Scanning Electron Microscopy

Volume 1986 | Number 2 Article 45

5-20-1986

Histochemistry of Colloidal Iron Stained Crystal Associated Material in Urinary Stones and Experimentally Induced Intrarenal Deposits in Rats

Saeed R. Khan
University of Florida

Raymond L. Hackett
University of Florida

SCANNING ELECTRON MICROSCOPY/1986/II (Pages 761-765)
SEM Inc., AMF O'Hare (Chicago), IL 60666-0507 USA

0586-5581/86$1.00+05

HISTOCHEMISTRY OF COLLOIDAL IRON STAINED CRYSTAL ASSOCIATED MATERIAL IN URINARY STONES AND EXPERIMENTALLY INDUCED INTRARENAL DEPOSITS IN RATS

Saeed R. Khan* and Raymond L. Hackett

Department of Pathology, College of Medicine
University of Florida, Gainesville, Florida 32610

(Received for publication January 21, 1986: revised paper received May 20, 1986)

Abstract

Organic material associated with the calcium oxalate crystals in urinary stones and experimentally induced nephrolithiasis was stained with colloidal iron and analysed by energy dispersive x-ray microanalysis using standard techniques. Iron was positively identified in the stained specimens indicating that some of the organic material is an acidic mucosubstance. The results also indicate that some of the organic material of urinary stones may originate in the kidneys.

Key Words: Stone Matrix, Urinary Macromolecules,Cytochemistry,Calcium Oxalate, Urolithiasis, Urinary Stones, Urinary Acidic Mucosubstances,Nephrolithiasis.

*Address for correspondence:
Department of Pathology,J-275, JHMHC,
College of Medicine, University of
Florida, Gainesville, Florida 32610
Phone No. (904) 392-3473

Introduction

Currently we are involved in a study of the organic material associated with the crystals in human urinary stones as well as the experimentally induced crystalline deposits in rat renal tubules. For histochemical identification of this organic material we decided to stain the specimens with colloidal iron and to localize the stain by energy dispersive x-ray microanalysis. This is a report of our results from these experiments.

Materials and Methods

The stones used in this study consisted of calcium oxalate and were obtained from Louis C. Herring & Co., Orlando, Florida. They were either sectioned using a diamond wafering saw or were fractured using a knife. A portion of each stone was decalcified by treating with a solution containing 0.25M EDTA at pH 7.2 in half strength Karnovsky's fixative [2]. Decalcified stone i.e. EDTA-insoluble residue was thoroughly washed in water and then rinsed in glacial acetic acid solution for 15 minutes. Part of it was stained in a working solution of Müllers colloidal iron [9] at pH 2.0 for 1 hour after which it was rinsed three times in glacial acetic acid solution for 15 minutes each. After glacial acetic acid rinse the specimens were thoroughly washed in distilled water and then processed for scanning electron microscopy: dehydrated through a graded series of alcohols, and critical point dried. They were examined after coating with gold/palladium or silver, or uncoated, using a Hitachi 450-S scanning electron microscope equipped with a Kevex 7000 Micro-X energy dispersive x-ray spectrometer. A part of the decalcified stone was processed for SEM without colloidal iron staining and examined. Part of each stone was

also stained without decalcification and examined by SEM.

Intrarenal calcium oxalate crystal deposition was induced by intraperitoneally injecting sodium oxalate in male Sprague-Dawley rats [4]. Sodium oxalate was injected at the rate of 7mg/ per 100g of rat body weight. Kidneys were fixed by perfusing them through the aorta with a mixture of paraformaldehyde and glutaraldehyde [5]. Fixed kidneys were stained, processed for scanning electron microscopy and examined similar to the decalcified stones. Unstained kidneys were used as control. Colloidal iron staining of the glomeruli also acted as the control.

Results

Calcium Oxalate Stones

As has been previously described [2] decalcified stones showed concentrically arranged fibrous material (Fig. 1) with occasional ghosts of calcium oxalate monohydrate and calcium oxalate dihydrate crystals. Cellular debris consisting of amorphous substances, vesicular material and distinct red blood cells was often present on the outer surfaces of the decalcified stones and occasionally even inside the stones. Microanalysis of the colloidal iron stained decalcified (Fig. 2A) as well as nondecalcified stones (Fig. 2B) showed distinct peaks for iron while microanalysis of unstained decalcified stones (Fig. 2C) showed total absence of iron. Stained nondecalcified stones were also positive for calcium.

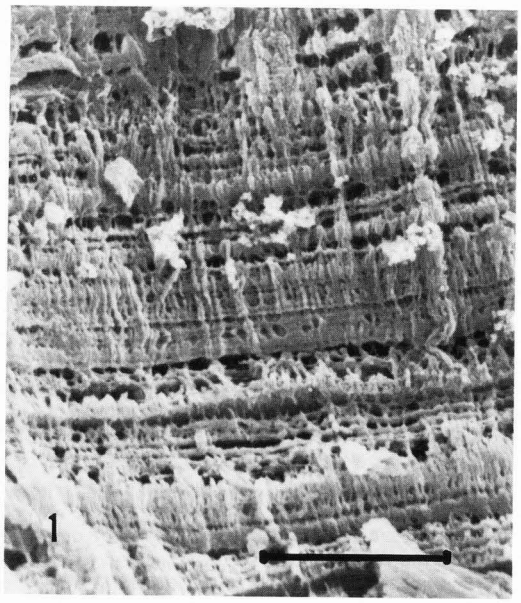


Fig. 1. Fractured surface of EDTA-insoluble residue showing concentrically arranged fibrous material. Bar = 5μm.

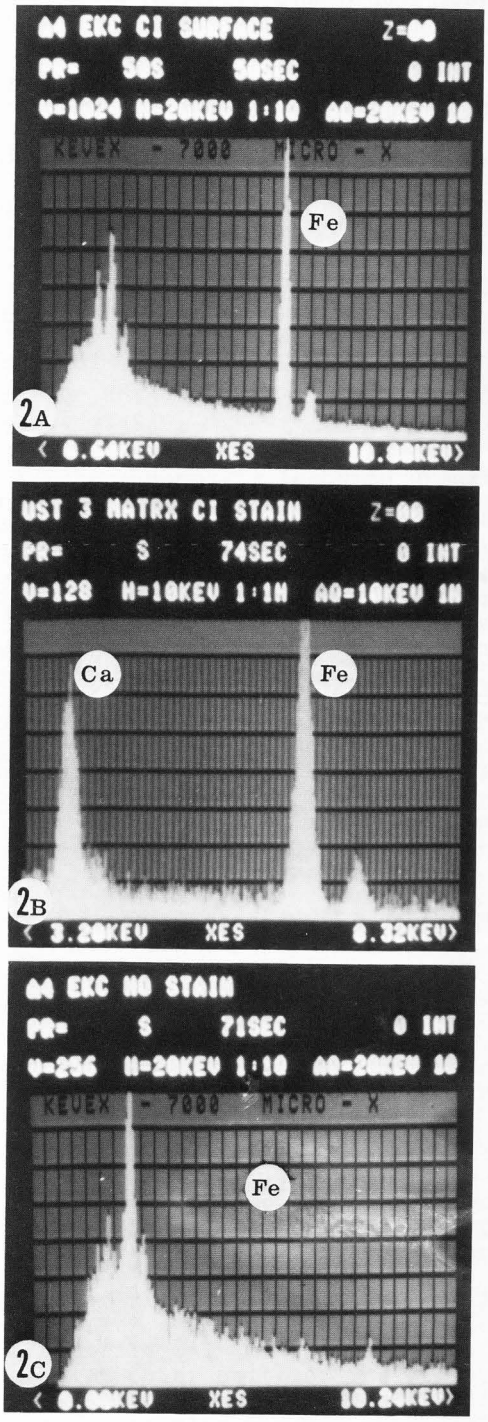


Fig. 2. Energy dispersive x-ray microanalysis of calcium oxalate urinary stones; Fig. 2A, Decalcified, colloidal iron stained; Fig. 2B,nondecalcified, colloidal iron stained; Fig. 2C, Decalcified, unstained. Ca, calcium; Fe, iron.

Calcium Oxalate Crystal Deposits in Renal Tubules:

Aggregates of plate-like crystals of calcium oxalate monohydrate were present in the lumina of the rat renal tubules (Fig. 3). Crystals were associated with amorphous as well as vesicular material. Microanalysis of the crystalline deposits without colloidal iron staining (Fig. 4A) showed the peaks for calcium but no peaks for iron while microanalysis of the deposits stained with colloidal iron (Fig. 4B) showed peaks for only iron, no calcium. Obviously the crystals were demineralized during 1 hour staining at a pH of 2.0. What remained were the ghosts of calcium oxalate crystals which maintained the

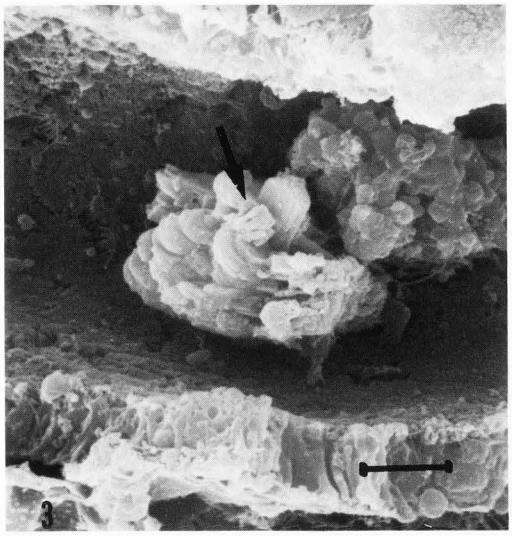
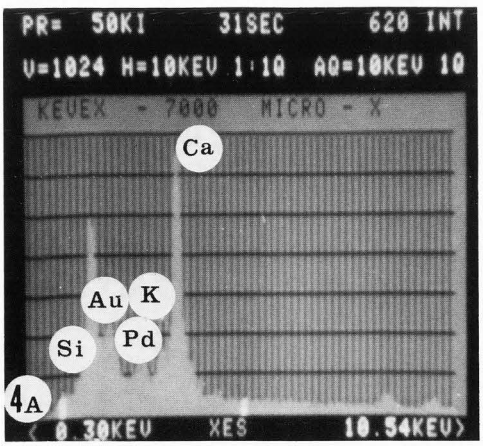


Fig.3. Aggregates of plate-like crystals of calcium oxalate monohydrate (arrow) present in the lumen of proximal tubule. Crystals are associated with cellular debris. Bar = 5μm.



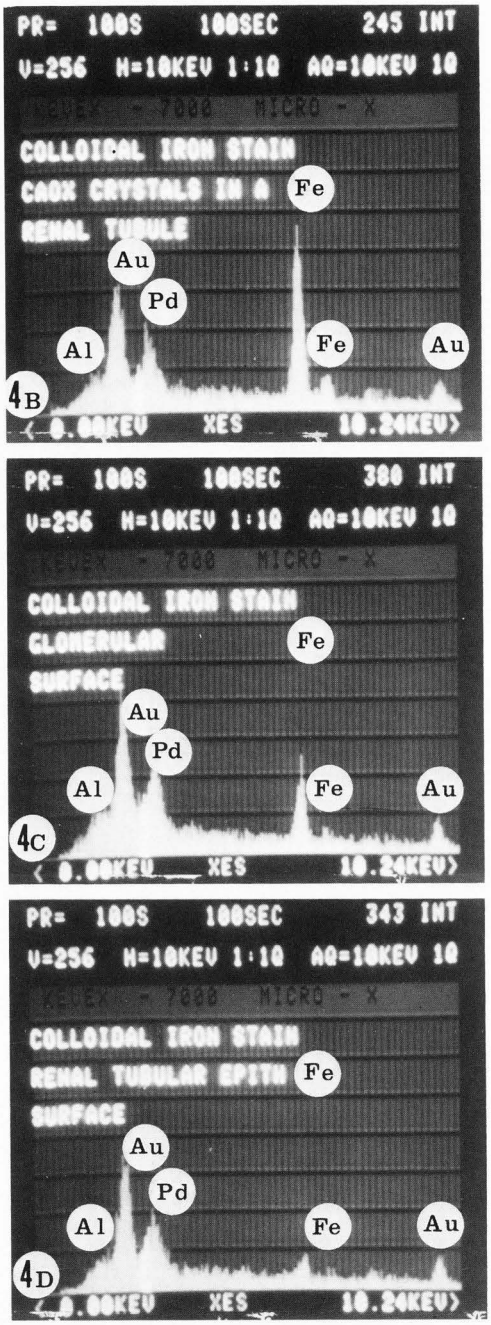


Fig.4. Energy dispersive x-ray microanalysis spectra of rat kidneys containing calcium oxalate crystals; Fig. 4A. Crystalline deposits not stained with colloidal iron; Fig. 4B. Crystalline deposits stained with colloidal iron; Fig. 4C. A glomerulus stained with colloidal iron; and Fig. 4D. Luminal surface of a proximal tubule. Ca, calcium; Fe, iron; K, potassium; P, Phosphorus; Si, silica from the detector; Al, aluminum from the stub; Au, gold and Pd, palladium from the coating.

plate-like habit of the crystals because of the adsorbed mucoid material and the associated cellular debris. In an earlier study using transmission and scanning electron microscopy of the calcium oxalate crystals we have shown that calcium oxalate crystals adsorb proteins from the solution and when the crystals are decalcified only the ghosts are left [6]. Glomeruli of the renal cortex were also stained by the colloidal iron as is evident by the presence of large peaks of iron in their x-ray microanalysis spectra (Fig. 4C). Microanalysis of the general epithelial surface of the renal tubules did not show large peaks for iron (Fig.4D).

Discussion

The presence of iron peaks in the energy dispersive x-ray microanalysis spectra of the colloidal iron stained specimens indicate a positive staining, and the positive colloidal iron staining of the organic material associated with the calcium oxalate crystals in the experimental model as well as in the urinary stones indicates the presence of acidic mucosubstances. Light microscopic histochemical studies of the decalcified urinary stones by Boyce and Sulkin [1] and Watanabe [11] also showed acidic mucosubstances in the organic material associated with the stones. Khan et al. [7] studied a non-decalcified stone removed from a human patient during an anatrophic nephrolithotomy and showed the presence of acidic mucosubstances associated with intratubular papillary deposits of calciumm oxalate crystals. Organic material associated with the urinary stones has been shown to be periodic acid-Schiff reaction (PAS) positive too [1, 7, 11]. Material associated with the experimentally induced intrarenal calcium oxalate deposits has also been shown to stain positive for the presence of acidic mucosubstances as well as being PAS positive [3, 8, 10]. These results indicate that organic material associated with the calcium oxalate crystals both in human as well as experimental urolithiasis contains acidic as well as neutral mucosubstances. Similarity of staining between the crystals present in the renal tubules and the urinary stones suggest that some of the organic material present in the urinary stones could originate in the kidneys.

Present study also demonstrates that light microscopic histochemical techniques utilizing dyes containing elements detectable by x-ray microanalysis can be successfully used in SEM histochemistry. We are planning to use other cytochemical techniques e.g. alcian blue staining, for SEM histochemistry of the urinary stone matrix.

Acknowledgements

We thank Dr. Henry Aldrich for the use of EM facilities and to Ms. Therese Ansman for providing colloidal iron stock solution.

References

1. Boyce WH, Sulkin NM. (1956). Biocolloids of urine in health and in calculous disease. III. The mucoprotein matrix of urinary calculi. J. Clin. Med. 35, 1067-1079.

2. Khan SR, Hackett RL. (1984). Microstructure of decalcified human calcium oxalate urinary stones. Scanning Electron Microsc. 1984; II:935-941.

3. Khan SR, Hackett RL. (1985). Calcium oxalate urolithiasis in the rat: Is it a model for human stone disease? A review of recent literature. Scanning Electron Microsc. 1985; II:759-774.

4. Khan SR, Finlayson B, Hackett RL. (1979). Histologic study of the early events in oxalate induced intranephronic calculosis. Invest. Urol. 17, 199-202.

5. Khan SR, Finlayson B, Hackett RL. (1982). Experimental calcium oxalate nephrolithiasis in the rat, role of renal papilla. Am. J. Path. 107, 59-69.

6. Khan SR, Finlayson B, Hackett RL. (1983). Stone matrix as proteins adsorbed on crystal surfaces: a microscopic study. Scanning Electron Microsc. 1983; I:379-385.

7. Khan SR, Finlayson B, Hackett RL. (1984). Renal papillary changes in patient with calcium oxalate lithiasis. Urology 23, 194-199.

8. McIntosh GH, Belling GB, Bulman FH. (1979). Experimental oxalate urolith formation in rats. Aust. J. Exp. Biol. Med. Sci. 57, 251-259.

9. Pearse AGE. (1985). Histochemistry theoretical and applied. Vol. 2. Churchill Livingstone Inc., London.

10. Rushton HG, Spector M, Rodgers AL, Hughson M, Magura CE. (1981). Developmental aspects of calcium oxalate tubular deposits and calculi induced in rat kidneys. Invest. Urol. 19, 52-57.

11. Watanabe T. (1972). Histochemical studies on mucosubstances in urinary stones. Tohoku J. Exp. Med. 107, 345-357.

Discussion with Reviewers

M. Resnick: Do authors have any experience studying non-calcium oxalate stones such as calcium phosphate, uric acid or magnesium ammonium phosphate.

Authors: To date, we have studied calcium oxalate stones only.

M. Resnick: What is the authors' opinion regarding the specificity of the incorporation of acid mucosubstances? Is this a specific process or is it non-specific in nature?

Authors: In common with many organic materials some of the mucosubstances are probably adventitiously acquired and thus non-specifically incorporated. Some of the mucosubstances may be specifically adsorbed on crystal surfaces or may act as heterogeneous nucleators of the crystals.

A. Hesse: The authors describe the staining of the stone matrix with colloidal iron. How many concrements were so treated and was positive staining achieved in all the stones?

Authors: We studied more than twenty stones for this study. All of them showed positive colloidal iron staining.

A. Hesse: What role does the purity of the stones (calcium oxalate monohydrate or calcium oxalate dihydrate) play with respect to the presence of the acidic mucosubstances?

Authors: None that we could tell.

A. Hesse: Under what conditions did the calcium oxalate crystals form in the proximal tubules in the experimental studies on the rat, and what part did the acidic mucosubstances play in this?

Authors: Lithogenic challenge with sodium oxalate results in hyperoxaluria and the formation of calcium oxalate crystals in the tubular lumina of the rat kidneys. Crystal deposition is associated with epithelial necrosis. We do not yet know which of the two, crystal formation or epithelial necrosis happens first. We think that under highly supersaturated conditions calcium oxalate crystals form which then induce cellular necrosis with release of cell debris into the tubular lumen, and incorporation of the cell material into the crystalline deposits. It is this material derived from the epithelial cells that makes up the bulk of the colloidal iron positive substance and induces crystal aggregation and retention. The specific activity of these substances in crystal formation is still debated and evidence for their inhibitory role as well as evidence that they may act as heterogeneous nucleators of the crystals can be found in the literature.

R. Tawashi: You indicated that calcium oxalate deposits in renal tubules were associated with amorphous material, what is the nature of this material and if they were subjected to EDX analysis?

Authors: They were PAS and colloidal iron positive indicating mucosubstances with adjacent glycol groups as well as acidic groups.

J.L. Meyer: Other than its staining properties, do you have any information to suggest that the kidney matrix and the stone matrix are chemically similar?

Authors: Not in our model. But in a study of experimental renal stone formation in rabbits, Wakatsuki et al. (J. Urol. 133, 319, 1985) measured glycosaminoglycans (GAG) in the stone matrix, renal tissue and urine by 2-dimensional electrophoresis and found hyaluronate as the sole GAG in the stone matrix, and a main component of the stone forming kidneys.

J.L. Meyer: Is it possible that the rather harsh staining procedure (i.e., glacial acetic acid) could have an effect on the apparent charge of the organic matrix?

Authors: Probably so. The technique of heavy metal staining for acid mucosubstances is usually performed at pH 2.0 or lower so that sulfate and phosphoric groups are dissociated permitting them to react with or be blocked by the heavy metal utilized. In the urine with its higher pH, many more groups are presumably dissociated.