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# New insights into the presence of sodium hydrogen urate monohydrate in Randall's plaque

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ABSTRACT

Calcium oxalate nephrolithiasis is a common ailment. Frequent risk factors associated with it are low diuresis, dietary imbalance, inherited or acquired metabolic disorders. Another important factor, namely the presence of a mineral deposit made of apatite at the surface of the papilla, named Randall's plaque (RP), has been recently underlined. In most cases, RP which serves as a nidus for kidney stone formation is made of calcium phosphate apatite (CA). However, RP does not seem to be composed exclusively of CA. We would like to assess the case of RP where sodium hydrogen urate monohydrate (NaUr) is also present in its chemical composition. To attain this goal, a set of experiments including Environmental Scanning Electron Microscopy (ESEM) and Synchrotron Radiation Fourier Transform Infra Red (SR-FTIR) has been performed to analyze papillae of six kidneys randomly selected after they were surgically removed for cancer. NaUr crystals were found in two samples. We show through ESEM a usual morphology of RP present at the surface of kidney stones in the presence of NaUr. Moreover, we discuss the presence of NaUr in the renal parenchyma and its spatial repartition with CA. The complete set of data indicates that different biochemical mechanisms are probably involved in the pathogenesis of RP. The next step will be to establish a significant relationship between these physicochemical data and the clinical and biochemical data of the patients.



## 1. Introduction

One of the main challenges for urology in the 21st century is to understand the biochemical mechanisms involved in the formation of Randall's plaque (RP) [1–4]. RP

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is a white deposit commonly made of calcium phosphate visible at the tip of papilla beneath the papillary epithelium. It was first reported by Alexander Randall in 1936, and it is considered today as a major factor for nucleation of calcium oxalate stones. From an epidemiologic point of view, a progression in the presence of RP at the tip of the papilla has been recently reported in industrialized countries [5–7]. More precisely, in US up to 80% of calcium stone formers present Randall's plaque at the tip of their papillae [8]. In France, more than 50% of stone formers exhibit Randall's plaque [9]. Stone formation is proportional to papillary surface coverage by RP, which may explain the significant increase of kidney stone prevalence observed over the past two decades in industrialized countries [10–14].

In the recent model proposed by A.P. Evan et al. [15,16], apatite plaque begins in the basement membrane of the thin loops of Henle, and spreads from there into the papillary interstitium. The starting point of our investigation is given by the observation that, if calcium phosphate apatite (CA) is the major component of most RP, other chemical phases can be found, such as amorphous carbonated calcium phosphate (ACCP) [17], whitlockite (WK), brushite, uric acid and sodium hydrogen urate monohydrate (NaUr) [18]. Even if NaUr is present in only 3.5% of RP [19], it represents a high number of patients, about 70000 subjects in France. Indeed, the occurrence of NaUr in urinary calculi is around 0.7%, i.e. 5 times less than in RP. Such chemical diversity of RP could indicate that RP begins not only in the basement membrane of the thin loops of Henle but in other parts of the kidney.

The aim of this study which follows our previous ones dedicated to RP [5,17,20,21] is to seek and discuss the presence of NaUr crystals in RP at the surface of kidney stones as well as in kidney tissues. Investigation regarding such pathological calcifications [22–25] calls for a set of in-lab techniques [26–39] or ones related to large scale instruments [40–49] capable of multi scale description [50,51]. Observations of the topology at the micrometer scale of RP present at the surface of calculi have been performed through an Environmental Scanning Electron Microscopy (ESEM) equipped with an energy-dispersive X-ray spectrometer (EDS) [52–54]. The chemical composition of such biological entities was obtained through classical Fourier Transform Infrared (FT-IR) spectroscopy [55]. Moreover, in order to obtain the spatial distribution of NaUr in kidney tissue slices, Synchrotron Radiation μFT-IR (SR-FTIR) data have been also collected [56–61].

## 2. Materials and methods

The biological samples including kidney tissue slices and kidney stones used in the present investigation came from Tenon hospital. As a referal center for stone analysis, the urolithiasis laboratory of AP-HP has had the opportunity to perform a morphoconstitutional analysis of more than 70,000 urinary stones over the past three decades coming from more than fifty hospitals in France. Such an analytical procedure based on the structure and the chemistry is of primary importance [62–64] and was previously described [65,66]. Because a number of stones are now removed from the urinary tract by various fragmentation techniques including ESWL, percutaneous nephrolithotomy or ureteroscopy, we selected only unfragmented stones, i.e. a cohort of 35,323 calculi.

Regarding ectopic calcifications, healthy parts of six kidneys removed by nephrectomy for cancer were used for seeking the presence of crystal deposits in the papillae.

All specimens were snap frozen and fixed with acetone. For light microscopy analysis, sections were stained with hematoxyline. For ESEM and SR-μFTIR analysis, four micron slices of the papillae's tip were deposited on low-e microscope slides (MirrIR, Kevley Technologies, Tienta Sciences, Indianapolis). Consent was obtained from Tenon hospital urology team according to the French legislation and samples were immediately anonymized. No data about patients were collected.

An FEI/Philips XL40 Environmental Scanning Electron Microscopy (ESEM) equipped with an energy-dispersive X-ray spectrometer (EDS) was used for a precise description of the sample topology [22–25]. An important feature of the ESEM compared to a conventional SEM is the fact that non-conductive materials can be imaged without any conductive coating, which permits a direct observation with no damage to the sample. Imaging was performed with a gaseous secondary electron detector, an accelerating voltage of 20 kV and a water pressure of 0.4 torr in the chamber. This low pressure was used to maintain a high spatial resolution for the X-ray analysis by minimizing the scattering of the primary electron beam. Also, a Zeiss SUPRA55-VP type scanning electron microscope was used for microstructure observation. This field effect gun microscope operates at 0.5–30 kV. High resolution observations were obtained using two secondary electron detectors: an in lens SE detector and an Everhart-Thornley SE detector.

SR-μFTIR experiments were performed at the infrared beamline SMIS of the Soleil-Synchrotron (St Aubin-Gif sur Yvette) operating in top-up mode at 300 mA [67]. The IR spectra were collected in reflection mode using an infrared microscope (Continuum-Thermo Electron Corporation) coupled to an FTIR spectrometer (Thermo Nicolet Nicplan IR microscope 32X/NA0.6 objective). The IR microscope is equipped with a motorized sample stage (precision 0.5 μm) and a liquid nitrogen cooled mercury cadmium telluride (MCT) detector (50 μm size). Each spectrum was acquired after 100 accumulations (1 mn) at 4 $cm^{-1}$ spectral resolution in the range of 650–4000 $cm^{-1}$. The spatial resolution was 11 × 11 $\mu m^2$. Data acquisition and processing were performed using Omnic software (Version 7.3, Thermo Electron Corporation).

## 3. Results

### 3.1. Epidemiologic investigation

From an epidemiologic point of view, among 35,323 unfragmented calculi referred to our stone laboratory over the past three decades, morphologic examination coupled with FTIR identified 10,462 (29.6%) umbilicated calculi initiated from a papillary deposit, namely a RP. Most of these stones (89.5%) were made of calcium oxalate