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J. A. Gokhale·M. D. McKee·S. R. Khan

Immunocytochemical localization of Tamm-Horsfall protein in the kidneys of normal and nephrolithic rats

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Abstract Studies using in vitro systems have indicated that Tamm-Horsfall protein (THP) can interact with calcium oxalate (CaOx) crystals during kidney stone formation. However, information regarding the nature of its participation in this process remains controversial and unclear. In order to better understand the putative interaction of THP and crystals in vivo, we compared the localization of THP in normal rats and in chronic and semi-acute rat models of nephrolithiasis. In these rats, CaOx crystal deposits were induced in the kidneys by administering ethylene glycol (EG) in drinking water. The formation of CaOx mono- and dihydrate aggregates in the urine was confirmed by scanning electron microscopy. Immunohistochemical localization, as well as protein A-gold labeling at the ultrastructural level, demonstrated that in addition to its normal distribution, THP specifically associated with the renal crystal deposits. The THP-containing, organic matrix-like material consisted of a fine, fibrillar meshwork surrounding individual crystals and their aggregates. In addition, THP also appeared in the papilla, where it is normally absent, concurrent with the appearance of crystal deposits in the kidneys. These observations indicate that in nephrolithic rats the normal localization of THP is altered. Such an alteration may indicate an important physiological event related to crystal aggregation and kidney stone formation.

J. A. Gokhale (✉)
Hospital for Special Surgery, Cornell University Medical College, 535 East 70th Street, New York, NY 10021, USA

J. A. Gokhale·S. R. Khan
Department of Pathology, University of Florida, Gainesville, FL 32610, USA

M. D. McKee
Department of Stomatology, Universite de Montreal, Montreal QC, H3C 3J7, Canada

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Introduction

Urine is a complex mixture of water, ions, salts, lipids, proteins and other metabolites, all of which can influence the process of calcium oxalate (CaOx) crystallization leading to stone formation. Of these components, proteins, which are a major constituent of the urinary stone matrix [7, 25], have the intrinsic ability to interact with crystals and can modulate crystallization effectively in a positive or a negative fashion. Based on its identification and isolation from human stones [14], it was suggested that Tamm-Horsfall protein (THP), the most abundant protein in normal urine, may have a role in stone formation. Although THP has been identified in other mammals, the majority of the structural as well as functional information on THP in relation to stone formation has been derived from human THP, and therefore via in vitro studies. For example, THP was shown to inhibit [8, 18, 36], promote [35, 42, 43] or have no effect [40] on CaOx crystallization. Because these studies have yielded controversial results, it is not clear to what extent THP is involved in stone formation. Therefore, to investigate further the role of THP in stone formation, we used a rat model of nephrolithiasis, to examine the THP-crystal interactions in vivo.

In the normal mammalian kidney, THP localizes in the thick ascending limb (TAL) of the loop of Henle, as demonstrated by immunoperoxidase [22] and immunofluorescent [39] techniques at the light microscopic level, as well as protein-A gold immunolabeling at the ultrastructural level [2, 30, 38, 39]. Furthermore, in situ hybridization study with a radioactively labeled THP-specific RNA probe has confirmed this specific

site of THP synthesis. This study has specifically demonstrated that along the TAL the message for THP is expressed in a gradient fashion, with the highest concentration of the signal in the cortical region, and gradually decreasing towards, and being completely absent from, the inner medulla and the papilla [3]. In the present study, we have compared this precise and specific location of THP in the normal nephron to its distribution in the kidneys of nephrolithic rats, in which stone-forming conditions were experimentally simulated by induction of hyperoxaluria, and CaOx crystal deposition in the kidney.

Materials and methods

Induction of CaOx deposits in the kidney

Chronic model

Male Sprague-Dawley rats (ten animals in one experiment) were housed in metabolic cages 2–3 days prior to the start of the experiment, for acclimatization. To induce a chronic, low-grade hyperoxaluria, animals were given 0.75% ethylene glycol (EG) in drinking water ad libitum and were sacrificed at 42 days.

Semi-acute (accelerated) model

The duration of this model was 10 days and a total of 24 rats were used. As a control group, four rats was given regular water and sacrificed after 10 days. A second control group of six rats was given water with 2% ammonium chloride (AC, to lower the urinary pH) only, for 10 days. From this group, three rats were sacrificed after 6 and after 10 days each. The remaining 14 rats were given 0.75% EG and 2% AC in their drinking water for 10 days, for the accelerated induction of crystal deposits in the kidney. From this group, three or four rats were sacrificed at 4, 6, 8 and 10 days.

In both models, at the time of sacrifice, the kidneys were removed and fixed in either 10% formalin or half-strength Karnovsky's fixative [23] for 24–72 h with or without prior perfusion. The tissue samples were processed for histology and embedded in paraffin, and 5-μm sections were used for localization of THP.

Crystal deposits

Kidney sections were examined at 42 days for the chronic model and at intervals of 4, 6, 8 and 10 days for the semi-acute model, for the presence of CaOx crystal deposits.

Examination of bladder aspirates by scanning electron microscopy

In addition to light microscopy, in order to obtain a profile of crystal deposition during the 10-day period of the semi-acute (accelerated) model, bladder aspirates were collected at the time of sacrifice, on 0.2-μm polycarbonate filters (Solution Consultants, Alpharetta, GA) by using a vacuum manifold. The dried filters were sputter coated with carbon and examined with a Hitachi 4000 scanning electron microscope.

Immunohistochemical staining of THP

Immunoperoxidase localization of THP was performed on rats killed at 42 days in the chronic model, and sections from all four time points (4, 6, 8 and 10 days) of death in the semi-acute model. The antibody and goat serum dilutions were made in 2% bovine serum albumin (BSA, Sigma, St. Louis, MO) in phosphate-buffered saline (PBS). In this study, one of the following antibodies were used at a dilution of 1:100: (1) a rabbit polyclonal antibody to rat THP was kindly provided by Dr. John Hoyer (Children's Hospital, Philadelphia, PA), the specificity for which has been shown previously [21]. (2) In addition, a similar antibody against purified urinary rat THP was made at our facility (in collaboration with Kel farms, Alachua, FL). This antibody specifically detected THP from rat urine on a Western blot (at a dilution of 1:10 000), and showed high reactivity in sensitive ELISA (data not shown). Nonimmune rabbit serum was used as the negative control.

For immunohistochemical staining, paraffin was cleared in xylene from the sections and the sections were re-hydrated through graded alcohol series. After two rinses in PBS of 5 min each, the endogenous peroxidase activity in the tissue was removed by incubation in 3% hydrogen peroxide for 30 min at room temperature. The slides were washed twice in PBS and then treated with 0.1% trypsin (Lipshaw Chemicals, Pittsburgh, PA) for 10 min at 37 °C. The nonspecific binding sites were blocked by incubation with normal goat serum (three drops in 2% BSA-PBS) for 5 min. The sections were then incubated with the primary antibody (anti-THP, 1:100 in 2% BSA-PBS) for 30–60 min at room temperature. After several rinses with PBS, sections were incubated with goat anti-rabbit-horse radish peroxidase conjugate (Fisher Scientific, Atlanta, GA) at a dilution of 1:200 in 2% BSA-PBS for 10 min. The color was developed with 3,3′-diaminobenzidine (DAB, Sigma, St. Louis, MO) as the substrate. After counterstaining with hematoxylin and re-hydration, slides were mounted with Permount (Fisher Scientific, Atlanta, GA) and examined by light microscopy.

Ultrastructural labeling of THP

Tissue processing

Kidney samples from rats treated with EG for 42 days were preserved by vascular perfusion followed by immersion in half-strength Karnovsky's fixative, and washed with 0.1 M sodium cacodylate buffer (pH 7.3), and decalcified for 2 weeks at 4 °C in 4.13% ethylenediaminotetraacetic acid (EDTA) containing 0.1% glutaraldehyde (pH 7.3). Following dehydration in ethanol, the samples were then infiltrated and embedded in LR White acrylic resin (Marivac, Halifax, NS), and the embedding medium was subsequently polymerized for 2 days at 55 °C. Semithin survey sections (1 μm) were cut with glass knives for light microscopy on a Reichert Ultracut E microtome and stained with toluidine blue. For electron microscopy, thin sections (80 nm) of regions identified as containing calculi by light microscopy were cut with a diamond knife, mounted on formvar- and carbon-coated nickel grids and processed for immunocytochemistry as described below. Sections were conventionally stained with uranyl acetate and lead citrate and examined by transmission electron microscopy using a JEOL JEM 2000FX-II transmission electron microscope.

Gold labeling procedure

The high-resolution, protein A-gold immunocytochemical technique [4] was applied to thin sections of kidney as described previously for bone [28]. Briefly, grid-mounted tissue sections from non-osmicated, decalcified specimens were floated for 5 min on a drop of

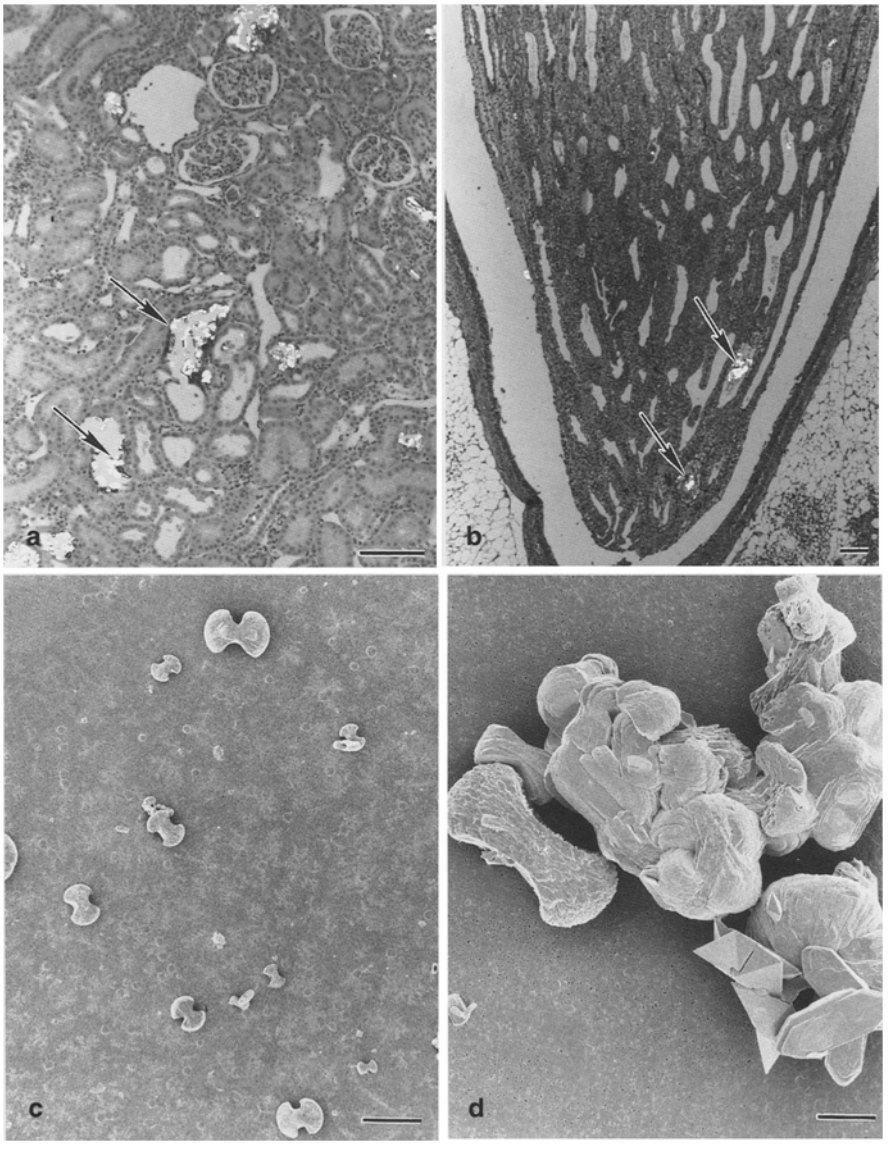

Fig. 1a–d CaOx crystal aggregates in rat nephrolithiasis models. In the rat kidney sections, typical birefringent crystal deposits are seen in the corticomedullary area in the chronic model (*arrows*, **a**). Typical deposits (*arrows*) in the papilla in the semi-acute model are shown in **b**. Scanning electron microscopic examination of bladder aspirates show single crystals (**c**) as well as some aggregates at day 4, and mostly numerous large aggregates (**d**) at day 10. *Bar* equals 100 μm (**a**), 500 μm (**b**), 50 μm (**c**) and 10 μm (**d**)

0.01 M PBS containing 1% ovalbumin (Sigma, St. Louis, MO) and then transferred and incubated for 1 h at room temperature on a drop of rabbit anti-THP diluted 1:20 in PBS containing 0.1% Tween detergent. After incubation, sections were rinsed with PBS, placed again on PBS-1% ovalbumin for 5 min, and incubated for 30 min at room temperature with protein A-gold complex (gold particle diameter approximately 14 nm). Controls consisted of the same sequence of incubation steps, with rabbit non-immune serum substituted for anti-THP antibody.

Results

Crystal deposition in rat kidney

In kidney sections, CaOx crystals were seen as birefringent deposits under the polarized light. Examples of typical crystal deposits are shown in Fig. 1a, b.

Chronic model of nephrolithiasis

The location and appearance of crystals in this model has been described in detail elsewhere [13]. Briefly, at the time of death, crystal deposition ranged from mild to severe in one or more of the following areas of the kidney: cortex, corticomedullary junction, papilla (either in tubules or externally), papillary tip and fornices.

Semi-acute model

In this model, the renal areas of crystal deposition were similar to those listed above for the chronic model. However, in contrast to the chronic model, described above, crystal deposition was accelerated and deposits

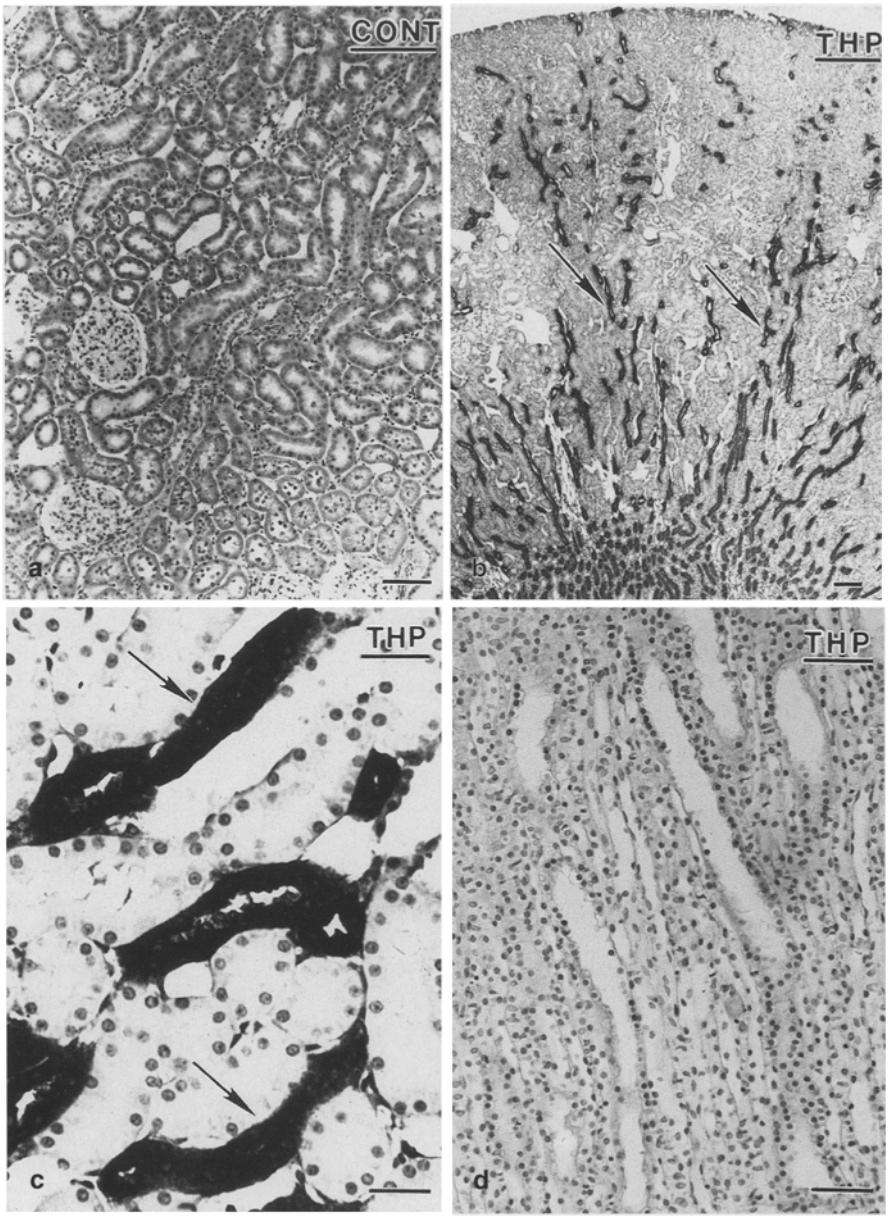

Fig. 2a–d Immunoperoxidase staining of THP in normal rat kidney. Sections incubated with non-immune rabbit serum and peroxidase-conjugated secondary antibody show an absence of labeling from the various elements of the kidney (**a**). With the polyclonal antibody, positive staining is seen in the cortex and outer medulla, specifically in the thick ascending limbs (TALs) of Henle's loop (*arrows*, **b**). A closer examination reveals that the cells lining the TALs stains intensely positive for THP (**c**). In contrast, the papilla stains negative for THP (**d**). *Bar* equals 100 μm (**a**, **d**) and 25 μm (**b**, **c**)

were more prevalent in the papilla. Examination of kidney sections over the 10-day period showed that typical crystal deposits appeared at day 6 (after 5 days of treatment) and were present thereafter.

Profile of crystal deposition in the urine

Examination of the bladder aspirates revealed that at day 4 in the semi-acute model, single crystals were present (Fig. 1c), as well as a few small aggregates. The number of aggregates increased gradually such that by day 10 most of the crystals were present in the form of large, numerous aggregates (Fig. 1d). Based on their morphology, aggregates were identified as containing CaOx monohydrate as well as dihydrate crystals.

Histochemical localization of THP by immunoperoxidase staining

Control rats

The specificity of the anti-THP antibody was demonstrated by a comparison of sections incubated with non-immune serum (Fig. 2a) or the antibody (Fig. 2b–d). As shown in Fig. 2a, incubation of sections with the non-immune serum showed no staining for THP. After incubation with the anti-THP antibody,

kidney sections from control rats showed a typical normal pattern of distribution (in the cortex and the medulla), with intense staining in the TALs (Fig. 2b). Examination of TALs at higher magnification revealed intense cytoplasmic staining of the epithelial cells lining the TALs (Fig. 2c). As reported previously by others [2], the papilla was negative for THP (Fig. 2d).

Nephrolithic rats

Chronic model. In addition to the normal staining pattern described above for the TAL, the darkly stained matrix-like THP was closely associated with the crystal deposits in the cortical and medullary regions of nephrolithic kidneys. Furthermore, it was seen that positive staining for THP in the papilla was significantly pronounced in kidneys with extensive crystal deposition.

Semi-acute model. In the EG+AC-treated rats, the appearance of THP in the papilla coincided with the formation of crystal deposits in the kidney. At day 4 (when no deposits were seen in the sections), there was no abnormal THP staining. In contrast, at day 6, when a few crystals were seen in the sections, some papillary tubules stained positive for THP. At day 8, in rats also with a few deposits, the THP staining in the papilla remained somewhat similar to that on day 6. In contrast, in rats with comparatively more deposits, the number of stained papillary tubules had increased significantly. In rats with 10 days of EG+AC treatment, THP was found associated with the crystal deposits in the papilla. In addition, some of the renal tubules contained deposits of the darkly stained matrix-like material, without any evidence of crystals. Examples of THP associated with crystals and present without crystals are shown in Fig. 3a and Fig. 3b, respectively.

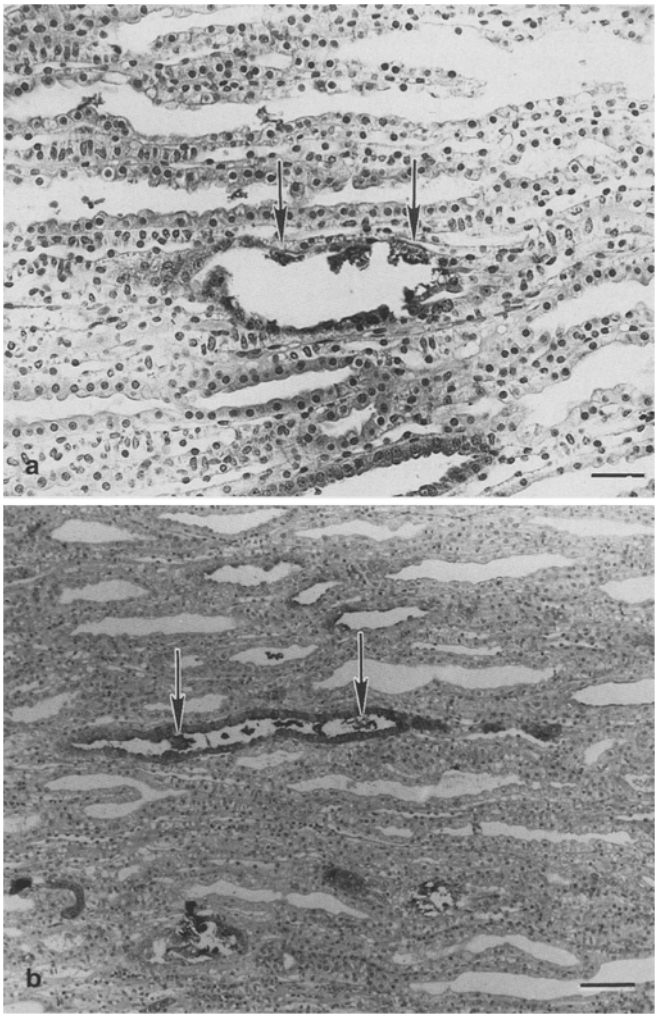

Fig. 3a, b Immunoperoxidase staining of THP in the nephrolithic rats. In addition to its normal distribution pattern, in the nephrolithic rats, THP is closely associated with the crystal deposits (*arrows*) found in the corticomedullary region (**a**). In addition, it appears in the papilla in association with the crystals, as well as without evidence of crystals, in the lumen of some papillary tubules (*arrows*, **b**). *Bar* equals 25 μm (**a**) and 100 μm (**b**)

Ultrastructural immunocytochemistry of THP

Sections incubated with non-immune serum (Fig. 4a) showed no labeling, whereas sections incubated with anti-THP antibody revealed a specific labeling pattern for THP over a meshwork of finely fibrillar, organic material in the lumens of the tubules (Fig. 4b–d). Gold particles were generally associated with small-clustered or attenuated masses of this fibrillar material often in the vicinity of, but not necessarily apposed to, single or aggregated crystal ghosts (Fig. 4b), or with extensive accumulations of the THP surrounding the crystal ghosts of the renal deposits (Fig. 4b, c). The fibrillar nature of this immunolabeled material is shown in greater detail in Fig. 4d.

Discussion

Proteins have long been known to be important components of biological crystallization systems. Due to their inherent property of binding to crystals, many proteins can act as efficient modifiers of the crystallization process [1]. In pathological crystallization such as observed in the urinary stone formation, they are a major constituent of the stone matrix [7] and may modulate the formation and subsequent growth of urinary calculi. At the ultrastructural level, the intimate association between organic matrix and CaOx crystals has been demonstrated with electron microscopy studies by Khan and Hackett [25].

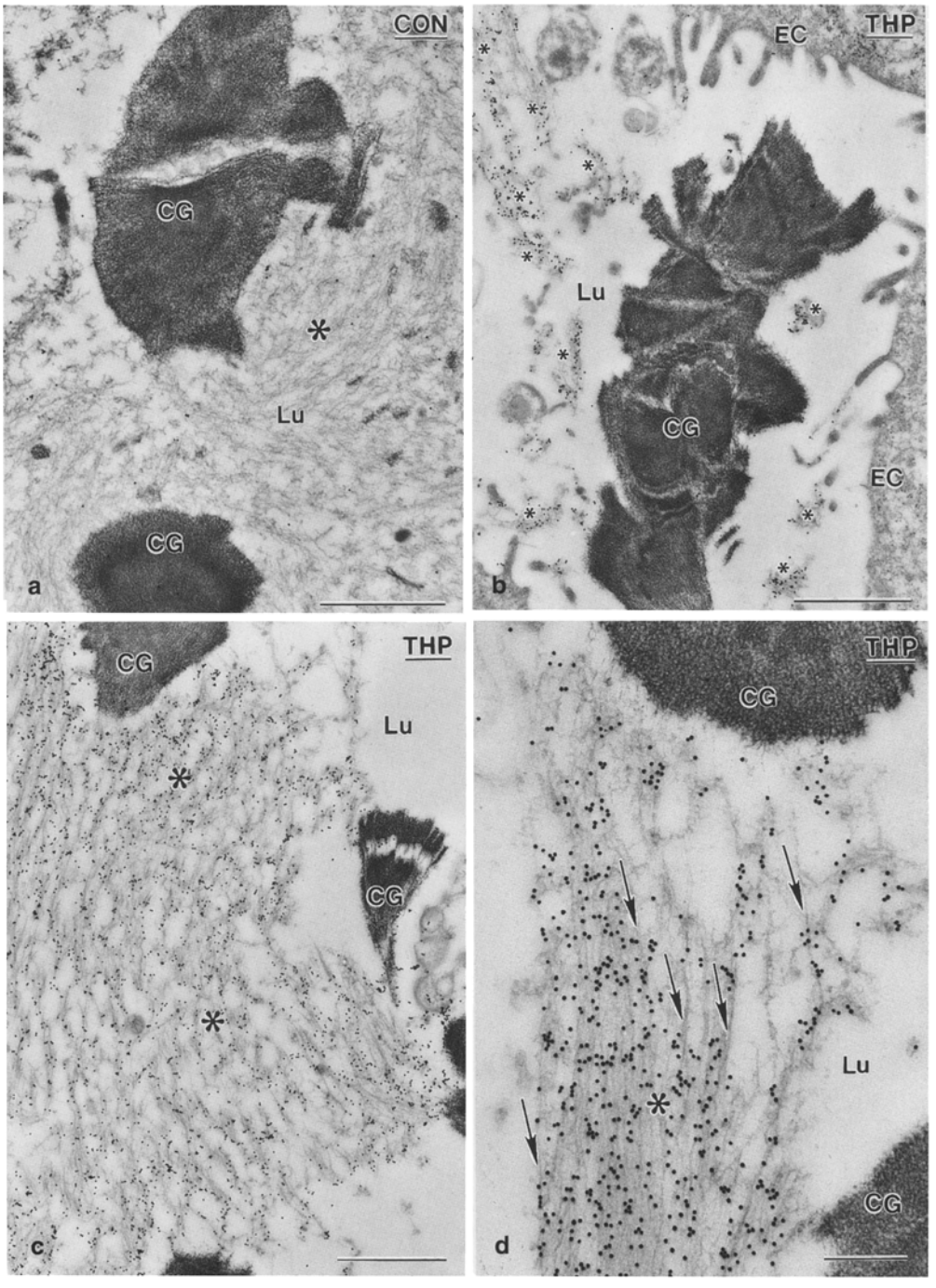

Fig. 4a–d Electron micrographs of kidney sections, from EG-treated nephrolithic rats forming renal calculi, after incubation with non-immune serum (**a**) and anti-THP antibody (**b–d**) followed by protein A-gold complex. In control (non-immune serum) incubations (**a**), only background gold particle labeling is observed over renal deposits – here represented by crystal ghosts (*CG*) in these decalcified samples – and over a finely fibrillar, organic material (*asterisk*) also found within the lumen (*Lu*) of the tubules. Following incubation with anti-THP and a protein A-gold (**b–d**), intense immunolabeling is observed over small clumps (*small asterisks*), or over larger attenuated masses (*large asterisks*), of this fibrillar material (*arrows*) that accumulates within the lumen (*Lu*) of the tubules, often in close association with the unlabeled CG profiles representing decalcified renal calculi. *EC*: tubular epithelial cell. *Bars* equal 1 μm (**a–c**) and 0.5 μm (**d**)

Most of the functional information on THP has been obtained from studies on human THP, and consequently this information is derived primarily from studies performed in vitro. These studies have used either urine processed by different methods, or using different buffers as the assay media, and together have demonstrated that the effect of THP on crystal aggregation can vary widely depending on experimental conditions. For example, Rose and Sulaiman [35], by using the technique of rapid evaporation, concluded that THP induced CaOx crystallization in ultrafiltered urine. In contrast, in a solution of calcium and oxalate, a 5-mg% solution of THP had no effect on crystallization and thus suggested that THP had no role in CaOx crystalluria [40]. In another study, Grover et al. [16] noted that by the evaporation technique THP (35 mg/l) promoted CaOx crystal deposition. In the same study, by the oxalate load method, THP significantly reduced the

size of precipitated CaOx particles. In experiments using undiluted, ultrafiltered human urine, THP significantly inhibited crystal aggregation [36]. Hess et al. [18] used a variety of buffers in a seeded assay system and demonstrated that THP inhibited CaOx aggregation. This property of THP was, however, negatively affected by an increase in NaCl and a lowering of the pH. In a later study, it was shown that addition of 3.5 M citrate reversed the property of promoting CaOx aggregation by THP from stone formers to that of inhibiting aggregation [20]. In summary, the assay conditions are of critical importance in determining the effect of THP on crystallization, and the discrepancy in the results from these in vitro assays may be partly attributable to the different interpretations based on these diverse techniques.

To the best of our knowledge, the association of THP with renal crystals has not been investigated in an experimental in vivo system. In the present study, by examining the interaction of CaOx crystals and THP in situ in intact renal tissue, certain issues regarding matrix-mineral relationships and crystal aggregation are addressed, issues that cannot be readily analyzed by in vitro methodologies. As discussed by Khan and Hackett [24], one can use various experimental protocols to induce either chronic or acute stone-forming conditions in in vivo models. These protocols are based on the induction of hyperoxaluria. Hyperoxaluria is a primary requirement for deposition of crystals, and it can be induced either indirectly by using any oxalate precursor, or by direct loading of oxalate salts via different routes. Based on the route and the precursor used, chronic [15, 27], semi-acute [6, 15] or acute [26] hyperoxaluria can be induced.

Since rats have an oxalate metabolism very similar to that of humans [24], they provide an ideal in vivo system to study processes involved in stone formation. As indicated in the introduction, this study has utilized two slightly different models of stone formation in rats. Oral administration of EG induces a low-grade persistent chronic hyperoxaluria ultimately leading to characteristic CaOx deposits in the kidney [33], a process that very much resembles stone formation in humans. The quantity of CaOx crystals deposited in the kidney is proportional to the concentration of EG used to treat the animals [5]. Based on the reports from other laboratories and from our own experience, a dose of 0.75% EG was selected as one which induced mild to severe crystal deposition after 42 days of treatment.

As an alternate protocol to using EG alone, Lyon et al. [27] found that, by using a combination of EG and AC in drinking water, crystal deposition in kidneys could be accelerated. A major advantage of this particular protocol is that because of the lowering of urinary pH by AC, the crystal deposition process is accelerated considerably. In addition, we observed that, in contrast to the chronic model, crystals were seen much more frequently in the papillary area in this model. By examination of bladder aspirates by scanning electron microscopy and in situ crystal deposition in the kidneys by light microscopy, this short-term accelerated model allowed us to precisely track the gradual course of crystal deposition over 10 days.

A similar accelerated model has been used by other researchers [6, 10], who described crystal deposition as occurring in various parts of the nephron. Using this accelerated model we found that, although hyperoxaluria was present as early as day 2, crystals were evident in the kidney commencing after 5 days of EG treatment and persisted thereafter. Positive staining for THP in the papilla was seen only after 5 days of treatment, concurrent with this crystal deposition. In contrast, prior to crystal deposition (day 4), no THP was found in the papilla. This indicates that the process of crystal deposition and the appearance of THP in the papillary tubules occurred somewhat simultaneously. As the extent of deposition increased (as indicated by the larger aggregates seen in the bladder aspirates and larger and more numerous deposits found in the kidneys), substantially more papillary tubules stained positively for THP.

The distribution of THP was an ideal parameter to study the alteration, if any, of protein expression in the nephrolithic kidney, since due to its specific site of synthesis in the TAL it is frequently used as a conserved marker for the TAL [34]. As demonstrated by the immunostaining at the light microscopic level, it was observed that the matrix-like THP was closely associated with the crystals deposited in the treated rats, and showed abnormal localization by appearing in the papillary tubules. Furthermore, at the ultrastructural level, immunogold-labeling technique confirmed this observation and additionally revealed that THP was a prominent component of a fibrillar, mesh-like material surrounding the crystal ghosts found in the tubular lumens. In contrast, this protein was not detected within the crystal ghosts. This indicates that THP primarily associates with the surface of individual crystals and the outermost aspect of crystal aggregates, and indeed is not a major internal constituent of the crystal ghosts themselves.

It is noteworthy to consider certain physicochemical properties of THP related to the ability of the protein to self aggregate. Physicochemical conditions that lead to THP aggregation include increasing the concentrations of various cations as well as increasing the concentration of THP itself (reviewed in [17]). In these cases, THP aggregation in vitro leads to the formation of large, macromolecular assemblies of this protein that form elongated fibrils [41] having dimensions in the range of similar fibrils observed here in the present study in vivo and reported previously [2]. We have demonstrated not only that these fibrils are rich in THP, but that they frequently accumulate, in somewhat regular and aligned patterns, in close proximity to renal calculi found within the tubular lumens of the

nephrolithic kidneys. It is interesting to consider that this meshwork of THP-containing fibrils, surrounding both individual CaOx crystals and small aggregates of these deposits, might in some physical and/or chemical way be related to the demonstrated capacity of THP to act as an inhibitor of crystal aggregation during urolithiasis [19, 36, 37]. Moreover, interposing fibrils of THP may physically deter crystal aggregation by spatially separating individual crystals and small deposits, and thus counteracting the potential of crystal-bound coatings ("crystal ghosts") comprising various macromolecules, such as found for osteopontin in renal calculi [29], to induce viscous binding [12, 32, 37] among these deposits.

The following conclusions can be made based on the data obtained in this study: Firstly, hyperoxaluria followed by deposition of CaOx crystals in the kidneys induced accumulation of THP around the crystals in both the cortex and the medulla. Secondly, THP also appeared in the papillary tubules, an otherwise abnormal site for its expression, where it was present in association with the crystals and also as what appeared to be matrix-like deposits in some tubular lumens, even where crystals were not readily visible. Taken together, these observations suggest that the normal localization of THP is altered in response to the process of nephrolithiasis. An increase in overall THP synthesis followed by its deposition has been reported in a variety of renal conditions [9, 11, 31]. It is possible that in these models of nephrolithiasis a similar, generalized tissue response involving abnormal and increased deposition of THP throughout the kidney takes place. However, it remains to be determined if this occurrence is the cause or effect of pathological crystal deposition in the kidney.

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References

1. Addadi L, Weiner S (1985) Interactions between acidic proteins and crystals: stereochemical requirements in biomineralization. Proc Natl Acad Sci 82:4110
2. Bachmann S, Koeppen-Hagemann I, Kriz W (1985) Ultrastructural localization of Tamm Horsfall glycoprotein (THP) in rat kidney as revealed by protein A-gold immunocytochemistry. Histochemistry 83(6):531
3. Bachmann S, Metzger R, Bunnemann B (1990) Tamm Horsfall m-RNA synthesis is localized to the thick ascending limbs of Henle's loop in rat kidney. Histochemistry 94:517
4. Bendayan M (1989) Protein A-gold and protein G-gold postembedding immunoelectron microscopy. In: Hayat MA(ed) Colloidal gold: principles, methods, and applications, vol. 1. Academic Press, New York, p 33
5. Blood FR (1965) Chronic toxicity of ethylene glycol in the rat. Fd Cosmet Toxicol 3:229
6. Boeve ER, Ketelaars GAM, Vermeij M, Cao LC, Schroder FH, De Bruijn WC (1993) An ultrastructural study of experimentally induced microliths in rat proximal and distal tubules. J Urol 149:893
7. Boyce WH (1968) Organic matrix of human urinary concretions. Am J Med 45:673
8. Coe FL, Nakagawa Y, Parks JH (1991) Inhibitors within the nephron. Am J Kidney Dis 17(4):407
9. Cohen AH, Border WA, Rajfer J, Dumke A, Glassock RJ (1984) Interstitial Tamm-Horsfall protein in rejecting renal allografts: identification and morphological pattern of injury. Lab Invest 50(5):519
10. De Bruijn WC, Ketelaars GA, Boeve ER, Soeber CW, Cao LC, Schroder FH (1993) Electron energy-loss spectroscopic and image analysis of experimentally induced rat microliths II. J Urol 149(4):900
11. Dziukas LJ, Sterzel RB, Hodson CJ, Hoyer JR (1982) Renal localization of Tamm Horsfall protein in unilateral obstructive uropathy in rats. Lab Invest 47(2):185
12. Finlayson B (1978) Physicochemical aspects of urolithiasis. Kidney Int 13:344
13. Gokhale JA, Glenton PA, Khan SR (1996) Localization of Tamm Horsfall protein and osteopontin in a rat nephrolithiasis model. Nephron 73:456
14. Grant AMS, Baker LRI, Neuberger A (1973) Urinary Tamm-Horsfall glycoprotein in certain kidney diseases and its content in renal and bladder calculi. Clin Sci 44:377
15. Gregory JG, Park KY, Burns T (1984) The interaction of urinary oxalate, calcium and pH in renal calculus formation as demonstrated in a rat model. In: Ryall, et al (eds) Urinary stone. Churchill Livingstone, New York, p 355
16. Grover PK, Ryall RL, Marshall VR (1990) Does Tamm-Horsfall mucoprotein inhibit or promote calcium oxalate crystallization in human urine? Clin Chim Acta 190:223
17. Hess B (1994) Tamm-Horsfall glycoprotein and calcium nephrolithiasis. Miner Electrolyte Meta 20:393
18. Hess B, Nakagawa Y, Coe FL (1989a) Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. Am J Physiol 26:F99
19. Hess B, Nakagawa Y, Kim BK, Coe FL (1989b) Functional heterogeneity of Tamm-Horsfall glycoprotein isolated from urines of normal subjects and idiopathic calcium oxalate stone formers and from human calcium oxalate kidney stones. In: Walker VR, et al (eds) Urolithiasis. Plenum Press, New York, p 173
20. Hess B, Zipperele L, Jaeger P (1993) Citrate and calcium effects on Tamm Horsfall glycoprotein as a modifier of calcium oxalate crystal aggregation. Am J Physiol 65:F784
21. Hoyer JR, Resnick JS, Michael AF, Vernier RL (1974) Ontogeny of Tamm Horsfall urinary glycoprotein. 30:757
22. Hoyer JR, Sisson SP, Vernier RL (1979) Tamm Horsfall glycoprotein: ultrastructural immunoperoxidase localization in rat kidney. Lab Invest 41(2):168
23. Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol 27:137a
24. Khan SR, Hackett RL (1985) Calcium oxalate urolithiasis in rat: is it a model for human stone disease? Scan Microsc 2:759
25. Khan SR, Hackett RL (1993) Role of organic matrix in urinary stone formation: an ultrastructural study of crystal matrix interface of calcium oxalate monohydrate stones. J Urol 150:239
26. Khan SR, Finlayson B, Hackett RL (1982) Experimental calcium oxalate nephrolithiasis in the rat. Role of the renal papilla. Am J Pathol 107(1):59

27. Lyon ES, Borden TA, Vermeulen CW (1966) Experimental oxalate lithiasis produced with ethylene glycol. Invest Urol 4(2):143
28. McKee MD, Nanci A, Landis WJ, Gotoh Y, Gersentenfeld LC, Glimcher MJ (1990) Developmental appearance and ultrastructural immunolocalization of a major 66 kDa phosphoprotein in embryonic and post-natal chicken bone. Anat Rec 228:77
29. McKee MD, Nanci A, Khan SR (1995) Ultrastructural immunodetection of osteopontin (uropontin) and osteocalcin (or osteocalcin-related gene/protein) as major matrix components of urinary calculi. J Bone Mineral Res 10:1913
30. Peach RJ, Day WA, Ellingsen PJ, McGiven AR (1988) Ultrastructural localization of Tamm Horsfall protein in human kidney using immunogold electron microscopy. Histochem J 20(3):156
31. Resnik JS, Sission S, Vernier RL (1978) Tamm Horsfall protein: abnormal localization in renal disease. Lab Invest 38(5):550
32. Robertson WG, Scurr DS, Bridge CM (1981) Factors affecting the crystallization of calcium oxalate in urine-critique. J Crystal Growth 53:182
33. Robinson M, Pond CL, Laurie RD, Bercz JP, Henningson G, Condie LW (1990) Subacute and subchronic toxicity of ethylene glycol administration in drinking water to Sprague Dawley rats. Drug Chem Toxicol 13:43
34. Ronco P, Brunisholz M, Legendre MG, Chatelet F, Verroust P, Richet G (1987) Physiopathologic aspects of Tamm-Horsfall protein: a phylogenetically conserved marker of the thick ascending limb of Henle's loop. Adv Nephrol 16:231
35. Rose GA, Sulaiman S (1982) Tamm Horsfall mucoproteins promote calcium oxalate crystal formation in urine: quantitative studies. J Urol 127:177
36. Ryall RL, Harnett RM, Hibbered CM, Edyvane KA, Marshall VR (1991) Effects of Tamm-Horsfall mucoprotein on calcium oxalate crystallization in undiluted human urine. Urol Res 19:181
37. Scurr DS, Robertson WG (1986) Modifiers of calcium oxalate crystallization found in urine. II. Studies on their mode of action in an artificial urine. J Urol 136:128
38. Sikri KL, Foster CL, Bloomfield FJ, Marshall RD (1979) Localization by immunofluorescence and by light and electron-microscopic immunoperoxidase technique of Tamm Horsfall glycoprotein in adult hamster kidney. Biochem J 181:525
39. Sikri Kl, Foster CL, Machugh N, Marshall RD (1981) Localization of Tamm Horsfall glycoprotein in the human kidney using immuno-fluorescence and immuno-electron microscopic techniques. J Anat 132:597
40. Sophasan S, Chatasigh S, Thanphaichitr P, Dhanamitta S (1980) Tamm Horsfall mucoprotein in urine of potential bladder stone formation. J Urol 124:522
41. Wiggins RC (1987) Uromucoid (Tamm-Horsfall glycoprotein) forms different polymeric arrangements on a filter surface under different physiocochemical conditions. Clin Chim Acta 162:329
42. Worcester EM, Nakagawa Y, Wabner CL, Kumar S, Coe FL (1988) Crystal adsorption and growth slowing by nephrocalcin, albumin and Tamm-Horsfall protein. Am J Physiol 255:F1197
43. Yoshika T, Koide T, Utsunomiya M, Itatani H, Oka T (1989) Possible role of Tamm Horsfall glycoprotein in calcium oxalate crystallization. Br J Urol 4:463