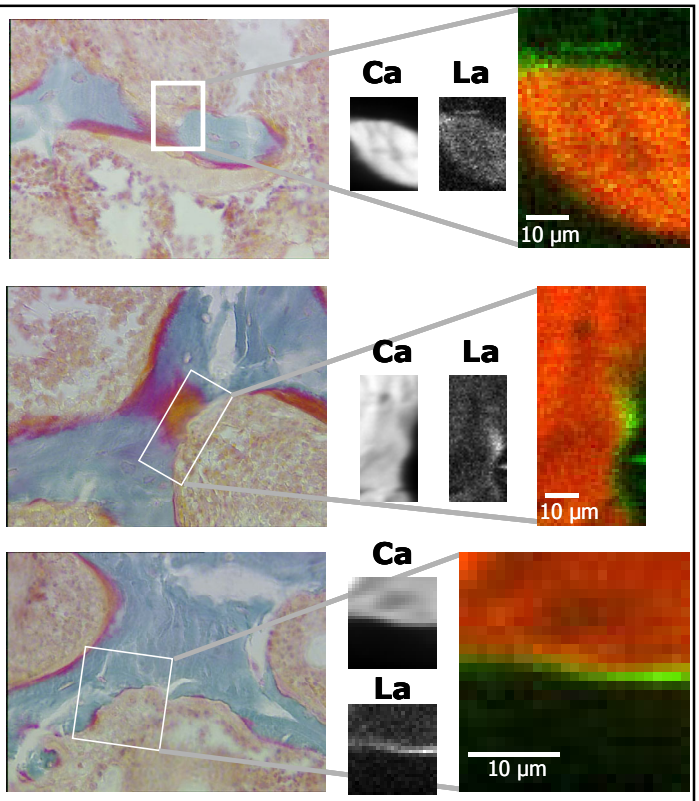
Experimental method:

Male Wistar rats with chronic renal failure were orally loaded with La carbonate at a dose of 2 g/kg/day for 12 weeks, followed by wash-out periods of 0, 2 or 4 weeks. Additional animals were loaded intra-venously with $LaCl_3$ for 6 weeks (5 mg/kg total dose). The proximal part of the tibia was fixed and embedded in methyl-methacrylate, and 10 µm sections were used for analysis. Adjacent sections were Goldner stained to visualise mineralised and unmineralised bone. Synthetic hydroxyapatite, doped with known La concentrations was used to estimate local La content.
Mapping of La distribution was performed using the ID21 Scanning X-Ray Microscope, operated in fluorescence mode. The beam was focussed to a microprobe (1 µm) using a Fresnel zone plate,

and the sample was raster scanned to acquire 2D images. Simultaneous mapping of Ca and La was performed by chosing appropriate energy windows (Ca: 3.53 to 4.12 keV; La: 4.92 to 5.93 keV) of the multichannel analyzer.

Results:

Figure 1: Left: Goldner stained adjacent sections, allowing easy identification of site of intrest. Right: Ca (red) and La (green) image of selected sites.
La could be localised at the outer edge of the mineralised bone, independent of the underlying type of renal osteodystrophy (high- or low-turnover). Furthermore, La was not solely co-localised with the active mineralisation front, but also found on quiescent surfaces (top panel) and resorption surfaces (bottom panel). Using the synthethic hydroxyapatite standards, local La/(La+Ca) ratios at sites of enrichment could be estimated, reaching values of up to 0.05 mol% La/(La+Ca).
In animals with high bone turnover, La was also found inside the calcified bone (top panel) at ratios of up to 0.01 mol% La/(La+Ca).



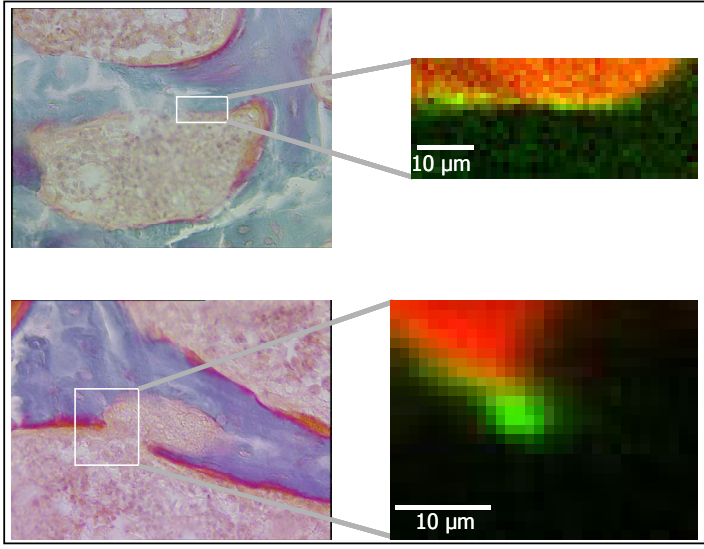


Figure 2: After 2 and 4 weeks of wash-out, La localisation remains similar: mainly the outer edge of the mineralised bone, independent of the underlying type of renal osteodystrophy.

Figure 3: In intra-venously loaded animals, La could also be found in the bone marrow (possibly in macrofages or as precipitates in the interstitium).