Les Actualités Néphrologiques
Jean Hamburger
Hôpital Necker
les lundi 26, mardi 27 et mercredi 28 avril 2010.
Maison de la Chimie
28 bis Rue Saint-Dominique - 75007 Paris

Nouvelles méthodes d’études des calculs et des plaques de Randall
New techniques of analysis of lithiasis and Randall’s plaque

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The ultrastructure of Randall’s Plaque (R’sP)

Epidemiological aspects
R’s P described at the mesoscopic scale through SEM
Role of oligoelements in the pathogenesis of R’s P
First Ex vivo Synchrotron radiation experiments on R’s P

Application of physical methods to KS characterisation

Calcium oxalate stones
Calcium phosphate stones
Uric acid stones

Conclusion & Perspectives

**Randall’s plaque: Definition**

At the surface of attached kidney stones, a particular deposit termed Randall’s plaque (R’sP) serves as a nucleus.

For a sake of clarity, we used the terms ‘nanocrystals’ and ‘crystallites’ according to Van Meerssche & Feneau-Dupont. In this simplified scheme, a collection of nanocrystals (measuring some hundreds of nanometres) constitutes crystallites (measuring some tens of micrometers).

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§ Epidémiologie, structure et physiopathologie

§§ Examination of whewellite kidney stones by SEM and PND techniques.

§§§ Diffraction techniques and vibrational spectroscopy opportunities to characterise bones,
D. Bazin et al., Osteoporosis Int. 20 (2009) 1065-1075.
Epidemiological aspects

Prof. O. Traxer (Tenon Hôpital) : From 2004 to 2007 (436 uretero-renoscopies)

289 lithiasic patients et 147 non lithiasic patients (tumeur, sténoses, …)
- Lithiasic: 164 patients with RP (57%)
- Non lithiasic : 48 patients with RP (27%)

Fréquence, topographie et classification des PR
Stone morphology suggestive of Randall's plaque
M. Daudon, O. Traxer, P. Jungers, D. Bazin
Renal Stone Disease 900, 26-34, 2007.
Field Emission Scanning Electron Microscope

The scanning electron microscope images the sample surface by scanning it with a high energy beam of electrons. Based on the interaction between these incident electrons and atoms at or near the surface sample, this technique is able to give relevant information on local details of the surface at the submicrometer scale.

Experimental aspects

Located at the solid state laboratory, a Zeiss SUPRA55-VP type scanning electron microscope (SEM) is used for microstructure observations. This field effect gun microscope operates at 0.5 to 30 kV. High resolution observations are obtained by two secondary electron detectors: in lens SE detector and Everhart-Thornley SE detector.

To maintain integrity of the kidney stones, measurements have been performed at low voltage (1.4 KeV) and without the usual deposits of carbon at the surface of the sample.
Observation at the mesoscopic scale through SEM

Randall’s plaques
M. Daudon, O. Traxer, J.C. Williams, D. Bazin
Chapitre de livre, Springer-Verlag, in press.

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Investigation @ the interfaces

Interface Papilla/ Randall’s plaque

Interface Randall’s plaque/ Kidney stones

KS

R’s P Apatite

KS

R’s P Apatite
Role of oligoelements in the genesis of R’sP

In a previous study, stones were investigated by -Synchrotron Radiation X-ray Fluorescence (-SRXF) at LURE (Laboratoire pour l’Utilisation des Rayonnements Electroniques, Orsay, France) in order to identify and quantify heavy elements.

Heavy elements in urinary stones
D. Bazin, P. Chevallier, G.; Matzen, M. Daudon, P. Jungers

With this experimental set up we can collect several fluorescence spectra for one sample

<table>
<thead>
<tr>
<th>Material</th>
<th>Zn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apatite KS</td>
<td>1059 ± 1056</td>
</tr>
<tr>
<td>RP</td>
<td>9550 ± 4758</td>
</tr>
<tr>
<td>Kidney tissue</td>
<td>15-32</td>
</tr>
<tr>
<td>Urine</td>
<td>0.002-0.1</td>
</tr>
<tr>
<td>Urine</td>
<td>0.3 ± 0.2</td>
</tr>
</tbody>
</table>

Are oligoelements potential risk factors for R’sP and kidney stone formation?
X. Carpentier et al., Submitted to J. of Trace elements in Medicine and Biology

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In order to obtain a cartography, we perform PIXE (Protons Induced X-ray Emission) experiments with a 1 µm probe.

Coll. H. Khodja LPSue (CNRS-CEA)
Role of oligoelements in the pathogenesis of R’sP - a PIXE investigation

Similar spatial distribution of Ca and Zn

Different spatial distribution of Ca and Zn

At least, two different Zn species on/in R’s P
First Ex vivo Synchrotron radiation experiments on R’s P

Chemical Composition of R’sP
Even if other phosphate phases such as whitlockite or brushite can be found as minor components (less than 5%), calcium phosphate apatite (CA) as well as amorphous carbonated calcium phosphate (ACCP) are the major components of most RPs.

Medical signification: ACCP comes from a calcium phosphate supersaturation with an excess of Calcium and/or phosphate and/or a high pH

Dr. X. Carpentier, CHU Nice, Hôpital Pasteur, Nice. PhD thesis end of 2010.
Experiments on Synchrotron Soleil

- Very first tests on SOLEIL regarding the Zn environment in pathological calcifications made of apatite determined by X-ray absorption spectroscopy.
  [http://www.lightsources.org/cms/?pid=1002029](http://www.lightsources.org/cms/?pid=1002029)

- Diffraction techniques and vibrational spectroscopy opportunities to characterise bones

- Revisiting the localisation of \(\text{Zn}^{2+}\) cations sorbed on pathological apatite calcifications made through Xas

- The pathogenesis of Randall’s Plaque: A papilla cartography of Ca compounds through an ex vivo investigation based on XANES spectroscopy

Highlight of Solid State Laboratory in 2010.
Characterisation of some points at the tip of the papilla

At either the K- or the L-edge, XANES contains both electronic and structural information, but can be difficult to interpret fully §.

Peculiar morphology of stones in primary hyperoxaluria


Peculiar Morphology of Stones in Primary Hyperoxaluria

TO THE EDITOR: Primary hyperoxaluria type 1 is a rare inherited disease leading to recurrent nephrolithiasis, nephrocalcinosis, pyelonephritis, and renal failure, usually requiring combined kidney and liver transplantation. Because of the rarity of this disease, the diagnosis is often missed or delayed by several years, especially when the disease occurs in adulthood, thus depriving patients of the benefits of therapeutic measures that have been implemented in a timely manner. Therefore, any method allowing early diagnosis is eagerly awaited.

However, although nephrocalcinosis is the prevailing symptom in the great majority of patients with this disease at any age, until now little attention has been paid to the analysis of stones as a possible diagnostic tool.

Over the past 20 years, we analysed stones obtained from 74 patients with a diagnosis of primary hyperoxaluria type 1 established on the basis of complete urinalysis biochemical tests and evidence of an enzyme defect. In addition to findings on nuclear spectroscopy, we examined the morphologic characteristics of the surface and sections of calculi by means of a scanning electron microscope. All primary hyperoxaluria type 1 calculi were composed of pure or virtually pure (99%) calcium oxalate monohydrate, or whewellite. As compared with idiopathic calcium stones with a similarly high whewellite content, all primary hyperoxaluria type 1 calculi showed very peculiar morphologic characteristics, including a whitish or pale-yellow surface and a loose, unorganized section, quite different from the dark-brown surface and well-organized, needle-like structure of common whewellite stones (Fig. 1). In addition, scanning electron microscopy confirmed a crystalline structure in the primary hyperoxaluria type 1 stone that was distinct from that of the common type of whewellite stone. This unusual morphologic characterstics and the ultrastructure of primary hyperoxaluria type 1 stones suggest a fundamental difference in the mechanism of stone formation, reflecting the very rapid and pronounced crystal formation induced by genetic hyperoxaluria. The peculiar morphologic character-
Relationships Between Carbonation Rate of Carbapatite and Morphologic Characteristics of Calcium Phosphate Stones and Etiology

The presence of bacterial imprints at the surface of KS is well known

Relationship between the carbonate rate of carbapatite, morphological characteristics of calcium phosphate stones and etiology
It is well known that uric acid stones are commonly more prevalent in male stone formers in most western countries. Recent epidemiologic investigations point out an intimate correlation between obesity, type 2 diabetes and uric acid kidney stone.

Distribution of UA stones with respect to diabetes status in male and female stone former

<table>
<thead>
<tr>
<th></th>
<th>With diabetes</th>
<th>Without diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M  F</td>
<td>M  F</td>
</tr>
<tr>
<td>Age</td>
<td>61.4 59.7</td>
<td>59.7 59.6</td>
</tr>
<tr>
<td>BMI</td>
<td>30.2 33.2</td>
<td>27.1 27.1</td>
</tr>
<tr>
<td></td>
<td>33.2% 42.5%</td>
<td>11.9% 9.5%</td>
</tr>
</tbody>
</table>

M. Daudon, O. Traxer, P. Conort, B. Lacour, P. Jungers
\[ \Phi = \frac{K\lambda}{\beta \cos(\theta)} \]

- \( \Phi \): Nanocrystal size
- \( \lambda \): wave length
- \( \beta \): Width
- \( \theta \): position

Coll. G. André, LLB CEA-CNRS
Non diabetic patients

\[ \Phi_{\text{nanocrystals (F)}} \gg \Phi_{\text{nanocrystals (M)}} \]
$\emptyset (F) \gg \emptyset (M) = \emptyset (Diab)$
Conclusion

The ultrastructure of R’sP

- R’P described at the mesoscopic scale through SEM

- oligoelements & R’sP

- First Ex vivo S.R. experiments on R’sP

Application of physical methods to KS charaterization

Calcium oxalate stones
Calcium phosphate stones
Uric acid stones

Understand the pathogenesis of RP
Use such techniques to perform diagnostic
To explain some epidemiologic data
Perspectives

Understand the pathogenesis of RP (JSR)
Use FESEM to perform diagnostic (NEJM)
To explain some epidemiologic data (Submitted)

I How drugs dissolve kidney stones: Case of cystine...

- Tiopronine
- Captopril

II Using Synchrotron Radiation to perform diagnostic
Coll. P. Dumas & Ch. Sandt
http://www.lewebzine.aphp.fr/spip.php?article408

III Other calcifications (Thyroid§, Cardiac valves§, Cartilage§)

§ submitted
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H. Khodja LPSue

S. Katelbach, Stage de Pharmacie, Hôpital Necker, Jan-Mai 2005.
Dr. X. Carpentier, Urologue, Master 2 Sciences et Technologie Mai-Sept 2007.
B. Hannache, stage, Maître de conférence, Jan 2009.
E. Mukuna, LI PGA de la Licence de Physique, Un. P. et M. Curie, Jan 2009.
Dr. Ch. Nguyen, Rhumatologue, Hôpital lariboisiere, Mai-Sept 2009.
M. Roméo, stage BTS, Technicienne de Laboratoire, Juin-Juillet 2009.

3 PhD thesis
ANR-09-BLAN-0120-02.
Ask for a ANR on cartilage (Prof. F. Lioté, Lariboisière) and other programs (Tassili, Un. Paris XI,Toulouse…)

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