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Lithofibrin. A New Remarkably Stable Substance Found in Renal Stones

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Besides inorganic constituents, renal stones contain organic matter known as stone matrix. We have examined such matrix obtained from stones that had been crushed by extracorporeal shock-wave lithotripsy, in order to determine its chemical properties.

Morphologically, the matrix was composed of fibers of different colors, most of them exhibiting autofluorescence. Proteins or any other well-known biochemical material were not present. It was remarkably stable against chemical degradation, and could not be dissolved in hot concentrated acids or bases or any common organic solvent. Elementary analysis revealed carbon (60.8%), oxygen (26.9%), nitrogen (7.6%), hydrogen (5.3%) and sulfur (0.77%) to be present. Furthermore, X-ray emission spectroscopy revealed the presence of titanium and chromium. Solid-phase ^{13}C NMR spectroscopy revealed the presence of aromatic and aliphatic carbon as well as carbon bound to O and/or N. The material was paramagnetic.

All findings indicated the matrix to be composed of a new class of organic compound, probably a polyaromatic heterocyclic organic material. We welcome suggestions on further methods that can ultimately elucidate the nature of lithofibrins.

For a long time,[1] the main part of renal calculi has been known to consist of inorganic components. However, in addition, it contains organic matter, called matrix. Several studies have revealed matrix to contain proteins as reviewed by Morse and Resnick.[2] When we obtained renal stones for analysis, from extracorporeal shock-wave lithotripsy (ESVL), matrix appeared as dust or textile fibers in the samples. In order to investigate the amino acid composition, we tried to hydrolyse them, but the fibers were resistant to hydrolysis. We also found them to be insoluble in all common organic solvents. In order to characterize the compound we then tried a lot of techniques. These experiments indicated that the fibers were not composed of protein but a high-molecular weight polyheterocyclic aromatic structure. We called it lithofibrin from λιθοσ=stone, and *fibra*=thread. Compounds such as this have not yet been described in living matter, and it is still not known how it is produced. We have observed lithofibrin in almost every renal stone, and also in kidneys at autopsy, indicating that it may play an important role in renal stone formation.

Results

Observations. At first we observed both intact renal calculi obtained by spontaneous passage or surgery, and material from ESVL with the aid of a stereo microscope (Zeiss) in ordinary light, and under ultraviolet illumination. Scanning electron microscopy (SEM) and X-ray energy spectroscopy (XES) were performed on a Jeol 840/Link system.

In ordinary light, fibers of different colors and shapes were observed. The most abundant form was white fibers about 3 μm in diameter and with a blue–white fluorescence. Red fibers (about 20 μm in diameter), some of which had red fluorescence, and black, green, blue, and violet fibers were also observed. Furthermore, coarse fibers, about 70 μm and several centimeters in length and brownish semi-transparent membranes were found. The latter two forms showed no fluorescence.

Microscopic examination showed that the fibers were present in almost all renal stones: not only those obtained by ESVL, but also from spontaneous passage or surgery. The fibers formed a framework throughout the whole stone (Fig. 1). Microscopic study of intact renal stones and ESVL material revealed that the fibers were included within the crystalline inorganic components.[3]

Purification. Lithofibrin was purified from renal stones obtained by ESVL. At first, the fibers were removed from most of the inorganic material with the aid of a pair of tweezers (the yield in this procedure may depend

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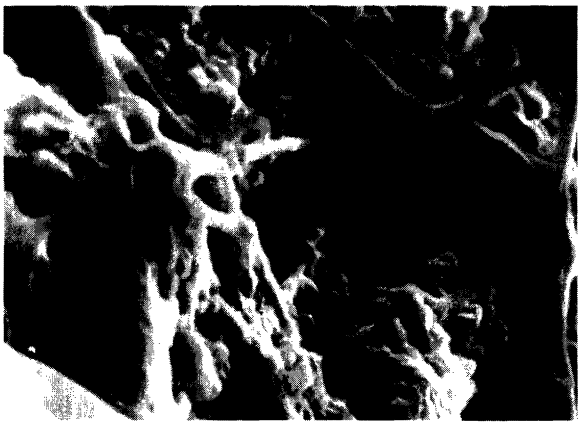

Fig. 1. Scanning electron microscopy of a piece of renal stone, showing a network of lithofibrin incorporated into the inorganic mass composed mainly of apatite. Magnification × 1080; 10.8 mm corresponds to 4.5 μm.

Table 1. Treatments of lithofibrin having no effect

HCl (6 mol dm^{-3}, 110 °C, 5 days)
HNO_3 (conc., 5 days)
NaOH (10 mol dm^{-3}, 100 °C, 1 h)
$FeCl_3$ (sat., 1 day)
Picric acid (sat., 3 days)
$KAg(CN)_2$ (1 mol dm^{-3}, 1 day)
H_4NOH (conc., 1 day)
$K_2Cr_2O_7$ (6%, 1 day)
NaOCl (techn., 1 day)
H_2O_2 (35%, 1 day)
Zn + HCl (6 mol dm^{-3}, 1 h)
Organic solvents: chloroform, acetone, acetic anhydride, ethanol, ethyl acetate, dichloromethane
Ester mixture (Panasolve^R) intended for destruction of epoxy resins

Table 2. Treatments having limited effect on lithofibrin

H_2SO_4 (conc.: white fibers became black)
$HClO_4$ (conc.: ruptures after 2 weeks)
Aqua regis (diminished fluorescence and ruptures after 3 days)
Heating to 450 °C (ruptures and disappearance of fluorescence after 2 h)

on the patience of the performer. Roughly about 90% of the total weight are removed by this procedure). Then soluble organic and remaining inorganic material was removed by reflux of about 4 g of raw material for 2 × 15 min in 250 ml of HCl (6 mol dm^{-3}) and filtration. The brown filtrate was discarded, and the insoluble material (26 mg) was retained. This material contained brown amorphous material (probably denaturated proteins) and lithofibrin, which was collected.

Chemical properties. We investigated the chemical stability of the purified lithofibrin by treating them with different solvents and observing the effect in a dissecting microscope. Reagents that had no or limited effects are listed in Tables 1 and 2, respected.

Elemental analysis revealed the presence of carbon (60.8%), oxygen (26.9%), nitrogen (7.6%), hydrogen (5.3%) and traces of sulfur (0.77%). Pyrolysis followed by gas chromatography and mass spectroscopy (PGCMS) revealed components (toluene, pyrrole, cresols, acrylonitrile, and 1,4-dicyano-2-butene) that confirmed lithofibrin to be an organic compound containing nitrogen. Despite a high (950 °C) pyrolysis temperature, the material was not completely destroyed.

X-Ray crystallography revealed no characteristic diffraction lines, indicating an amorphous structure, however a low degree of crystalline order may be present.

Spectroscopic examinations. Spectroscopic investigation consisted of fluorimetry, infrared spectroscopy, X-ray emission spectroscopy (XES), ^{13}C NMR spectroscopy, and EPR spectroscopy.

Excitation and emission wavelengths were measured with a Turner 430 spectrofluorimeter (bandwidth 15 nm). Peak excitation and emission wavelengths were 395 and 428 nm, respectively. Fig. 2 shows the infrared spectrum

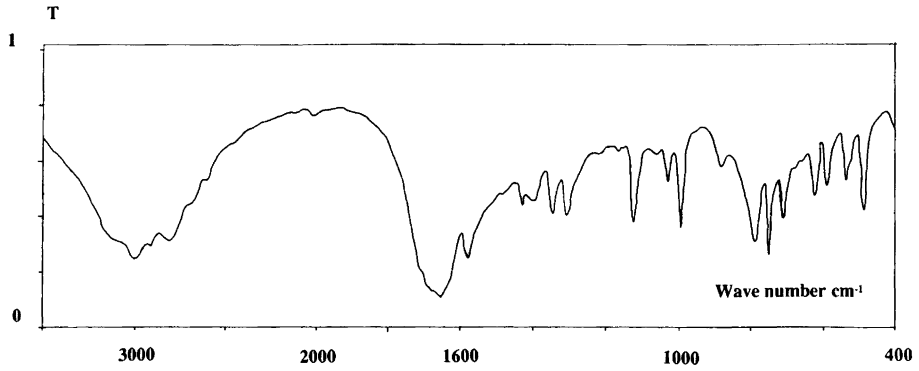

Fig. 2. Infrared spectrum of lithofibrin in KBr. The peak at 1650 cm^{-1} indicates the presence of carbonyl group(s) probably in the vicinity of aromatic structures.

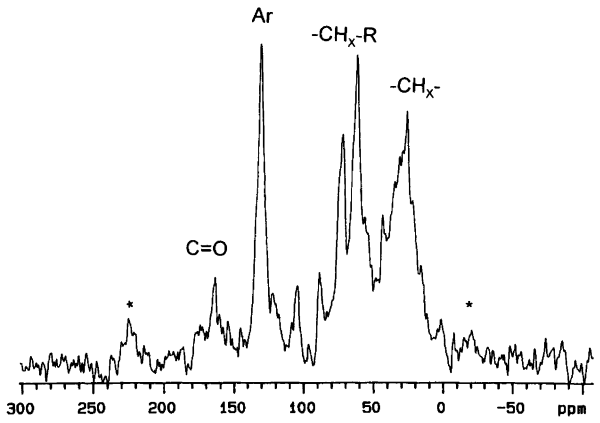

Fig. 3. Solid-phase ^{13}C NMR spectroscopy of lithofibrin, using the CPMAS (Cross Polarization Magic Angle Spinning) technique at 7 kHz with a Varian VXR300 instrument. The peaks indicated with * are artifacts (spinning sidebands) caused by the rotation of the sample cell. The small peak at 164 ppm should be carbonyl (C=O), and that at 131 ppm aromatic carbon (Ar). At about 26 ppm there are several overlayered peaks probably derived from aliphatic carbon ($-CH_x-$). In between the Ar and $-CH_x-$ peaks there are peaks probably derived from different carbon–nitrogen and carbon–oxygen bonds ($-CH_x-R$, where R=O or N, and $x=1,2,3$).

of purified lithofibrin in KBr. It was not possible to relate the fingerprint section of the spectrum to any known substance.

Under the stereomicroscope, different morphological types of fiber were separated with a pair of tweezers. Each type was fixed with glue on a stub covered with aluminium foil, and then muffled at 450 °C for 3 h. An XES spectrum was obtained from the remaining material after coating with aluminium under vacuum. White fibers contained titanium, and black fibers chromium. Background XES spectra were run on spots where no fibers were present. Apart from aluminium from the coating, only traces of iron were present there, probably an impurity of the aluminium foil. The XES equipment was calibrated with pure titanium, aluminium, and copper and zinc in the brass sample holder.

Sodium, potassium, chloride, sulfur, and phosphorus could be detected, however their amounts varied between different fibers. XES spectroscopy is only qualitative, and no quantitative conclusions could be drawn from the spectra. As the sum of the elementary analysis revealed about 100% recovery (101.4% including sulfur) the mean content of these elements should be very small. Silicon was not detected.

Solid-phase ^{13}C NMR spectroscopy revealed the presence of aromatic and aliphatic carbon atoms plus bands probably derived from carbon–oxygen and carbon–nitrogen bands (Fig. 3).

Purified lithofibrin had a paramagnetic shift both at room temperature, and at 77 K (Fig. 4). The shift ($g=2.0067$) was not typical for Ti^{III}, but indicated an *N*-centered free radical to be present.

Discussion

Obviously lithofibrin does not belong to any common class of organic compounds. Morphological characterization indicated variability in the composition, but the insolubility of lithofibrin prevented the use of common chemical separation methods. Although we used a lot of techniques, it was not possible completely to characterize lithofibrin. However, all tests indicate that it is composed of a polyaromatic heterocyclic ring system. The presence of titanium and chromium may be explained by complexation to an organic ring system. The paramagnetic shift may be attributed to some radical in such a ring system, or to Ti^{IV} in a complex.

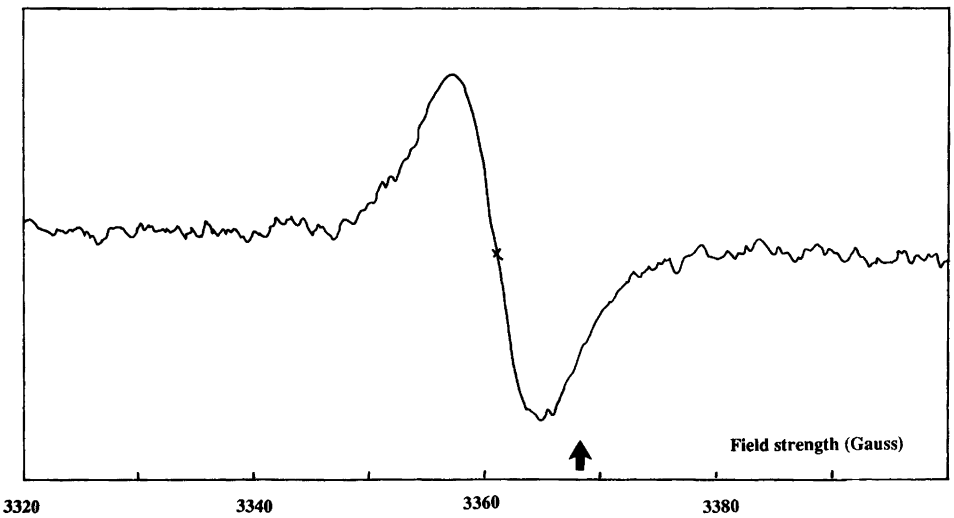

Fig. 4. EPR spectrum (1st derivative) of lithofibrin at 77 K. The radiofrequency was 9.4395 ± 0.0001 GHz. EPR for lithofibrin ($g=2.0067$) was symmetrical around 3361 G. Free electron EPR ($g=2.002322$) was calculated to be 3368 G (arrow).

The fibers were found in kidneys at autopsy,[4] but not in urine, indicating lithofibrin to be derived from the kidney tissue and not from urine. One hypothesis[4] is that lithofibrin is produced in the renal papillas, and then protrudes into the renal pelvis where it acts as centers for crystallisation of the inorganic components. This hypothesis is in accordance with that of Randall[5,6] stating that the stone formation starts with a plaque ('Randall's plaques') at the renal papilla.

Conclusion. To date, we do not know why lithofibrin is formed. As far as we can see, no similar compound has been reported in living matter. This fact and the possibility that lithofibrin is important in stone formation should initiate further studies on this matter. We welcome suggestions on further methods that may ultimately elucidate the nature of lithofibrin.

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