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A. PHYSICAL CHEMISTRY: SUPERSATURATION AND CRYSTALLIZATION

A-1
DETERMINATION OF THE BONN-RISK-INDEX IN THAWED URINES

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This study was performed for quantification of the effect of refrigerator storage on a urine's calcium oxalate crystallization risk. In total, 49 urine samples were collected without use of any preservatives. During the collection period, the urine samples were stored at + 4 °C. Upon completion of collection, an aliqout of the sample was immediately stored in a refrigerator at –24 °C. In the remaining urine, the BONN-Risk-Index (BRI) was immediately determined after collection, and the urinary osmolality, OS, was measured. After one week, both parameters were determined from the thawed samples to obtain BRI_F and OS_F. From the ratios BRI/BRI_F and OS/OS_F, mean, SEM, and SD values were computed. Both methods were tested for equality by statistical methods. Linear regression of BRI vs. BRI_F and OS vs. OS_F revealed correlation coefficients of 0.897, and 0.976, respectively. Method comparison for both data sets revealed that i) no significant deviation of linearity exists, and ii) that both methods are statistically identical ($p \geq 95\%$). No relevant linear correlation between BRI/BRI_F and OS/OS_F exists. However, testing of clinical relevance of the observed differences revealed that the statistical fluctuations between the results of BRI and BRI_F amount to approximately 25%. This variation exceeds the clinically acceptable limit. In conclusion, BRI determination from thawed urines cannot be recommended.

A-2
COMPARISON OF THE BONN-RISK-INDEX TO OTHER METHODS OF URINARY CALCIUM OXALATE CRYSTALLIZATION RISK EVALUATION

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Regular risk evaluation and risk monitoring during stone therapy are recommended measures to ensure reduction of recurrence of crystal formation. This strategy optimizes the patient's treatment by a more individual approach and reduces expensive overtreatment. The presented study was designed to evaluate the BONN-Risk-Index (BRI) through data actualization and evaluation refinement. The BRI was compared to the most common methods of risk evaluation: (1) the calculation of the model value of the relative urinary calcium oxalate (CaOx) supersaturation (RS) and (2) the calculation of the APCaOx, estimating the urine's activity product with respect to CaOx. 201 urine samples were collected from 95 normals and 106 CaOx stone-formers (SF). The BRI, RS, and APCaOx were determined. The data were index-individually grouped into eight classes and frequency distributions were plotted. Furthermore, Receiver Operator Curves (ROC) of the three methods were computed. The log-arranged BRI-groups from both, the normals' and SFs' data, show Gaussian frequency distributions. The ROC of the BRI is almost in all instances higher than ROC of the two other indices. Compared to RS and APCaOx, BRI allows optimum distinction between normals and SF. Although RS and APCaOx require much more analytical efforts for determination, their results show lower reliability.

A-3
THE INFLUENCE OF VARIABLE DIFFERENTIAL VOLUME FUNCTION OF KIDNEYS ON STONE GROWTH RELATED URINARY DEPLETION EFFECT

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Recently, we showed that urine passing growing stone material on its way through the urinary tract becomes systematically depleted of its lithogenic substances. Depending on, e.g., the stone's growth rate, the depletion effect can be of such an order of magnitude that it gains clinical relevance, and thus has to be considered in urinalysis interpretation. Just as any model which tries to describe real complex physico-chemical processes, the "depletion model" also requires simplifications of the true situation. Apart from geometric and physiological simplifications, our recent model assumes that both kidneys, the stone free and the stone forming one, contribute equally to the bladder urine volume; i.e. split function, SF, amounts to 50 %. However, even in healthy individuals, SF varies by ± 6 %. A number of parenchymal defects and hereditary disorders can lead to dramatic decline in renal function resulting in reduction of filtration rate. Patients suffering from these diseases often present with calcium nephrolithiasis. Variable SF has been included in our model. The computations' results demonstrate that the influence of a variable SF on the extent of the urinary depletion effect should be considered in patients whose SF > 56 % or SF < 44 %. In these cases, the relative error of estimation of the depletion effect's extent exceeds 10 % in relation to the value calculated for the ideal situation SF = 50 %. The resulting difference is of clinical relevance.

A-4
A NEW METHOD FOR MEASURING URINARY OXALATE AND CITRATE USING A CHEMILUMINESCENCE ANALYSIS SYSTEM

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It is important for diagnosis and treatment of urinary stones to know urinary oxalate, citrate and other chemistry data relating urolithiasis. However, conventional measuring methods for oxalate and citrate had some disadvantages that are time consuming, complicated and expensive. We developed a new analysis system for the measurement of urinary oxalate and citrate. The system was consisted of a column chromatographic apparatus with a chemiluminescence circuit containing tris (2,2'-bipyridyl)ruthenium(II). A 100-microliters aliquot of sample was injected for analysis. Urinary oxalate and citrate were simultaneously measured by chemiluminescence method using the ruthenium complex and an oxidizing reagent within 20 minutes. These results were compared to a capillary electrophoresis method for oxalate and enzyme method for citrate. The data from the new detection technique for urinary oxalate and citrate showed a strong correlation with conventional methods. This chemiluminescence diagnostic assay will be a time and cost-effective analyzing technique for the determination of urinary oxalate and citrate.

A-5
SERUM CONCENTRATION OF OXALATE BY DIRECT UV DETECTION

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Serum concentration of oxalate is important, because CaOx can be seen in about 70% of urinary tract stones. Several analytical methods are available, however those have problems in cost, sensitivity or rapidity for routine examination. The present study was conducted to examine the sensitivity of direct UV detection for serum concentration of oxalate in healthy volunteers. Separation was obtained on a fused silica capillary, 90 cm long x 100 μm (i.d.)filled with buffer containing 90 mM NaH_2PO4, 90 mM Na_2HPO4 and 0.25MC ethyltrimethylammonium Chloride (pH 6.86) running at a constant voltageof −10 kv. Oxalate was detected by direct UV detection with the signal at 190 nm. Injection was used electrical method running at a constant voltage of −3kv.Using this method, we examined serum concentration of oxalate in 5 healthy male volunteers. Standard curve was liner by using aqueous solution containing 0.11–112 μM ($r_2 = 0.999$). The serum oxalate level was 7.21 μM in one healthy male volunteer, however the remaining 4 could not be detected. These results suggested that direct UV detection would be useful in the determination of serum concentration of oxalate in the screening checkup.

A-6
CHARACTERIZATION OF CALCIUM OXALATE CRYSTAL SURFACES AT NEAR MOLECULAR LEVELS

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Atomic force microscopy (AFM) has been used to obtain real-time in situ images of crystal growth on various different faces of calcium oxalate monohydrate (COM) crystals to elucidate the role of COM surface structure and macromolecules in the regulation of kidney stone formation. Dynamic, real time AFM imaging has revealed dramatically different morphologies for different COM faces, and characterization of the growth modes and rates along different crystallographic directions has allowed us to determine the influence of relevant molecular or macromolecular additives on those rates. We also have used AFM to measure directly the adhesion forces between tipimmobilized molecules and COM surfaces in the same solutions with the same additives. These measurements confirmed the important role of carboxylate groups in both growth and adhesion processes. In addition, these measurements have revealed critical influences of other details of chemical structure, best exemplified by differences in effects seen with polyaspartate and polyglutamate as additives. Comparable differences were demonstrated between commonly studied urinary macromolecules, such as osteopontin, albumin, and chondroitin sulfate. The combination of dynamic imaging of crystal growth and adhesion on various COM crystal faces, correlated with bulk crystallization results, will provide the tools necessary to produce a comprehensive structural model for the interactions of biomolecules with COM related to stone formation.

A-7
THE "FREE-PARTICLE" MODEL OF STONE INITIATION? DEAD OR ALIVE?

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For 25 years, the "Fixed-Particle" model of stone-formation has been considered to be the only viable model of stone initiation. This paper reexamines the "Free-Particle" model taking into account certain dynamic factors ignored by previous authors. A mathematical model of the kidney was set up in Excel designed to mimic the kidney in terms of filtration, reabsorption and secretion of water, calcium and oxalate. The model utilised the same values for the lengths of the various sections of the tubule, their diameters, the number of nephrons/kidney, the number of papillae/kidney and the number of ducts of Bellini/papilla, as described by Kok and Khan. The new factors included in this model were (a) the difference in flow rates of fluid layers in tubules in relation to their distance from the central axis of the tubule, (b) the frictional drag of the tubular walls on particles of different sizes in relation to the diameter of the tubule and (c) the effect of gravity on particles in upwardly-draining tubules. The relative supersaturation of the tubular fluid with respect to CaOx was calculated throughout the length of the tubule. The model showed that, under conditions of hypercalciuria and mild hyperoxaluria, it was possible for CaOx to be nucleated at the end of the descending limb of the loop of Henle. The transit times of crystals forming there, calculated at different distances (r) from the central axis of the tubule segment (radius R), showed that the particles in the range $r/R > 0.95$ were markedly delayed and could stop moving. As the concentration of urinary oxalate increased, the earlier in the tubule were the crystals of CaOx trapped. The model shows that the "Free-Particle" model of stone initiation is still possible.

A-8
FACTORS AFFECTING THE UPPER LIMIT OF METASTABILITY (ULM) FOR CALCIUM OXALATE (CAOX) AND CALCIUM PHOSPHATE (CAP) IN NORMAL URINE

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Urine inhibits nucleation of CaOx and CaP. In vitro assays show that the supersaturation (SS) with respect to these salts at which crystals begin to form (the ULM) is usually higher than the urinary SS, and is reduced in stone formers. The nature of the urinary constituents responsible is not known. 24 hr urine samples were collected from 8 normal males. Urine was dialyzed against 10 mM or 100 mM NaCl using a membrane with a molecular weight (MW) cutoff of 8 kD. Aliquots of dialyzed urine were adjusted to pH 5, 5.7 and 6.5, and calcium concentration adjusted to 5 mM. The ULM for CaOx or CaP was determined by adding aliquots of NaOx or sodium dihydrogen phosphate, and measuring turbidity after 3 hr at 620 nm. SS was calculated using EQUIL. The mean ULM for CaOx in the undialyzed urine (12) was significantly above that for urines dialyzed against 100 mM NaCl (5.5); both were above that of simple buffer. The difference was less marked for CaP (2 and 1.5, respectively). pH had no effect on the ULM of CaOx, while Ca ULM decreased markedly as pH rose. In both assays, ULM was 2-fold higher in urines dialyzed against 100 mM NaCl, compared with 10 mM NaCl. Small MW substances contribute significantly to the nucleation inhibition found in urine, but may contribute more to CaOx than CaP inhibition. Ionic strength also affects the ability of urine to inhibit nucleation.

A-9
FOURIER TRANSMISSION INFRA RED ANALYSIS (FTIR) VS OPTICAL MICROSCOPY IN URINARY STONE ANALYSIS

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This study was done to find out the utility of Fourrier transmission infrared analysis as against Optical Microscopy in the analysis of stone composition. 473 stone samples were analysed using both optical microscopy and FTIR. The delineation of components of stone in either method was compared. FTIR showed that the COM – 146 (30.11%) constituted the majority followed by Mixed stones – 208(42.89%), COD – 60 (12.37%), Uric acid – 48 (10%), Phosphorus – 8 (1.64%) and Cystine – 1 (0.2%). Optical microscopy showed the predominant component in each area studied to be either COM, COD, Uric Acid or Phosphates. The

presence of frond formation, spicules, bosselations and crystal morphology reported by optical microscopic study had individual variations and were observer dependent. The differentiation between COM / COD and Cystine was very difficult. In FTIR due to the avalability of plenty of peaks in the finger print region (400–1200) the differentiation between COM, COD and Cystine stones become easier. The differentiation between different phosphates was also very effective with the FTIR analysis, whereas this was not possible with optical micros copy to a magnification of 100. Comparing the findings of various other investigative modalities for the detection of stone components like qualitative analysis, quantitative analysis, CT Scan, SEM, TEM, Thermogravimetric analysis, Spectroscopic analysis and IR analysis, the FTIR analysis supported by Optical microscopy appears to be the best and cost effective. The analysis reports are scientific and reproducible.

A-10
IS THE MEASUREMENT OF PH IN 24-H URINE SAMPLES USEFUL IN THE SCREENING OF PATIENTS WITH UROLITHIASIS?

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There has been considerable controversy over the years as to whether or not it is useful to measure pH in 24-h urine samples collected from stone-formers. Indeed, many laboratories do not carry out this measurement in their screening of patients with stones. In this paper, the value of 24-h pH in the assessment of patients with urinary stones is re-examined. Twenty-four hour urines and 7-day Diet Diaries were collected from 600 patients with urolithiasis. Urinary pH was correlated (a) with quantitative stone analysis and (b) with the urinary pH from the same patients calculated from the composition of their diets using the techniques described by Remer and Manz (1995). Results showed a strong correlation between quantitative stone composition and urinary pH. Below pH 5.5, stones consisted mainly of UA, sometimes mixed with CaOx. Between pH 5.5 and 6.0, stones consisted mainly of ''pure'' CaOx. Between pH 6 and 7.3 stones consisted of a mixture of CaOx and CaP although the proportion of CaP increased logarithmically over this range. Above pH 7.3, stones consisted of a mixture of CaP and MAP in varying proportions depending on the calcium concentration in the urine. There was a very good correlation ($P < 0.001$) between urinary pH estimated from the composition of the diet and measured 24-h urinary pH in calcium stone-formers. Patients with infection stones or with dRTA had higher urinary pH values than expected from their diet and patients with UA stones had lower urinary pH values than calculated from their diets. It is concluded that it is useful to measure urinary pH in stone-formers.

A-11
INTRACRYSTALLINE MACROMOLECULES IN COM: A SYNCHROTRON X-RAY DIFFRACTION (SXRD) STUDY

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Inclusions in mineral crystals create stacking defects, increase crystal disorder, raise non-uniform strain and reduce crystallite size. The aim of this study was to determine the effect of increasing concentrations of intracrystalline macromolecules on calcium oxalate monohydrate (COM) crystallite size and non-uniform strain. Crystal matrix extract (CME) was isolated from CaOx crystals deposited from healthy urine and added to distilled H_2O, and also to ultrafiltered (10 kDa) human urine to give solutions with final protein concentrations of 0 – 11.0 mg/L and 0 – 5 mg/L, respectively. Aqueous solutions of human prothrombin (PT) were prepared with final concentrations ranging from 0 – 21 mg/L. CaOx crystals were formed by addition of Ca and/or oxalate, and non-uniform strain and crystallite size were determined using synchrotron X-ray diffraction with Rietveld whole-pattern peak fitting and profile analysis. In all cases, non-uniform strain increased and crystallite size decreased relative to protein concentration, reaching plateau values with CME but not with PT. This suggests that the COM crystal can accommodate larger quantities of PT within its structure than CME, which consists of an undefined mixture of urinary macromolecules. These data show unequivocally that intracrystalline macromolecules, especially PT, increase COM crystal disorder in a dose-dependent fashion and support the concept that high endogenous concentrations of intracrystalline proteins may assist stone prevention by increasing the likelihood that urinary crystals phagocytosed by renal epithelial cells will be destroyed by intracellular degradation and dissolution.

A-12
INVITRO STUDY ON THE EFFECT OF DIFFERENT PH VALUES OF NATURAL URINE ON STONE FRAGILITY BY EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY

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Urinary stones removed after surgery were cut into four equal halves. Each piece was made to run in three different pH values of natural urine and one piece was kept as control. After 30 days run, fragility studies were done in an invitro model in extracorporeal shock wave lithotripsy (ESWL) and the results analysed for different types of stones treated with different pH values. The effect of pH on stone fragility was studied for twenty stones, ie., for 80 individual pieces (20×4). Of the total 20 stones, 10 stones were calcium predominant, nine stones were predominant with carbonate apatite (CA) and magnesium ammonium phosphate hexahydrate (MAPH). One stone was of the type uric acid (UA) and ammonium urate (AU). The mean and SD values of fragility were calculated for control and pH treated stones. The comparison of the results between control and pH treated samples for calcium oxalate stones using student 't' test showed no correlation between them, proving calcium oxalate stones did not change in fragility values with any of the pH values selected. Student 't' test performed on control and pH4 treated CA and MAPH stones showed that the difference in fragility values were statistically significant. The fragility values were more at pH4 compared to control and samples of other pH. The fragility value was less for pH8 treated stone when compared to control and pH4 treated and the difference was statistically significant. These results imply that at pH4 stones of the type CA and MAPH become more fragile. Effect of pH was studied for one stone of uric acid and it was seen that uric acid stone became more fragile at pH8.

A-13
BONN RISK INDEX (BRI) IN CALCIUM OXALATE STONE FORMERSBEFORE AND AFTER STONE SURGERY

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To determine the value of Bonn Risk Index in Calcium Oxalate stone formers before and after stone removal. Twenty four hours urinary and stat urine samples were collected from 66 patients with Calcium Oxalate stones before and one month after stone removal with dietary and medical interventions. BRI was estimated in 2 ml of native urine at 37. Ammonium Oxalate 40 mmol/L was added at minute interval with constant mixing in 0.050 ml increments and absorbance measured at 620 in spectrophotometer. Nucleation was recorded with the increase of absorbance. Ionic Calcium was measured by an Ion selective electrode. BRI was calculated by the formula: $BRI = [Ca + 2]$

mmol/L/[Ox-2]. BRI > 1/L was taken as risk and BRI of < 1/L as low to no risk for stone formation. Urinary calcium, magnesium, oxalate, citrate, sodium, potassium, phosphate and pH were recorded in all samples. The mean BRI before surgery in 24 hour urine was 3.4 ?1.8/L as compared to post-operative mean value of BRI 1.4 ?1.4/L (p = .002). Mean urinary calcium before surgery was 1.8 ?0.6 mmol/L vs 1.2 ?0.4 (p = 0.01) after surgery. Mean oxalate added was 0.8 ?0.3 vs 1.3 ?0.5 mmol after surgery. Urinary magnesium showed a hyperbolic correlation with BRI. No correlation was found with citrate. In comparison to 24 hour urine the mean BRI in 14 stat samples was 4.6 ?5.29 vs 2.1 ?1.8 in stat urine post-operative. BRI is a valuable tool to assess risk of Calcium Oxalate stone formation and clearly reflects the influence of dietary and medical intervention post operatively. It can be used to monitor effects of dietary and medical interventions.

B. CRYSTALLIZATION MODULATORS AND MACROMOLECULES

A-14
HYPOCITRATURIA - NORMAL URINE PH IS AN ENTITY INCREASING THE RISK FOR RECURRENCE IN STONE FORMERS

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Citrate is one of the most important inhibitors in urolithiasis. Hypocitraturia is a common risk factor in stone formers. Citrate excretion is regulated – amongst others – by acidosis and protein intake. A considerable number of stone formers, however, show hypocitraturia in the presence of normal urine pH levels. This is potentially due to defects in the renal tubular citrate carriers (NaDC 1 and 3) which may be genetically determined. N = 350 consecutive stone formers were examined. Exclusion criteria were: urinary tract infection, hypokalemia, and steatorrhea. The following parameters were measured: serum: creatinine, calcium, potassium, uric acid; urine: pH-profiles, citrate, calcium, uric acid, ammonia, urea, and creatinine. 83/350 patients were hypocitraturic (48 males, 35 females). 14/83 had low urine pH (< 6), 69/83 showed normal levels (> 6). In the latter group there was a significantly higher recurrence rate (29 vs. 7 %). The two groups were not different in serum parameters. In urine, only pH was significantly lower in the first group. Citrate did not correlate with urine pH, urea, ammonia and creatinine in the hypocitraturia-normal pH group. In the hypocitraturia-low pH patients, there was a significant correlation between citrate and pH, in females also with urea and ammonia. Hypocitraturia-normal urine pH is an entity indicating a high risk for recurrence. Since there was no correlation between citrate and pH, urea, ammonia respectively, citrate excretion is not regulated in these patients as usually. Potentially, they have defects in the renal tubular citrate carriers which may be genetically determined. Genetic examinations should be performed to elucidate a potential genetic disorder in hypocitraturia-normal pH stone formers

A-15
AGGREGATION PROMOTION IN CALCIUM OXALATE STONE FORMATION

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All stones form as aggregates of crystals, and prior research has shown that the urine from many calcium oxalate (CaOx) stone formers demonstrates reduced ability to inhibit aggregation. We have characterized the affect of urinary macromolecular mixtures from idiopathic stone forming patients (SFU) and normal, healthy adults (NU) by measuring changes in the particle size distribution (using an Accusizer 780) of preformed COM crystals in the presence of these urine extracts at near equilibrium solution conditions (0.25 mM $CaCl_2$ and $Na2O_x$ in 150 mM NaCl and 10 mM HEPES, pH = 7.5). We have also tested several individual macromolecules for regulation of COM aggregation. Urinary macromolecules were isolated from random urine samples in the presence of protease inhibitors, using ultradiafiltration against a 10 kD cutoff membrane. Preexisting aggregates in our COM seed crystals were partially dissociated by urinary macromolecules from all NU (n = 25) and most SFU samples, while 7/28 SFU samples induced aggregation, which correlated with disease in a family grouping. Individual macromolecules, including osteopontin, Tamm-Horsfall Protein, albumin and synthetic polyanions (polyD and polyE), again caused COM aggregate dissociation, whereas polycations and chondroitin sulfate had no effect. However, mixtures of polyD with polyR induced aggregation, suggesting that aggregation promotion in urine samples may result from the combination of polyanions and polycations in urine.

A-16
ASSESSMENT OF MACROMOLECULAR INHIBITOR FUNCTION IN KIDNEY STONE FORMERS

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We have characterized the influence of isolated urinary macromolecules from stone formers and healthy adults on calcium oxalate monohydrate (COM) crystallization kinetics, and correlated differences with stone formation. Urinary macromolecules were isolated from random urine samples, collected from 28 stone forming (SFU) and 25 healthy adults (NU). Constant composition assay (CC) at supersaturated conditions (s = 2.2) and aggregation assay (AA) at near saturation conditions (s = 1.1) were used to characterize the inhibitory effects. Inhibitory behaviors were categorized by plotting the ratio of final to initial average particle diameters from the CC assay against those from the AA assay. We, thereby, separated growth related aggregation from static effects and nucleation. Consistent with previous observations, no differences were detectable between SFU and NU population mean values for measured parameters, with little variation between NU individuals. On the other hand, striking differences were noted between specific parameters when comparing samples from selected individual SFU with NU mean values, and these differences were consistent with disease in a family cohort. The most common difference observed was weak aggregation inhibition in exactly half of SFU samples. We conclude that there are several possible crystallization inhibitor defects that lead to stone disease, but weak aggregation inhibition was the most common defect. Averaging effects in population mean values have frequently obscured these differences in earlier studies.

A-17
EFFECT OF URINARY INHIBITORS IN HISTOLOGY OF RAT KIDNEYS

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This experimental study was conducted to assess the protective effect of inhibitors on the histological damages produced by calculogenic agents. Disodium hydrogon citrate, potassium citrate and sodium bicarbonate were the agent tested. Calculogensis was initiated using oral sodium oxalate. Histology of the control group was used for comparing with the other appearances. Over and above the normal appearances of the kidney tubules and glomeruli occasional protein cast was seen in the tubules suggestive of proteinuria. In the group two (sodium oxalate) the collecting tubules showed blood cast. The tubules were dilated. Proximal

convoluted tubules showed hobnailing. Distal tubules showed round bodies. Blood casts were seen in large numbers in the proximal and distal tubules. In group three (sodium oxalate and disodium hydrogen citrate) haemoglobin casts were seen indicating haematuria. Blood casts were seen in the collecting tubules and Bowman's capsule. Focal degeneration was seen in the proximal convoluted tubules. Interstitial inflammation was also seen. In group four (sodium oxalate and potassium citrate) blood casts were seen PCT showed degenerative changes. Glomeruli were congested and cellular. Group five (sodium oxalate and sodium bicarbonate) collecting tubules were dilated and contained blood. Glomeruli were congested. PCT showed degeneration, with hobnailing. Administration of sodium oxalate produced significant degenerative and obstructive changes in the tubules and glomeruli indicating bleeding and urothelial damage. Administration of sodium oxalate along with the inhibitors sodium citrate potassium citrate or sodium bicarbonate reduced the intensity of urothelial damage and presence of blood casts. The protective effect on the damages produced by sodium oxalate was maximum seen in the rats given potassium citrate along with the sodium oxalate. It is concluded that potassium citrate has maximum inhibitor effect on calculogenic changes in the kidneys of experimental rats.

A-18
OXALATE STIMULATES IL-6 SYNTHESIS AND SECRETION IN HK-2 CELLS, A LINE OF HUMAN RENAL PROXIMAL TUBULAR EPITHELIAL CELLS

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Oxalate is a metabolic end product, excreted primarily by the kidney and associated with several pathological conditions. The most common pathological condition involving oxalate is the formation of calcium oxalate stones in the kidney. Several stimuli have been implicated in the development of glomerular and tubular injury in various forms of immune-mediated renal diseases. The elevated level of IL-6 has been reported in the urine of kidney stone forming patients. In the present study we investigated role of oxalate, a major constituent of calcium oxalate kidney stone disease in the production of IL-6 in normal human HK-2 kidney cells. Confluent cultures of HK-2 cells (a renal epithelial cell line of human origin) were exposed to various concentrations of oxalate (0.2 to 2.0 mM), and LPS (0.1 and 10 μg/ml) for various time points (4–24 hours) under serum free conditions. The conditioned mediums were collected and an IL-6 protein level was measured by enzymelinked immunosorbent assay (ELISA). The total cellular RNA was isolated from the cells and subjected to relative quantitative RT-PCR to determine the expression of IL-6 mRNA. The statistical analysis of the results was carried out using the Student's *t* test. HK-2 cells express IL-6 mRNA and Protein. Oxalate increased secretion of IL-6 protein in HK2 cells in concentration dependent fashion. Oxalate exposure to HK-2 cells also induced transcriptional up-regulation of IL-6 gene as determined by increased level IL- 6 mRNA expression following treatment with oxalate. Moreover the effects of oxalate on IL-6 expression were time and concentration dependent. This is the first report demonstrating regulation of IL-6 by oxalate. This study provides first direct evidence that oxalate up-regulates the expression and secretion of IL-6 in renal epithelial cells. The increased IL-6 expression and secretion by renal epithelial cells may play a critical role in the progression of urolithiasis.

A-19
POSSIBLE PROTECTIVE ROLE FOR CITRATE DURING CRYSTAL INDUCED INJURY

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Citrate is a known inhibitor of calcium oxalate (CaOx) crystallization and is one of the most common medicines used by kidney stone patients. Since citrate supports NADPH production to maintain glutathione:glutathione disulfide (GSH/GSSG) redox potential, the central component of the antioxidant system, we decided to investigate citrate's role as an antioxidant during renal epithelial exposure to oxalate (Ox) and CaOx. LLC-PK1, NRK-52E and MDCK cells were exposed to 500 uM/ml oxalate or 150 ug/cm^2 calcium oxalate crystals for 30, 60 and 180 minutes with or without 3 mg/ml. Cell viability was determined by lactate dehydrogenase (LDH) release and trypan blue exclusion. Production of free radicals was determined as hydrogen peroxide and existence of lipid peroxidation was established by ELISA specific for 8-Isoprostane (8-IP). Presence of citrate was associated with a significant decrease in LDH release ($p < 0.001$) and increase in number of cells which excluded trypan blue ($p < 0.05$). Production of hydrogen peroxide ($p < 0.05$), and 8-IP ($p < 0.0005$) secretion into the media also decreased. We conclude that citrate protects renal epithelial cells from injury by preventing lipid peroxidation through the decrease in production of free radicals. In lowering the free radical presence, citrate helps prevent cellular injury and death, risk factors for crystal formation and their retention. Thus citrate retards stone formation by not only reducing ionic calcium concentration through complex formation and inhibiting crystal nucleation, growth and aggregation but also by reducing oxalate and crystal induced oxidative injury to the renal epithelium.

A-20
PROTEIN, MINERAL, AND THE SUSCEPTIBILITY OF CALCIUM OXALATE TO PROTEOLYSIS *IN VITRO*

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Intracrystalline proteins incarcerated within calcium oxalate (CaOx) crystals deposited from human urine may help to prevent stones by facilitating lysosomal destruction of crystals internalised by renal epithelial cells. This study aimed to determine the effect of urinary protein concentration on the proteolysis of CaOx crystals *in vitro*. CaOx crystals were deposited, by addition of oxalate, from ultrafiltered (10 kDa) urine containing organic matrix isolated from demineralised CaOx crystals generated from healthy urine, at final protein concentrations of 0, 0.05, 0.5, 1.0 and 5.0 mg/L. Crystals were fractured and incubated in buffered saturated CaOx solutions of cathepsin D (pH 6.0) or Proteinase K (pH 7.5) for 16 hours at 37°C, prior to field emission scanning electron microscopy and analysis of their intracrystalline proteins by SDS-PAGE and Western blotting for osteopontin (OPN) and prothrombin fragment 1 (PTF1). Irrespective of protein concentration, all untreated crystals, as well as those precipitated at protein concentrations of 0 and 0.05 and 0.1 mg/L and then treated with protease, were smooth. Protease-treated crystals deposited at 1, and especially 5 mg/L, were markedly eroded and degraded. OPN and PTF1 increased directly with the concentration of added crystal matrix: thus the extent of protease damage correlated directly with the amount of protein in the mineral. We conclude that the vulnerability of urinary CaOx crystals to proteolytic attack depends upon the ratio of intracrystalline protein to the amount of mineral precipitated. Urinary crystals deposited at low protein concentrations will resist invasion by lysosomal proteases and consequently, will be more refractive to intracellular destruction.

A-21
TAMM-HORSFALL GLYCOPROTEIN: A GUILTY BY-STANDER IN CALCIUM OXALATE CRYSTALLIZATION

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The aim of this study was to determine whether Tamm-Horsfall glycoprotein (THG) binds irreversibly to the CaOx crystal surface, and thereby, becomes trapped inside the mineral bulk during growth. THG, isolated from healthy human urine, was added to distilled H2O at final concentrations of 0, 8, 21 & 34 mg/L, and to fresh ultrafiltered (10 kDa) urine at a final concentration of 35 mg/L. CaOx crystals were formed in these samples by addition of Ca and/or oxalate, and non-uniform strain and crystallite size were determined using synchrotron X-ray diffraction with Rietveld whole-pattern peak fitting and profile analysis. Binding of THG was also studied using SDS-PAGE and Western blotting of organic extracts of urinary crystals that had been washed with H2O or with dilute NaOH to remove superficial protein. Irrespective of THG concentration, non-uniform strain and crystallite size values of *all* crystals were indistinguishable from those of pure, inorganic CaOx crystals, demonstrating unambiguously that THG is not intracrystalline. Demineralised extracts of crystals washed only with H_2O contained large amounts of THG, while the protein was undetectable in organic extracts of crystals washed with NaOH. THG does not bind irreversibly to the CaOx crystal surface, which is consistent with its weak inhibition of CaOx crystal growth in urine and inorganic media. Its potent inhibition of aggregation must result from steric hindrance, which prevents inter-crystal collision and cohesion. Scrupulous washing must be performed to remove proteins caught loosely between single crystals in aggregates when assessing whether or not they are intracrystalline components of CaOx.

A-22
CHARACTERISATION OF *N*-LINKED GLYCANS ON URINARY PROTHROMBIN FRAGMENT 1 FROM WHITE AND BLACK SOUTH AFRICANS

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South Africa's black population is virtually free from kidney stones compared with the white population. Urinary glycoproteins are important inhibitors of crystallisation and their glycosylation may alter their function and activity. *N*-linked glycans on UPTF1 from healthy white and black subjects were characterised to assess their role in the protection of South African blacks from stone formation. CaOx crystals containing UPTF1 were precipitated from the urine of healthy white and black subjects. The crystal matrix extracts were electrophoresed and the *N*-linked glycans were released from gel bands of UPTF1 by in-gel deglycosylation. The glycans were fluorescently labeled and characterised using exoglycosidase digests, normal phase and anion exchange HPLC, and mass spectrometry. The *N*–glycans on UPTF1 were mostly complex biantennary structures with a high proportion of α2–6 linked sialic acid residues (95%). The *N*-glycans from black subjects had a significantly greater proportion of disialylated structures than from the white subjects. The high proportion of sialylation on UPTF1 could be important in directing the attachment of CaOx crystals to negatively charged renal cell surfaces. Increased disialylation of black subjects' UPTF1 glycans may provide a larger repulsive force to the renal cell surface, thereby diminishing crystal adhesion and subsequent stone formation in this population group.

A-23
ISOLATION AND SEMIQUANTITATIVE ANALYSIS OF URINARY BIKUNIN FROM THE BLACK AND WHITE POPULATION GROUPS IN SOUTH AFRICA

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South Africa's black population is relatively immune to kidney stone formation. Bikunin, a 30 kDa peptide of the protease inhibitor inter-α-inhibitor (IαI), has been found to inhibit calcium oxalate (CaOx) crystal nucleation and growth. The main objective of this study was to isolate and quantify bikunin from black and white South Africans. 24hr urine specimens were obtained from black and white healthy subjects and CaOx crystallization was induced. X-ray powder diffraction confirmed the formation of the monohydrate. Bikunin was isolated from matrix extract proteins using immunoaffinity chromatography (protein G sepharose column) which has not been previously used for the isolation of bikunin. The protein was identified by western blotting and its purity was verified by gel electrophoresis. Bikunin was isolated from the CaOx matrix extract of black and white subjects and found in trace amounts of 18 μg/mg and 12 μg/mg respectively. A method has been established for the purification of urinary bikunin using immunoaffinity chromatography. Semiquantitative analysis suggests that it is found in greater amounts in black subjects' matrix extracts. Since bikunin is an inhibitor, this may play a contributory role in the extremely low incidence of urolithiasis in the black population.

A-24
EFFECT OF SEX HORMONES ON KIDNEY SULFATE/OXALATE EXCHANGER (SAT-1, SLC26A1) MRNA EXPRESSION IN RATS

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The renal tubular secretion of oxalate is thought to be controlled primarily by the basolateral sulfate/oxalate exchanger (Sat-1, Slc26a1) of proximal tubules. In this study, we examined kidney Sat-1 mRNA expression by Northern Blot analysis in male and female rats with sex hormone manipulation. Ten rat groups with different sex hormone manipulation were used for the study (Table). A DIG labeled cDNA probe was used for the Northern Blot analysis of kidney Sat-1 mRNA expression

Group	Sex	Castration	Implant	Sat-1density	*% change
1	M	No	No	14.684	
2	F	No	No	2.930	−52% vs G1
3	M	Yes	No	10.514	
4	F	Yes	No	9.743	−10% vs G3
5	M	Yes	Testosterone	18.204	
6	M	Yes	Estradiol	11.944	−5% vs G5
7	F	Yes	Testosterone	6.764	
8	F	Yes	Estradiol	3.088	−59% vs G7
9	M	No	Estradiol	10.688	
10	F	No	Testosterone	6.307	−40% vs G9

* % change after standardized with β-Actin

Kidney Sat-1 mRNA expression is modulated by sex hormones: male hormones increase Sat-1 mRNA expression, whereas female hormones decrease its expression. In turn, increased expression of Sat-1 mRNA in males may partly explain why urolithiasis is a male predominant disease.

A-25
EFFECT OF CITRATE ON THE GROWTH OF CALCIUM STONES

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Except for rare matrix stones, urinary calculi are primarily crystalline with calcium oxalate, followed by calcium phosphate being the predominant species. As prevention of crystallisation will necessarily prevent stone formation, the processes of calcium precipitation in urine are frequently investigated and citrate has been shown to inhibit growth and aggregation of calcium crystals in simple salt solutions, urine-like liquors and in urine. Along with epidemiological and clinical trial evidence, this has been used to infer that urinary citrate is a natural inhibitor of calcium stone

formation whose activity can be enhanced by therapeutic manipulation. A crucial gap in this chain of reasoning is the distinction between formation of crystals and formation of stones; the latter does not necessarily follow from the former as is evidenced by crystalluria in healthy individuals. We have developed a method of growing multiply calcium stone simultaneously in vitro, which is used here to test the effect of citrate. The stone farm consists of twelve small (20 ml) crystallisation chambers arranged as six pairs. These first of each pair continuously receives two input solutions, one containing calcium, the other oxalate. The second chamber of each pair receives the output of the first. Inside each chamber, a small fragment of urinary calculus is suspended. The system is operated continuously for many hours (> 300) and the change in stone weight monitored. Three of the pairs of chambers received solutions containing 2 mM citrate and the other three pairs received 6 mM citrate. In artificial urine, stone growth rate was significantly reduced in the presence of 6 mM citrate compared to 2 mM (mean ± s.e. 0.063 ± 0.006 mg/h compared to 0.289 ± 0.045 mg/h, $p = 0.004$). In artificial urine supplemented with macromolecules prepared from the urine of healthy male controls, the growth rate was reduced from 0.117 ± 0.016 mg/h (2 mM citrate) to 0.041 ± 0.006 mg/h (6 mM citrate) ($p = 0.001$). In artificial urine supplemented with macromolecules prepared from the urine of recurrent male calcium stone formers, the growth rates were similarly inhibited by citrate; 0.088 ± 0.004 mg/h (2 mM citrate), 0.036 ± 0.008 mg/h (6 mM citrate) ($p = 0.001$). These results show that citrate inhibits calcium stone growth in vitro, adding further to the evidence supporting therapeutic use of citrate to slow the growth of new stones or residual fragment remaining after lithotripsy.

A-26
EFFECT OF MACROMOLECULES ON THE GROWTH OF CALCIUM STONES

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Some urinary macromolecules (UMM) are modifiers of calcium oxalate crystallisation and some are selectively included within urinary calculi but it is unclear if these activities have any moderating action on the process by which microscopic crystals become macroscopic stones. Our stone farm provides an *in vitro* model in which this can be investigated. The stone farm comprises twelve (20 ml) chambers operated as mixed suspension mixed product removal continuous crystallisers, arranged as six parallel pairs with each pair in series. Input feed solutions make up an artificial urine which is supersaturated with calcium oxalate. A fragment of urinary stone is suspended in each chamber and grown continuously for > 300 hours. A number of procedures are in place to prevent bacterial contamination. Urine (free of blood and not infected) was collected from male stone formers and healthy male controls and urinary macromolecules (UMM) were prepared by tangential flow ultrafiltration (5 kD cut-off) and diafiltration. Control (Ctl) stones had no additions to their feed solutions, others had 100 μg/ml of human serum albumin (HSA) or bovine submaxillary gland mucin (BSM), or UMM at approximately native concentration added. In each of two experiments, 6 Ctl stones were matched against 6 HSA treated stones; in one experiment, 6 Ctl stones were matched against 6 BSM treated stones; in one experiment, 12 stones were grown with HSA; in each of three experiments, 6 stones were grown with UMM; 18 Ctl stones from unrelated experiments are also included for comparison. There was no significant difference ($p > 0.05$) in the growth rates between Ctl and HSA treated stones or between Ctl and BSM treated stones ($p > 0.05$). The mean (± s.e.) growth rate of all Ctl stones was 0.263 ± 0.018 mg/h which was significantly greater than the UMM treated stones (0.093 ± 0.007) ($p < 0.05$). As albumin is found in stones and mucin (a glycoprotein) has been shown to have moderating activity towards calcium oxalate crystallisation processes, it is clear that neither of these properties necessarily leads to a change in rate of calcium stone growth in this system. UMM on the other hand is here shown to inhibit stone growth by about 65%.

A-27
EFFECT OF PHYTIC ACID ON THE GROWTH OF CALCIUM STONES

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Because of the potential that dietary modification or phytate supplementation might be an effective means to reduce calcium stone recurrence rates, we have chosen to investigate the action of phytate in our stone farm. The stone farm is an in vitro model of stone growth in which 12 fragments of human urinary calculus are grown in continuously replenished media for many (> 300) hours. To control the ionic conditions, we use artificial urine, supersaturated with calcium oxalate. In each experiment the feed solutions for 6 of the stones was supplemented with phytic acid at 5 μM, 2.5 μM or 0.5 μM. In one experiment, the media for all 12 stones was also supplemented with urinary macromolecules (UMM) at approximately native concentration. Urine (free of blood and not infected) was collected from male stone formers. UMM were prepared by tangential flow ultrafiltration (5 kD cut-off) and diafiltration. Phytate at 5 μM or 2.5 μM almost completely abolished stone growth and at 0.5 μM the growth rate was inhibited by about 50%. Compared to the control conditions (no phytate), inclusion of UMM has an inhibitory effect. When 0.5 μM phytate as well as UMM were present, no further inhibition was observed (mean growth rate ± s.e. (mg/h) without phytate was 0.093 ± 0.012 and with 0.5 μM it was 0.090 ± 0.008, $p > 0.05$). The potent effect of phytate on calcium stone growth inhibition in artificial urine is confirmed. At a low dose, this inhibition is abolished by the inclusion of UMM. It is too early to say if this mitigating effect of UMM on the inhibition by phytate will only be exhibited at low concentrations and/or growth rates.

A-28
URINARY PROTEINS AND CALCIUM OXALATE CRYSTALS

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The role of matrix in urinary stone remains controversial. A helpful investigative approach is to examine proteins associations with calcium oxalate crystals formed in vitro using urine as the crystallisation medium. Such studies have led to suggestions that specific proteins associate with crystals; that this specificity extends to particular proteins and hydromorphs; that the proteins may become internalised within the crystal structure and act as an ultrastructural element. As some of the urinary proteins which interact with CaOx crystals are also potent in vitro inhibitors of CaOx crystal growth there is a body of opinion which holds that these proteins have a clear functional role as agents which offer protection from crystals and stone formation. In a series of studies, we have investigated urinary protein interactions with CaOx crystals formed *in vitro*. The tools used include electron microscopy, X-ray diffraction, thermogravimatric analysis, crystal density measurements and quantitative and qualitative protein estimations. We found that only a small proportion (up to 10%) of available urinary proteins associate with the crystals and that they contribute no more than 5% to the crystal volume. Most of this protein is surface bound and easily removable, leaving an even smaller proportion which may be intracrystalline. The pattern of proteins found to be surface bound was not identical to that of the occluded proteins but we did not find major differences between the proteins which would associate with mono- and dihydrate crystals. Using six methods to generate crystals from the

same pooled urine suggested that the stabilisation of particular hydromorphs and induction of some morphological changes were protocol dependent and not, as previously suggested, tributable to specific protein interactions. We conclude that while some urinary proteins interact with CaOx crystals and modify crystallisation processes, they do so through non-specific actions. We have not found evidence that these proteins are present in urine as teleological inhibitors of crystal/stone formation.

A-29
EFFECT OF ORTHOSIPHON GRANDIFLORUS ON CALCIUM OXLATE CRYSTALLIZATION IN URINE

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The effect of *Orthosiphon grandiflorus (OG)* on calcium oxalate crystallization in urine was investigated. When aqueous extract of OG was added to the urine (1 mg/ml), it promoted calcium oxalate crystal formation. The crystals, however, were small and poorly aggregated. Proteins of 26 kD disappeared from the crystal matrix in the presence of OG extract, suggesting the extract plays a role in crystallization and aggregation. Consumption of OG tea also affects crystallization of calcium oxalate in urine. After drinking OG tea for one week, urine samples collected from four healthy subjects showed a significant increase in the amount of induced crystals ($p < 0.0001$). The amount of protein extracted from the crystals, however, was significantly decreased ($p < 0.0001$). Our results suggest that the observed therapeutic effects of OG in stone treatment are associated with the inhibition of calcium oxalate crystal growth and aggregation. Urinary proteins of 26 kD might be involved in these processes.

A-30
PROTEIN MODULATORS OF UROLITHIASIS IN PAKISTANI PEDIATRIC STONE-FORMERS

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Kidney stone disease is the commonest urological problem in Pakistan. In SIUT alone, over 11,000 patients were treated for this condition during the period 1990–98. The magnitude of the problem warranted scientific research to gain a better understanding of stone formation in Pakistan, and SIUT took the initiative to launch its Program on Urolithiasis. In order to define the role of the modulators, one has to investigate crystallization in vitro in the presence or absence of individual macromolecules. It is clear that inorganic solutions do not replicate the complex urinary environment, and so we analyzed proteins extracted from calcium oxalate and calcium phosphate crystals induced *in vitro* in the urine of normal children and pediatric stone formers. The crystals were collected by centrifugation and then were washed, freeze-dried and analyzed by SDS-polyacrylamide 7.5–22% gradient gel electrophoresis. Proteins were extracted by demineralization with EDTA solution. Anti-sera against the above mentioned proteins reacted with the crude and partially purified crystal matrix and stone matrices proteins. Low molecular weight stone matrix proteins (8–16 KD) of struvite stones have been partially purified. These proteins have bacteriostatic antifungal activity. Albumin was found to be the major component of the organic matrix of calcium oxalate and calcium phosphate crystals, leading us to conclude that albumin appears to play a crucial role in kidney stone formation, at least in our Pakistani pediatric stone-formers. The precise role of albumin is yet to be elucidated, and similar studies are being planned in adult patients at a later stage.

C. CELL/TISSUE – CRYSTAL INTERACTION

A-31
PROTECTIVE EFFECTS OF HEPARAN SULFATE PROTEOGLYCAN (SYNDECAN-1) ON THE RENAL EPITHELIAL CELL LINE DURING CALCIUM OXALATE MONOHYDRATE CRYSTAL ATTACHMENT

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We have reported that heparan sulfate (HS)/heparan sulfate proteoglycan (HSPG, syndecan-1) expression significantly increased in rat kidney during calcium oxalate (CaOx) nephrolithiasis. Although exact mechanism remains unclear, HSPG is thought to have some important role in the CaOx crystal formation. Mardin-Darby canine kidney (MDCK) cells are most commonly used in kidney stone research. It was reported that MDCK cells do not express syndecan-1 gene. So we established a novel MDCK cells (KIC-synd-1 cells) that express human syndecan-1 gene. We confirmed stable expression of syndecan-1 gene. Immunohistochemical study revealed positive staining of syndecan-1 monoclonal antibody in the basolateral and cytosolic area of the KIC-synd-1 cells. We also investigated the composition of glycosaminoglycans side chains in MDCK cells and KIC-synd-1 cells by using high-performance liquid chromatography. Total amounts of HS was significantly increased in the KIC-synd-1 cells compared with that in the MDCK cells ($p < 0.05$). Although scanning electron microscopy revealed no significant differences between cell surface of MDCK cells and that of KIC-synd-1 cells in normal condition, CaOx crystal attachment was apparently decreased in KIC-synd-1 cells compared with that in MDCK cells by using scintillation counter ($p < 0.05$). These results suggested that cell surface HS/syndecan-1 has preventive role for CaOx nephrolithiasis against COM crystal attachment.

A-32
CRYSTAL ADHESION TO RENAL EPITHELIAL CELLS BY URINARY MACROMOLECULES

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Adhesion of urinary crystals to renal tubular cells could be a critical event in kidney stone formation. Since macromolecules (MM) in normal urine can decrease calcium oxalate monohydrate (COM) crystal binding to cultured renal cells (MDCKI line), the present study was performed to determine if this property is abnormal in stone forming (SF) individuals. A sample of first morning whole urine (WU) was obtained from 6 SF subjects and 6 age-, race- and sex-matched controls (C). Urine was centrifuged and an ultrafiltrate free of MM > 10 kDa (UF) and 10X concentrate (Uconc) were prepared. Supplementing UF or artificial urine (AU) with increasing amounts of SF or C Uconc to return the MM concentration to 0.25X, 0.5X, or 1X of baseline progressively decreased crystal binding to cells. This effect was blunted in the SF group when Uconc was added to either UF or AU ($P < 0.05$ SF vs C for 1X MM). In order to identify the responsible MM(s), COM crystals were incubated with Uconc and adherent proteins were released and probed by Western blot for known urinary crystallization inhibitors. COM crystals coated with C Uconc contained significantly more Tamm-Horsfall Protein, whereas those coated with SF Uconc bound more Inter-alpha-trypsin inhibitor. In conclusion, urinary MM > 10 kD can coat COM crystals and block their adhesion to renal cells. This capacity appears to be decreased in SF individuals, perhaps due to abnormalities in the quantity and/or function of specific proteins.

A-33
EXTRACT FROM *HERNIARIA HIRSUTA* COATS CALCIUM OXALATE MONOHYDRATE CRYSTALS AND BLOCKS THEIR ADHESION TO RENAL EPITHELIAL CELLS.

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The interaction of calcium oxalate crystals with renal epithelial cells is a critical event in kidney stone formation. In this study, we assessed the effect of aqueous extract from *Herniaria hirsuta* on adhesion of calcium oxalate monohydrate (COM) crystals to cultured renal cells (MDCKI line). COM crystal binding to cells was inhibited by the extract in a concentration-dependent manner. Prior exposure of crystals but not cells to the extract blocked crystal binding, suggesting that plant molecules can coat and exert their effect at the crystal surface. Crystal attachment appeared related to membrane fluidity since crystal adhesion ncreased at higher temperatures (37° C) compared to lower temperatures (0° C), and *Herniaria* extract altered crystal adhesion only under conditions of increased fluidity (increased temperature). The extract also displaced a significant portion of pre-bound crystals without apparent effects on cell function or the morphology of preexisting calcium oxalate crystals. *Herniaria* extract exerted no adverse or toxic effect on cells, which proliferated normally in its presence, even at relatively high concentrations. Our present data suggest a mechanism whereby *Herniaria hirsuta* extract, used in traditional medicine, might prevent, and possibly eliminate pre-existing kidney stones. Further characterization of the active compound(s) could identify a new candidate drug for patients with nephrolithiasis.

A-34
THE EFFECTS OF OXALATE ON THE GROWTH OF HUMAN RENAL EPITHELIAL CELLS – HK2 CELLS

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Oxalate is not only considered to be one of the main factors composing urinary stones, but also have toxic effects on renal tubular epithelial cells, affecting the pathogenesis of nephrolithiasis. Human proximal renal tubular epithelial cells (HK-2) were incubated with different concentrations of oxalate for various intervals, and the effect of oxalate on the growth of the cells was assessed by MTT assay. The caspase-3 activity assay was performed in order to evaluate apoptosis. Immunoblot analysis of Bax, Bcl-2, Bcl-xL, and caspase-9 were performed. MTT assay showed that the growth of HK-2 cells significantly decreased as the concentration of oxalate increased. The cytotoxic effect of oxalate on HK-2 cells was considered to be induced by apoptosis because the active caspase-3 activity increased according to the treatment of HK-2 cells with oxalate. The expression of Bax and caspase-9 protein significantly increased and the expression of Bcl-2 protein decreased as oxalate concentrations increased. There is no significant difference in the expression of Bcl-xL protein expression in each concentrations of oxalate. Our results show that oxalate has the cytotoxic effect on HK-2 cells and it occurs by apoptosis. The apoptosis of HK-2 cells induced by oxalate is considered to result from the activation of caspase-9 after the increased expression of Bax protein and simultaneous decrease of the expression of Bcl- 2 protein. These data suggest that oxalate may affect the pathogenesis of nephrolithiasis by promoting apoptosis in human renal tubular epithelial cells.

A-35
DICARBOXYLATE TRANSPORTER: ROLE IN CALCIUM OXALATE MEDIATED MITOCHONDRIAL O2? PRODUCTION

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We have reported that exposure to calcium oxalate (COM) results in the leakage of electrons from mitochondrial electron transport in renal distal/collecting duct epithelial cells (MDCK). However, other crystals present in tubular fluid do not elicit the same response. We have investigated the role of different mitochondrial transporter systems of oxalate on this phenomenon. MDCK cells were treated with COM or free oxalate and assayed for mitochondrial O2? Formation by lucigenin chemiluminescence, following digitonin permeabilization in respiration buffer. Mitochondrial oxalate transport was probed using nigericin to inhibit the K +/H + antiporter, 9- dicyclohexylcarbodiimide to inhibit the inner membrane anion channel (IMAC), and either mersalyl or phosphate (PO4-) omission to inhibit the dicarboxylate transporter. The presence of a calcium chelator in the respiration buffer was an absolute requirement for COM mediated O2? formation. However, free oxalate could not fully reinstate the effect of crystals. Neither COM nor oxalate mediated O2? formation was altered by IMAC or K +/H + antiport inhibition. However, both were decreased to control levels by mersalyl or the omission of PO4- ($p < 0.001$). This implies that oxalate entry into mitochondria via the dicarboxylate transporter is fundamental to the enhanced O2? formation observed with the free ion or COM. However the importance of mitochondrial Mg + in this mechanism cannot be excluded at this time, as calcium chelators and the lack of PO4- will both have an impact on this important mitochondrial mediator.

A-36
OXALATE EXPOSURE STIMULATES COX-2 IN MDCK RENAL EPITHELIAL CELLS

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Exposure of cultured renal epithelial cells to oxalate, a common constituent of kidney stones, elicits a cascade of effects including membrane lipid rearrangement, oxidant stress, changes in gene expression, limited cell proliferation and cell death. Many of these effects are mediated by arachidonic acid (AA) and lysophosphatidyl choline (LysoPC), lipid signals generated by phospholipase A2 (PLA2) activation. The present studies tested the hypothesis that oxalate exposure would stimulate MDCK cell expression of cyclooxygenase-2 (COX-2), an oxidoreductase that converts AA to PGH2, precursor to prostaglandins, thromboxanes and prostacyclins. Exposure to 350 μM free oxalate increased COX-2 mRNA and COX-2 immunoreactivity within 2 hr. Mepacrine a nonspecific PLA2 inhibitor, but not AACOCF3, a selective inhibitor of cytosolic (c)PLA2, inhibited oxalate-induced COX-2 mRNA, suggesting that another PLA2 isoform may mediate COX-2 induction. Exogenous Lyso-PC mimicked the response to oxalate, suggesting that this lipid byproduct of PLA2 activation plays a role in COX-2 mRNA induction. In contrast, exogenous AA blocked both basal and oxalate-induced COX-2 mRNA, consistent with our previous demonstration of an AA-induced end-product inhibition of PLA2 activity in MDCK cells. In concert with PLA2 activation, COX-2 may play a role in the cascade of responses triggered in renal epithelial cells by oxalate exposure.

A-37
GENOMIC EFFECTS OF OXALATE: GLOBAL CHANGES IN GENE EXPRESSION BY OXALATE IN HK2 CELLS: A LINE OF HUMAN KIDNEY EPITHELIAL CELLS

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Oxalate exposure to the renal epithelial cells results in a program of events that include cell re-initiation of DNA synthesis and

cellular apoptosis. We reported first evidence of alterations in gene expression in renal cells upon oxalate exposure and showed that oxalate exposure resulted in the transient transcriptional activation of c-*myc* gene. Since that time we and others have found evidence of induction of c-*jun*, *egr*, *MCP* and recently *HSP70* and *IL-6* following oxalate exposure. These data suggest effects of oxalate might involve changes in the expression of several other genes. However, oxalate induced changes in global gene expression in renal epithelial cells have not been fully explored. In this study we set out to evaluate the effects of oxalate on global gene expression profile in HK2 cells, a line of human proximal tubular renal epithelial cells. Cells were exposed to oxalate for 4 hr and RNA was isolated. Effects of oxalate on expression profile of all the gene (54675) known to be expressed in human tissues using DNA micro-array chip technology. We used HG_U133_plus2 gene chip which represents 11 independent replicate probe sets for each gene. Genes expression was statistically compared among replicated as per Affimatrix statistical program. Results from these studies demonstrate that growth arrested human kidney epithelial cells express 26211 genes. Oxalate exposure results in expression of ~1491–1644 genes that are not at all expressed in control cells. We also observed that oxalate exposure resulted in complete blockade of ~2525–2593 genes that are normally expressed in control cells. In addition to these changes oxalate exposure was associated with up-regulation of ~299 genes that are mildly expressed in control cells and down regulation of ~350 genes that are strongly expressed in control cells. These data demonstrate for the first time global gene expression pattern of entire known human genome in HK2 cells and also provide a first birds eye-view of the genomic effects of oxalate in renal epithelial cells.

A-38
OXALATE EXPOSURE PROVOKES THE IMMEDIATE EARLY GENE AND HSP70 RESPONSE IN LLC-PK1 CELLS, A LINE OF RENAL EPITHELIAL CELLS

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Oxalate interaction with renal epithelial cells results in a program of events consistent with cellular stress. Recent studies from our laboratory using inhibitors of transcription and translation indicated that the cellular adaptations to oxalate toxicity are dependent on new gene expression and protein synthesis. However, the adaptive response of renal epithelial cells following oxalate exposure remains poorly understood. Induction of HSP confers resistance to subsequent various stresses including heavy metals, hypoxia, arsenate, ethanol, and hyper tonicity and hence the more general name "stress proteins". We investigated the effects of oxalate on immediate early genes and stress protein HSP70, commonly induced genes in response to a variety of stresses. LLC-PK1 cells were exposed to oxalate for various time points. Gene transcription and translation were monitored by Northern and Western Blot analysis. RNA and DNA synthesis were assessed by 3H-uridine and 3Hthymidine incorporation, respectively. Oxalate exposure selectively increased the levels of mRNA encoding IEG s c-*myc* and c-*jun* as well as stress protein HSP 70. While expression of c-*myc* and c-*jun* were rapid (within 15 min to 2 h) and transient, HSP 70 expression was delayed (~8 h) and stable. Furthermore, oxalate exposure resulted in delayed induction of generalized transcription by 18 h and re-initiation of the DNA synthesis by 24 h of oxalate exposure. To the best of our knowledge this is the first study to demonstrate the heat-shock response to oxalate toxicity. These results suggest that IEG expression may regulate additional genetic responses to oxalate and increased HSP70 expression would serve as protective role during oxalate stress thus providing a new insight into cellular adaptation to oxalate toxicity.

A-39
ROLE OF p38 MAP KINASE SIGNAL TRANSDUCTION IN OXALATE AND CALCIUM - OXALATE MONOHYDRATE (COM-) CRYSTAL INDUCED APOPTOSIS AND SURVIVAL OF RENAL EPITHELIAL CELLS

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p38 MAP kinase signal transduction pathway is activated by various forms of cellular stress, including osmotic stress, chemical stress and oxidative stress. While, in many tissues activation of p38 MAP kinase is associated with apoptosis, in some tissues p38 activation is critical for survival under these stresses and is even required for growth and proliferation of the tissues. Oxalate and calcium oxalate deposits are associated with several pathological conditions, including kidney stone disease, benign neoplasm of the breast and in thyroid hypertrophy. Many of these conditions are associated with aberrant proliferation and cellular apoptosis. Previously, we and others have shown that exposure of the renal epithelial cells to oxalate and Calcium oxalate crystals (COM-crystals) is associated with re-initiation of the DNA synthesis, alterations in gene expression as well as cellular apoptosis. Studies from our laboratory demonstrated that exposure of renal epithelial cells to oxalate as well as COM-crystals) resulted in activation of p38 MAP kinase pathway. Moreover, the inhibition of p38 MAP kinase pathway by SB 203580 or by dominant negative expression of kinase dead mutant of p38 MAP kinase resulted in the inhibition of oxalate as well as COM-crystal induced re-initiation of the DNA synthesis. These data also demonstrate that the effects on inhibition of p38 MAP kinase correlated with inhibition of the re-initiation of DNA synthesis in a dose dependent manner. Additional unpublished data from our laboratory demonstrate involvement of p38 MAP kinase in regulation of gene expression and cellular apoptosis following oxalate exposure. These results demonstrate a critical role for p38 MAP kinase pathway in pathological conditions involving oxalate and suggest that p38 MAP kinase signal transduction pathway might serve as a novel target in treatment of these disorders.

A-40
EFFECTS OF OXALATE ON IMCD CELLS, A LINE OF MOUSE INNER MEDULLARY COLLECTING DUCT CELLS

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Oxalate, a metabolic end product and a major constituent of majority of renal stones has been shown to be toxic to renal epithelial cells of cortical origin. However, cells of inner medullary collecting duct (IMCD), which are physiologically exposed to higher levels of oxalate, have not been evaluated. In the present study, we examined the effects of oxalate on IMCD cells were maintained in DMEM: F12 media supplemented with FBS and antibiotics. Cells were exposed to oxalate (0.2–10 mM) for various time points. Trypan blue exclusion criteria was used to assess membrane integrity, cell morphology was assessed by H&E staining, while crystal violet staining was used to measure cell density. Exposure of IMCD cells to oxalate produced time and concentration dependent changes in the light microscopic appearance of the cells. The long-term exposure to oxalate resulted in alterations in cell viability, with net cell loss following exposure to high oxalate concentrations. The effects of oxalate were time and concentration dependent. However no significant cell damage was observed up to an oxalate concentration of up to 2 mM. In comparison to IMCD cells, LLC-PK1 cells as well as HK2 cells, showed significant toxicity starting at lower oxalate concentrations (0.4 mM and above). These results demonstrate the IMCD cells are resistant to oxalate toxicity as compared to

renal epithelial cells of cortical origin. The results provide first direct demonstration of toxic effects of oxalate in IMCD cells, a line of renal epithelial cells of inner medullary collecting duct; and suggest that the cells lining the collecting duct are relatively resistant to oxalate toxicity.

A-41
POTENTIAL ROLE OF FIBRONECTIN IN STONE FORMATION

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Attachments of newly formed crystals to renal epithelial cells appear to be a critical step in the development of kidney stones. We recently reported that fibronectin (FN: 230 kDa) was oversecreted from the renal tubular cells as a result of the stimulation of calcium oxalate monohydrate(COM) crystals, and inhibited the adhesion of COM crystals to renal tubular cells in MDCK cells. However, the exact mechanisms of inhibiting the adhesion of COM crystals are not yet understood. Therefore, to examine the role of FN during early stage of stone formation, we established stable transfectants of FN in MDCK cells. To investigate the role of FN in renal epithelial cell injury, we examined the effects of FN in transfected MDCK cells. First, we synthesized reverse transcription product (801 bp) from RL-65 (ATCC; established line of normal rat lung cells) which produces FN. To produce FN expression vector, rat FN cDNA was inserted into the EcoRI and EcoRI sites pTargeT expression vector plasmid. An expression vector containing rat FN was transfected into MDCK cells via highly efficient lipofection method. The effects of oversecreted FN in MDCK cells were examined in stone formation. We concerned that FN was transfected in MDCK cells via reverse transcription- polymerase chain reaction. The established transfectants were isolated and characterized. The stable transfectants overexpressing the FN exhibited relatively higher levels of FN and inhibited the adhesion of COM crystals to renal tubular cells than the control. The results indicate that the deposition of FN in transfectants serves to prevent apoptosis induced by the stimulation of COM crystals, and hence inhibits stone formation of MDCK cells.

A-42
NADPH OXIDASE AS A SOURCE OF REACTIVE OXYGEN SPECIES IN OXALATE AND CALCIUM OXALATE CRYSTAL INDUCED INJURY OF RENAL EPITHELIAL CELLS

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Reactive oxygen species (ROS) are currently considered major mediators of oxalate and crystal induced injury of the renal epithelium and mitochondria have been shown to be a key source of ROS when renal epithelial cells are exposed to high levels of oxalate and calcium oxalate (CaOx) crystals. We investigated the possibility of the involvement of plasma membrane NADPH oxidase in the process. NRK 52-E cells in confluent cultures were exposed to 500 μm oxalate, or CaOx monohydrate crystals at 66.7 $\mu g/cm^2$ in the presence or absence of NADPH oxidase inhibitor diphenelineiodium chloride (DPI). Release of lactate dehydrogenase (LDH), and production of hydrogen peroxide (H_2O_2) as well as product of lipid peroxidation 8-isoprostane (8IP) were measured. Exposure to oxalate and crystals of CaOx monohydrate resulted in the release of LDH. In addition all treatments induced the production of H_2O_2 and lipid peroxides 8IP. This increase was significantly reduced in the presence of DPI. However an exposure to CaOx monohydrate crystals was more injurious and resulted in significantly higher release of LDH and higher production of H_2O_2 and 8IP. Results indicate that oxalate as well as the other calcific crystals at the concentrations used here is injurious to renal epithelial cells and cause lipid peroxidation through H_2O_2 production, as we have previously known. Results also show that DPI provides a protection against these injuries by reducing the production of reactive oxygen species suggesting an involvement of the NADPH oxidase.

A-43
FILLERS IN THE MIDST: INTRACRYSTALLINE PROTEIN CONCENTRATION AND THE DISSOLUTION OF COM AND COD CRYSTALS IN MDCK II CELLS

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Intracrystalline proteins may assist lysosomal destruction of crystals internalised by renal epithelial cells. The aim of this study was to determine the effect of urinary protein concentration on the degradation of COM and COD crystals by cultured MDCK II cells. The organic matrix was isolated from demineralised CaOx crystals deposited from healthy urine and added to ultrafiltered (UF: 10 kDa) urine to give final protein concentrations of 0–3 mg/ml. The urine was divided, and the [Ca] adjusted to give two samples of 2 mM and 10 mM, from which were precipitated COM and COD, respectively, by ^{14}C-oxalate addition. Crystals were incubated with MDCK cells for varying times. Crystal dissolution and degradation were assessed by ^{14}C-oxalate release and scanning electron microscopy, and intracrystalline protein content by SDS-PAGE and Western blotting for osteopontin (OPN) and prothrombin fragment 1 (PTF1). Intracrystalline protein content was proportional to the concentration of organic matrix added to the UF urine. Except for inorganic control crystals, which dissolved slowly, dissolution of all other crystals was virtually complete in all cases within 2 days. COD dissolved more rapidly than COM. The rate of dissolution was directly related to the quantity of intracrystalline protein, as was the degree of physical degradation as shown by scanning electron microscopy. Thus, high endogenous urinary concentrations of intracrystalline proteins, such as OPN and PTF1, may protect against stones by facilitating intracellular proteolytic digestion and destruction of any crystals that attach to and are phagocytosed by renal epithelial cells.

A-44
β-tubulin is the major cell membrane COM-crystal binding protein in renal epithelial cells.

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Crystal formation in the renal tubules and crystal retention by the renal epithelial cells are the two critical determinants in nephrolithiasis. In this study we set out to characterize the major protein in COM-CAR complex. (COM crystal associated receptor). Brush border membranes vesicles were prepared from renal epithelial cells (MDCK cells and HK2 cells). COM-crystals were added to these vesicles and following crystal binding, the vesicles were solubilized with a combination of ionic and non-ionic detergents to achieve maximum solubilization. COM-crystals were separated by centrifugation and washed with two more changes with detergent mixture to remove loosely associated molecules. Proteins firmly associated with COM-crystals were separated following dissolution of COM-crystals and SDS-PAGE electrophoresis. We used N-terminal micro-sequencing and western blot analysis and immuno-fluorescence microscopic techniques for protein characterization. N-terminal sequence determination identified β-tubulin as the major COM-CAR molecule in the brush border membrane. Results from Western blot analysis confirmed these results. Using fluorescent labeled antibodies we demonstrate that β-tubulin was indeed present on the renal epithelial cell surface and that COM-crystals co-localized with β-tubulin on the renal cell surface. Electron micrographs demonstrated that COM-crystals were associated

with long flagellar structures on the intact renal epithelial cells and immuno-fluorescent microscopic examination revealed that these flagellar processes contained β-tubulin. Moreover pre-treatment of renal epithelial cells with anti-tubulin antibody blocked COM-crystal binding. Taken together these studies demonstrate β-tubulin as the major cell surface COM-crystal binding protein in renal epithelial cells.

A-45
THE EFFECT OF OSTEOPONTIN TO EPITHELIAL CELLS FOR CRYSTAL INTERACTION AND PROTECTION FROM EXCESSIVE OXALATE EXPOSURE

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The association of crystals with renal tubular cells is considered a potentially important factor in the process of renal stone formation. And, excess oxalate ions (OX) stimulate an array of responses inducing localized injury and inflammation in the kidneys. We previously reported a strong expression of osteopontin (OPN) on renal tubular cells in the stone-forming kidney. In the present study we examined the biological consequences of inhibition of OPN expression at the translational level on (1) the formation and adhesion of crystals and (2) the cell injury from oxalate. We synthesized antisense OPN expression vector (pTet-OPNas) using the Tetracycline-Regulated Expression System. The pTet-OPNas was constructed using a mouse OPN cDNA sequence in an inverted (antisense) orientation. Two clones (NRK-52E/ASs) were identified by transfection of pTet-OPNas into NRK-52E cells, and they showed a marked reduction of OPN synthesis in the absence of tetracycline. (1) Calcium oxalate (CaOx) crystal suspension was spread homogeneously on top of the NRK-52E cells. After incubation, the association of CaOx crystals and cells was visualized by scanning electron microscopy (SEM). (2) Confluent cultures of NRK-52E/ASs were exposed to OX, with or without tetracycline. Cell death was determined by the MTT assay. (1) NRK-52E cells were associated with CaOx crystals. In contrast, the expression of antisense OPN prevented the association of CaOx crystals with NRK-52E cells. (2) The level of OPN mRNA and protein expression significantly increases the following treatments with OX. The MTT assay showed that the NRK-52E/ASs with depressed OPN synthesis by the expression of antisense OPN were injured dependent on OX concentration, more severely than intact NRK-52E cells and tetracycline-treated NRK-52E/ASs. The results suggests that OPN plays a crucial role in the adhesion process of CaOx crystals to renal tubular cells in stone formation and has a protective effect on renal epithelial cells against excessive OX exposure. We suggest that even though OPN expression protects renal epithelial cells from excessive OX concentration in urine, OPN promotes the adhesion of CaOX crystals to renal tubular cells. We suspect that in this way OPN changes its role in stone formation from defense to formation.

A-46
GLOBAL ANALYSIS OF EXPRESSED GENES IN RENAL EPITHELIAL CELLS EXPOSED TO CALCIUM OXALATE CRYSTALS

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The binding and internalization of calcium oxalate monohydrate (COM) crystals, the most common crystal in renal stones by renal epithelial cells may be a critical step leading to kidney stone formation. Exposure to COM crystals alters the expression of various genes, but pervious studies on gene expression have generally been limited. To obtain more detailed insight into gene expression, we examined gene expression profiles in renal epithelial cells exposed to COM crystals using cDNA macroarray. NRK-52E cells were exposed to COM crystals for 60 and 120 minutes. Poly (A) + RNA was isolated and converted into 32P-labeled first-stand cDNA, then the cDNA probe was hybridized to the membrane. Hybridization images were scanned and the signal intensities were quantified. Expression of mRNA of 1176 genes were analyzed with global sum normalization methods. Exposure to COM crystals altered the expression of some of the genes reported previously. Furthermore, novel genes were also identified. Over twenty genes were found to be regulated at least 2-fold. We performed a large-scale analysis of gene expression in renal epithelial cells exposed to COM crystals, and identified the genes differentially regulated. cDNA macroarray is a useful tool for evaluating gene expression in urolithiasis research.

D. PATHOPHYSIOLOGY AND METABOLIC DISORDERS

A-47
URINARY CITRATE CONCENTRATION IS INCREASED FOLLOWING LONG-TERM SAMPLE STORAGE AT -20° C

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Hypocitraturia is a frequent biochemical disturbance among patients with nephrolithiasis. In addition, citrate salts are often used for the prevention of stone recurrence. Thus, it is important to assess urinary citrate concentration in the stone-forming patient. Under most circumstances, samples are preserved, refrigerated or frozen until assay can be performed. We have observed that the type and extent of storage conditions can significantly alter urinary citrate concentration. In order to address this concern in more detail, we assessed the effect of acidification and storage at 4° C or at -20° C for periods up to six weeks in 24-hour collections from 12 patients with urolithiasis. Urinary citrate was determined enzymatically using an automated platform (Cobas MIRA CCPlus). Refrigerated acidified specimens showed no significant change over 6 weeks of storage. Two non-acidified specimens became infected despite refrigeration resulting in low citrate values. Frozen specimens, both plain and acidified, displayed a marked increase in urinary citrate (32.9 ± 41.3 SD in non-acidified, and 19.0 ± 26.6% in acidified after 6 weeks), when compared to the corresponding values obtained in fresh urine. This observation suggests that freezing of urine may result in erroneous elevation of citrate. Studies are underway to ascertain why such a change occurs with long-term freezing.

A-48
THE ASSOCIATION OF NEPHROLITHIASIS AND METABOLIC SYNDROME

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Nephrolithiasis (NL) has been associated with elevated body mass index (BMI), hypertension (HTN), and diet, suggesting that kidney stones may be linked with the metabolic syndrome. Therefore, we tested the hypothesis that the prevalence of diabetes (DM) is increased in patients with NL, using a case-control study. Two hundred forty-eight cases were randomly selected from all Rochester (RST), MN residents diagnosed with NL at the Mayo Clinic between 1980–1999. Age at NL diagnosis ranged from 7 to 90 years. One control was randomly selected for each case from all RST residents seen at Mayo Clinic within 1 year of diagnosis with NL, matched for age, sex and length of medical record. Medical records were reviewed to obtain relevant information including DM, HTN, serum cholesterol (chol), stone analyses, and BMI. Using conditional logistic regression, HTN (OR = 1.62, 95% CI 1.02–2.58) and BMI (OR = 1.05, 95% CI 1.02–1.09) were both linked to a diagnosis of NL.The prevalence of DM (9% vs 7%) and chol levels were similar in cases and controls. In 114 patients with known stone type, the prevalence of

DM in 9 patients with uric acid (UA) stones was markedly increased (44% vs 10% for all other stone types, $p = 0.047$), suggesting that DM favors UA stone formation. Therefore, our study confirms that HTN and elevated BMI increase the risk of NL. Although DM is not associated with NL prevalence, we cannot exclude a co-association of subclinical insulin resistance, and DM does appear to influence stone composition towards UA.

A-49
POSSIBLE ROLE OF LIVER MITOCHONDRIAL ALANINE: GLYOXYLATE AMINOTRANSFERASE- 1 IN THE CONTROL OF OXALATE FORMATION FROM L-HYDROXYPROLINE OR GLYCOLATE

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Alanine:glyoxylate aminotransferase-1 (AGT-1) is largely located in mitochondria (Mt) in carnivores, whereas it is entirely found within peroxisomes (Ps) in herbivores and humans. In rat liver, AGT-1 is found in both of these organelles, and only the mitochondrial enzyme is markedly induced by glucagon. Glyoxylate formation from glycolate and that from L-hydroxyproline (L-Hyp) have been shown to occur in Ps and Mt, respectively. We have investigated whether mitochondrial AGT-1 plays a role in removing L-Hyp- or glycolate derived glyoxylate from oxidation to oxalate. After oral administration of 630 mg L-Hyp or 100 mg glycolate to glucagon-treated and control rats in fasting conditions, 24 h-urine was collected, and urinary excretions of glycine, oxalate, glycolate and other amino acids were determined. The AGT-1 activity in rat liver homogenates was determined. We found that urinary excretion of oxalate was markedly increased when a large dose of L-Hyp or glycolate was administered to rats. Oxalate formation from L-Hyp but not that from glycolate was significantly reduced when mitochondrial AGT-1 had been induced by glucagon. There is a significant inverse correlation between the AGT-1 activity and urinary oxalate or glycolate produced from L-Hyp. These results suggest that an important role of mitochondrial AGT-1 in carnivores is to convert L-hydroxyproline-derived glyoxylate into glycine *in situ*, preventing undesirable overflow into the production of oxalate.

A-50
RENAL TUBULAR ALTERARATION BY CRYSTALLURIA INITIATES CALCIUM OXALATE STONE FORMATION – AN EXPERIMENTAL STUDY BY MEANS OF MDCK-CELLS

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The mechanism behind stone formation is still unclear. Physicochemical properties alone cannot explain why urinary stones form. Detection of cellular enzymes in the urine, papillary calcifications and the short transit time of the urine in the renal tubule are factors that do not permit crystals to grow to calculi and thus speak for an involvement of the renal tubule in the process of stone formation. In addition animal experiments with a rat model suggest that alteration of the distal renal tubular cell triggers stone formation. Experiments were done by means of a cell culture model (Madin Darby Canine Kidney cells, MDCK- cells). MDCK- cells were cultured in DMEM with 10 % fetal calf serum (FCS) at 37 degrees Celsius und 5% CO_2. 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM glutamine were added. Subculturing was performed with 0.025% trypsin. Cultures were prepared by plating 2.5×10^4 cells/well in 96-well dishes. Calcium oxalate crystals (COC) with a diameter of 2 µm were added and cytotoxic effect was determined by photometrical measurement of Lactate-Dehydrogenase (LDH) in cell supernatant. In the study group parathormone (PH), vitamin D_3 (VD), allopurinol, oxipurinol and selenium were added. PH and VD had no altering effect on the tubule in contrast to the experimental findings from the animal model. On the other hand, a reproducible tubular cell impairment was induced with COC, expressed as a 27%-rise in the LDH concentration in the cell supernatant. Allopurinol, oxipurinaol and selenium inhibited this effect. Oxalate concentration and pH did not influence renal tubular alteration by COC. The observations show that COC in the urine can directly damage the renal tubule. These findings indicate that an interaction between COC and the renal tubular cell is significant for urinary stone formation. This effect was inhibited by allopurinol and selenium, a fact that may speak for the use of both substances in the scope of stone metaphylaxis.

A-51
HEPATOCYTE GROWTH FACOR HAS AN INHIBITORY EFFECT IN CRYSTAL-CELL INTERACTION.

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Studies in cultured cells have shown that high oxalate level induced cell injuries and increased adhesion of calcium oxalate monohydrate (COM) crystals to renal tubular cells. Hepatocyte growth factor (HGF) is initially identified as the most potent growth factor for hepatocytes and is well known as a mesenchyme-derived pleiotropic factor on various types of cells. We investigated whether HGF has a role on apoptosis and crystal-cell interaction. MDCK cells were exposed to potassium oxalate or COM crystals in the presence or absence of HGF. Exposure of MDCK cells to both KOX and COM crystals resulted in a significant increase in LDH release and apoptotic cells in a time-dependent manner. These effects were reduced by HGF in a dose-dependent manner. HGF had inhibitory activity against the adhesion of COM crystals to MDCK cells. These findings suggest that HGF might play an important role in stone formation, although further studies are necessary regarding the effect of HGF both *in vitro* and vivo.

A-52
RECURRENCE OF PRIMARY RENAL DISEASE IN A KIDNEY TRANSPLANT DUE TO ADENINE PHOSPHORIBOSYLTRANSFERASE(APRT) DEFICIENCY

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A 63 years old Caucasian male was given a kidney transplant on September 2003. He had been suffering of systemic hypertension, overweight and dyslipidemia for more than ten years. Initial renal disease was unknown despite a late renal biopsy in October 2002 wich displayed intense vascular lesions and the presence of some undefined crystals. Due to a protracted post transplant renal failure, a graft biopsy was performed (day 26). A tubular necrosis was observed but intense crystals deposits were present in tubular lumen and epithelial cells. No tubulitis was observed (Dr Doucet). Crystalluria analysis exhibited 2, 8- dihydroxyadenine (2, 8-DHA) crystals wich was confirmed by infrared spectrometry of urine (Dr Bigot) and renal parenchyma (Dr Daudon). APRT activity was completely absent in the lymphocytes (Dr Ceballos Picot). DNA study is ongoing. Of possible interest the patient dysplayed permanent low level of alkaline phosphatasemia without obvious etiology to explain it. Allopurinol was started 100 mg/day then 200 mg/day in accordance with renal function. One month later urine crystals have disappeared however they were still present in parenchyma. Nevertheless renal function was improving. To conclude; crystals deposition due to 2, 8-DHA in this patient highlights the tremendous importance of crystal identification to avoid early recurrence of this rare renal disease. In patients whose renal disease is not explained, the presence of scarce crystals must lead to infrared spectrometry in kidney parenchyma. Increased physicians awareness may hasten diagnosis and limit the morbidity associated with this disease.

A-53
25OH VIT D DEFICENCY: A MAJOR PROVOCATEUR IN PRIMARY HYPERPARATHRYOPIDISM [PHT] GENESIS?

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Our past experience in Pakistan (J Pak Med Assoc 1999 49:194-8) showed that PHT patients are younger (38.4 +/- 13.2 years); and present with preponderance of bone disease (59.4%), either alone or in combination with stone disease (SD). 35% had isolated SD. Those with BD had higher urinary (Ur) calcium (Ca) levels than SD [482 +/− 340 vs 265 +/− 89 mg/24 hours ($p = 0.013$)] and longer hospitalization. In order to explain this difference we evaluated the frequency of hypovitaminosis D in 16 PHPT, seen in the surgical clinic in 2002/3, through measurement of 25OH Vit D. Mean age (41.5 ± 13.7 years) and frequency of SD/BD was similar (43.8/50%). There was a greater referral from the endocrine service. Serum 25OH Vit D levels were low (median:7.47, range 5.4–38.87 ng/ml). Ninety four percent of the patients had levels under 18 ng/ml. Mean serum Ca was 11.46 ± 1.25 mg/dl; s Phosphate: 2.3 ± 0.55 mg/dl; s alkaline phosphatase: 1868 ± 399 mg/dl, Ur Ca : 310 ± 173 mg/24 hours, Ur citrate 173 + 32 mg/24 hours. DEXA scan: T score Radial: [−2.9 ± 1.57], Femoral [−2.36 ± 1.24] Lumbar spine [−2.5 ± 1.46]. There was one hyperplasia and one carcinoma in this group. No correlation was found between 25OHVit D and sCa, Ur Ca and sPTH. Given this frequency and extent of Vitamin D deficiency, hypovitaminosis D might be the major provocateur in onset of PHT, in our patients.

A-54
IMPROVEMENT IN RENAL TUBULAR DAMAGE AND ANTIOXIDANT STATUS AFTER TREATMENT OF RENAL STONE PATIENTS WITH POTASSIUM - MAGNESIUM CITRATE PLUS VITAMIN C AND VITAMIN E

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Hypokaliuria, hypomagnesiuria, hypocitraturia as well as increased renal tubular damage and low antioxidant status were frequently reported in renal stone patients. Thirteen renal stone patients were treated with KMgCit (42: 21: 63 mEq) plus vitamin C (200 mg/day) and vitamin E (400 units/day) for a period of one month. The 24-h urine and heparinized blood samples were collected before and after treatment and analyzed for parameters related to stone risk indices, tubular damage and antioxidant status. Following the treatment, urinary excretions of potassium, magnesium and citrate were significantly increased. There was a correction in antioxidant status (plasma TAS, Vitamin E, and erythrocyte glutathione) with a decline in plasma and urinary malondialdehyde and an increase in creatinine clearance with a subsequent decline in FE Mg.

A-55
REGULATORY EFFECTS OF RENAL CARBONIC ANHYDRASE ACTIVITY ON THE URINARY RISK FACTORS OF CALCIUM OXALATE STONE FORMATION – A NEW PHYSIOLOGICAL CONCEPT

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In a previous study, carried out in an in vivo rat model, we were able to demonstrate a regulatory influence of carbonic anhydrase on the oxalate transport process in the proximal tubule. Now we investigated these effects in human beings. The present study verifies the regulation of oxalate-, calcium- and citrate excretion under physiologic conditions in humans. 10 healthy volunteers underwent a standardized procedure. Carbonic anhydrase inhibition markedly increased renal oxalate excretion rate (control: 2.44 µmol/l vs. 3.28 µmol/l 30 min after acetazolamide dose, 3.34 µmol/l after 60 min, 2.79 µmol/l after 90 min and 2.60 µmol/l after 120 min). Calcium excretion rate rose similar to oxalate (control: 4.50 µmol/l vs. 10.80 µmol/l 30 min after acetazolamide dose, 9.24 µmol/l after 60 min, 10.00 µmol/l after 90 min, 9.87 µmol/l after 120 min). In contrast citrate excretion decreased (control: 4.17 µmol/l vs. 3.34 µmol/l 30 min after acetacolamide dose, 3.37 µmol/l after 60 min, 2.79 µmol/l after 90 min and 2.60 µmol/l after 120 min). Our study is the first to show that renal carbonic anhydrase has a direct influence on the excretion rates of the lithogenic factors in humans. Renal oxalate and calcium excretion increased simultaneously, while the excretion of citrate, one of the most important inhibitors, decreased. Based on these preliminary results we assume a central role for renal carbonic anhydrase in idiopathic calcium oxalate stone disease. Future studies must reveal if the activation of this enzyme in the proximal tubule is useful for kidney stone prevention.

A-56
RENAL OXALATE TRANSPORT DEPENDS ON LOCAL CARBONIC ANHYDRASE ACTIVITY IN THE PROXIMAL TUBULE - FROM THE ANIMAL MODEL TO HUMAN PHYSIOLOGY

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Elevated urinary oxalate levels increase markedly the risk of calcium oxalate stone formation. Micropuncture and clearance studies were carried out in male Sprague-Dawley rats. On the cellular level ^{14}Coxalate transport was characterized with the *in situ stopped flow microperfusion technique*. Changes in renal 14C-oxalate excretion were measured in separate *clearance experiments*. Each animal underwent a control and an acetazolamide (ACA) period. In a next step *net oxalate excretion* was determined in 10 healthy volunteers without metabolic disorders. Net oxalate excretion of these persons was measured before and after oral administration of 500 mg acetazolamide. *Micropuncture studies* showed that ^{14}C-oxalate transport in the proximal tubule was significantly inhibited after CA inhibition with ACA (control 21.47% vs. ACA 9.71%, $p \leq 0{,}001$). In the *clearance trial* CA inhibition increased significantly the renal oxalate excretion rate (control 43.47 ± 4.34 p mol/min vs. ACA 62.15 ± 2.81 p mol/min, $p < 0.001$). Net oxalate excretion in the *healthy volunteers* rose after CA inhibition significantly (control 0.39 ± 0.18 nmol/min vs. ACA 0.59 ± 0.27 nmol/min, $p = 0.001$). Renal oxalate excretion is influenced by the local CA activity in the proximal tubule. The existence of this physiological mechanism was demonstrable in the animal model and in humans, respectively. Dysfunction of CA in the proximal tubule could be a cause for mild hyperoxaluria.

A-57
WHICH IMPORTANCE SHOULD WE ATTACH TO PTH ON OXALATE TRANSPORT IN THE PROXIMAL TUBULE?

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A defect in the renal oxalate transport has been proposed to play a major role in the pathogenesis of calcium oxalate stone disease. In previous *in vivo* studies we were able to characterize the transcellular oxalate transport at the renal proximal tubule. The transport activity of these oxalate carriers is influenced by local hormones. PTH is known to regulate calcium and phosphate transport in the proximal tubule. The goal of the present study

was to evaluate the effect of PTH on the renal oxalate transport processes. Luminal transport was characterized *in vivo* (rat model) at the proximal tubule using the in-situ-stopped flow microperfusion method. For a time period of 3 minutes peritubular preperfusion with different concentrations of PTH (10^{-12} – 10^{-3} mol/l) was performed. Then luminal transport of ^{14}C-oxalate was determined by luminal micropuncture. Peritubular perfused PTH inhibited ^{14}C-oxalate transport significantly. The inhibitory effect on cellular ^{14}C-oxalate uptake was dependent to the perfused PTH concentration: control 20.71% vs. PTH 10^{-12} mol/l 12.97% ($p = 0.022$), PTH 10^{-9} mol/l 14.70% ($p = 0.037$), 10^{-6} mol/l 11.46% ($p < 0.001$) and 10^{-3} mol/l 7.32% ($p = 0.001$). PTH is commonly known to regulate the calcium homoeostasis. Our study demonstrated for the first time that PTH influences additionally the oxalate transport processes in the proximal tubule. Even very low blood concentrations of PTH inhibited the luminal oxalate transport significantly. Probably this effect is quite enough to increase the risk of calcium oxalate stone formation without pathological PTH levels.

A-58
REGULATION OF EXPRESSION OF RENAL CALCIUM TRANSPORT PROTEINS BY ESTROGEN IN AROMATASE DEFICIENT MICE

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In the mammalian kidney, although most of the calcium reabsorption occurs in the proximal tubule and cortical thick ascending limb by diffusion, the active transcellular transport of calcium in distal convoluted tubule under hormonal control has an important role in the body's calcium homeostasis and in nephrolithiasis. The reabsorption of calcium here involves transport across the apical membrane by epithelial calcium channels protein, specifically ECaC-1, cytosolic calcium binding proteins (calbindin-D28 k and calbindin-D9 k) diffusing to basolateral membrane and calcium secretion into the blood by the plasma membrane calcium ATPase and sodium-calcium exchanger protein. Previous reported results indicate that the expression of calbindin-D28 k in other organs is regulated by estrogen. In this study we examined urinary calcium excretion and the expression of genes involved in calcium transport in the distal convoluted tubule in a model of estrogen deficiency, namely the aromatase deficient (ArKO) female mouse. Female ArKO mice had higher urinary Ca/Cr ratios than wildtype (WT) mice despite higher PTH levels. The kidneys of WT, ArKO and ArKO treated with low dose (20 μg/kg) and high dose (20 μg/mouse) estrogen were used for protein and RNA extraction. Quantitative RT-PCR results showed expression of ECaC1 and the calbindin genes was down in ArKO compared to WT, but upregulated at the mRNA level in the kidney of ArKO mice treated with high dose estrogen. The analysis of expression of the calbindin-D28 k protein by western blot or immunohistochemistry revealed lower expression in ArKO samples but higher expression of the protein in kidney of ArKO mice in response to high dose estrogen. These results may explain the increased incidence of renal stones in women after the menopause. The estrogen mediated regulation of expression of other proteins involved in transcellular renal calcium transport is underway.

A-59
URINARY EXCRETION OF PROXIMAL TUBULE-DERIVED ENZYMES IN NORMAL AND STONE FORMING SUBJECTS FOLLOWING AN ORAL OXALATE LOAD

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An elevated urinary excretion of renal proximal tubule-derived enzymes is an early indication of renal injury. There have been reports that the excretion of such enzymes is elevated in a significant fraction of calcium oxalate stone formers. *in vitro* and animal experiments suggest that oxalate itself causes proximal tubular damage. To determine whether the urinary excretion of two proximal tubule-derived enzymes, & # 947; -glutamyl transpeptidase (GGT) and N-acetyl- & # 946; -glucosaminidase (NAG) is elevated in calcium oxalate stone formers we have compared fasting levels in normal subjects and stone formers and examined their acute responses to an 8 mmole oral oxalate load. To avert dietary influences on these measurements, subjects consumed a strictly controlled metabolic diet, including control of dietary oxalate, for one week prior to the loading studies. Results indicated that stone formers had an elevated excretion of enzymes in their fasting urines and that, in contrast to normal subjects, enzyme levels increased in the period 2–6 hrs following the oxalate load. These results suggest that early signs of renal proximal tubular injury are evident in some stone formers and that dietary oxalate may be a contributing factor.

A-60
XANTHINE OXIDASE AS INHIBITORS OF COM CRYSTALS ADHESION TO CELLS

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It is known that exposure to oxalate and COM crystals results in renal tubular epithelial cell injury via free radical. It is also reported that this free radical induced cell injury can be protected by antioxidants such as xanthine oxidase inhibitors. However, the effect of xanthine oxidase inhibitor on crystal – cell interaction is unknown. Therefore we investigated the relationship between the xanthine oxidase inhibitos and COM crystal adhesion to renal tubular epithelilal cells. Briefly, renal epithelial cell lines were incubated at the presence or absence of 0.2 or 2 mmol. of allopurinol or oxypurinol for four hours as a pretreatment. To investigate the effect of oxalate on the adhesion of COM crystal, the cell lines were coincubated with oxalate and allopurinol. One mg/ml of 14C COM seed suspension were added and incubated for 10 minutes. Non-adherent seeds were washed in PBS. The amount of adherent radioactive seeds was measured by liquid scintillation counter. The cytotoxicity induced by COM crystal and oxalate were measured by LDH release assays. Allopurinol and oxypurinol significantly reduce the COM crystal adhesion to cells in dose dependent manner. Inhibitory rates of 2 mmol of allopurinol or oxypurinol were up to 40% and that of 0.2 mmol of allopurinol and oxypurinol were up to 20%. Allopurinol and oxypurinol did not prevent the release of LDH from COM crystal injured cell lines. Oxalate facilitate the adhesion of COM crystals to cell lines and this adhesion effect was also suppressed with allopurinol. Xanthine oxidase inhibitors inhibit crystal-cell adhesion. One of the mechanisms for this inhibitory effect may be explained by the prevention of cell cytotoxicity. Therefore xanthine oxidative inhibitor such as allopurinol and oxypurinol may be expected as potent agents for urolithiasis.

A-61
CALCIUM OXALATE MONOHYDRATE CRYSTAL COATING PROTEINS (COM-CCP) PREVENT CRYSTAL ADHERENCE TO MDCK CELLS

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Crystal retention in the kidney is the critical step in calcium oxalate stone formation. Majority of people do not form stones despite crystaluria, suggesting that renal epithelial cells are capable of handling crystalline waste. These studies focused on understanding the mechanisms involved in renal handling of crystalline waste. We tested our hypothesis that renal cells secrete calcium oxalate monohydrate crystal coating proteins (COM-CCP), which interact with crystals and prevent their binding to each other (crystal

growth) and to the renal cells: the two commonly proposed mechanisms involved in crystal retention. For these studies, we used MDCK cells (a line of canine renal epithelial cells) were grown to confluence, washed several times in serum free media and incubated with serum free Media. The spent media were collected and COM-CCP were isolated by affinity adsorption, crystaldemineralization and Amicon-filtration. Effects of COM-CCP on crystal growth and crystal binding to cells were evaluated using 14C-labelled COM-crystals. Using a novel approach for the isolation of crystal interacting proteins, MDCK cells were found to secrete an array of COM-CCP, which inhibit crystal growth, as well as binding of the COM crystals to MDCK cell monolayers. These data demonstrate the secretion of COM-CCP as cellular defense against the crystal retention. The exposure of the COM-CCP to calcium increases the interaction of COM-CCP with the COM crystals, whereas the exposure to oxalate decreases the crystal-protein interaction, suggesting that calcium may potentate defenses against crystal retention while oxalate may compromise these defenses. These data demonstrate renal epithelial cells secrete COM-CCP as defense against crystal retention and suggest opposing influences of calcium and oxalate on the cellular defenses against crystal retention.

A-62
BETEL-CHEWING AS A POTENTIAL CAUSE OF CALCIUM STONE-FORMATION

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Betel-chewing is a common practice amongst the indigenous populations of the Indian sub-continent, South-East Asia and East Africa. In our database of nearly 1800 patients, 7 patients (six male and one female, all Bangladeshis), aged 39–62 years, were found to have multiple, recurrent Ca-containing stones and a history of betel-chewing (3–5 times per day). On screening, all were found to be hypercalciuric ($>$ 6 mmol/day), 6 were found to pass an average 24-h urine of pH $>$ 6.3, and 6 had low urinary citrate excretions ($<$ 2.2 mmol/day). All had a moderate renal leak of calcium (TmCa/GFR $<$ 2.00 mmol/l GF). All had moderate-to-high PSF values for calcium phosphatecontaining stones in spite of passing good urine volumes. Betel-chewing involves the addition of chuna [consisting of lime (Ca(OH)2)] to the crushed nut of the areca palm wrapped in the leaf of the betel pepper. The alkali stimulates the release of arecoline, one of the active constituents of the areca palm nut. If the patient chews betel more than 3–4 times per day then he/she consumes a significant quantity of additional calcium and alkali since each preparation contains approximately 4 mmol of calcium and twice that quantity of OH^- ions. At much higher consumption levels (30–50 times per day), betelchewing has been reported to cause milk-alkali syndrome. None of our patients showed any radiological signs of milk-alkali syndrome. The study shows that, in our multicultural society, it is important to take into account different ethnic customs in our screening of patients at our Stone Clinics.

A-63
NEPHROLITHIASIS IN KIDNEY TRANSPLANT PATIENTS (KTP):THE CHICKEN AND EGG STORY REVISITED

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Nephrolithiasis (NL) is rarely seen in KTP. However, cadaveric or living kidney donors with NL have been reported to have 'donated' a stone to the recipient at the time of grafting. In 2003, we identified stones in both kidneys of a 67 year-old cadaveric donor that had been grafted into 2 women (55 and 63 years) with end stage renal disease, but no history of NL. Fasting blood and urine samples revealed normal values for U-Ca/U-Cr (0.04 and 0.1 mM/mM), FE urate (6.6 and 4.9 %) and U-pH (5.7 and 5.4) in both recipients, and low TmP/GFR (0.43 mmol/l GF) in one case with high S-PTH. 24-h urine excretions of the major risk factors were normal in both cases, except for Ucitrate (9 and 31 mg/24 h). PSF indices for the risk of forming CaOx, CaP and urate stones were normal in each patient. Eleven female KTP aged 56 to 67 years with C_{Cr} $>$ 40 ml/min. and no history of NL served as controls: mild hyperoxaluria was observed in 2 cases, hypercalciuria in 1 case, hypomagnesiuria in 4 cases, overt hypocitraturia in 9 cases and low citraturia in another 2 cases. The PSF indices were above normal ($>$ 0.6) in 4 cases and very high ($>$ 0.9) in 2 cases. So far none of these patients has become a clinical stone former. We conclude that grafting of one kidney containing a stone does not usually lead to a transfer of renal stone risk or of other renal tubular defect to the recipient. However, based on PSF indices, KTP are at increased risk of NL, in part because of severe hypocitraturia. This study raises the question as to whether or not the prevalence of NL in KTP has been previously underestimated.

A-64
KILLER-SEEGMILLER SYNDROME IN A KIDNEY TRANSPLANT RECIPIENT:CASE REPORT

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A 36-year-old man with chronic renal failure underwent kidney transplantation in March 2003.The immunosuppresion was tacrolimus, mizoribine, prednisolone, and ALG. Doses of tacrolimus were adjusted to maintain blood levels between 10 and 15 ng/ml. Postoperatively the patient developed progressive elevation of serum uric acid. And on the 2nd postoperative day, it elevated up to 44.7 mg/dl. Therefore we carried out hemodialysis, tried to increase urinary output, started low purine and several medicines. The level of serum uric acid was gradually improved. The point mutation of HPRT gene was found and HPRT activity decreased to 62.5%. On the other hand, APRT activity was increased. No insanity was observed, so we diagnosed as Kelley-Seegmiller Syndrome.

A-65
EFFECT OF MANIPULATING SEX HORMONES ON KIDNEY OSTEOPONTIN MRNA EXPRESSION IN RATS

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In-vitro studies have shown osteopontin (OPN) to be a strong inhibitor of calcium oxalate (CaOx) crystallization and crystal adhesion to renal tubular cells. The present study examined kidney OPN mRNA expression by Northern Blot analysis in normal male and female rats before and after sex hormone manipulation. OPN mRNA expression was studied in five male and five female adult SD rats groups with different sex hormone manipulations. A DIG labeled cDNA probe was used for Northern Blot Analysis

Group	Sex	Castration	Implant	OPN density	*% change
1	M	No	No	5.352	
2	F	No	No	6.329	87% vs G1
3	M	Yes	No	3.783	
4	F	Yes	No	5.686	49% vs G3
5	M	Yes	Testosterone	4.596	
6	M	Yes	Estradiol	6.847	50% vs G5
7	F	Yes	Testosterone	4.131	
8	F	Yes	Estradiol	2.686	51% vs G7
9	M	No	Estradiol	7.540	
10	F	No	Testosterone	5.214	−60% vs G9

*% change after standardized with β-Actin

OPN mRNA expression is weaker in make or testosterone treted kidneys compared to female or estradiol treated kidneys. These results support the hypothesis that estrogens inhibit and restosterone promotes kidney calcium oxalate stone formation.

A-66
THE EFFECT OF OXALATE LOADING OR ACIDIFIED MEDIA ON THE EXPRESSION OF MRNA ENCODING CANDIDATE OXALATE TRANSPORTERS IN CACO-2 MONOLAYERS

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Oxalate is thought to be absorbed through transcellular as well as paracellular pathways in the intestine. In the present report, we evaluated the effects of luminal oxalate and [H+] on the mRNA expression patterns of a variety of anion exchanger genes that may mediate transmembrane oxalate flux by Caco- 2 cells. The existence of candidate oxalate transporters was first established in Caco-2 cells grown to confluence using RT-PCR. Confluent monolayers were challenged with media containing 0.5 or 1.0 mM sodium oxalate or with media buffered to pH 6.6, 6.8 or 7.4 for 6 or 24 hours. Changes in the expression profiles of the -candidate and housekeeping genes (β-actin and GAPDH) were then assessed by real-time PCR. Seven genes encoding for members of the SLC26 gene family (SAT-1, DTDST, PDS, DRA, CFEX) and the SLC4 gene family (AE2 and AE3) were expressed by Caco-2 cells. mRNA abundance of these candidate transporters was unaffected by luminal oxalate challenges for 24 hours. In contrast, mRNA encoding SAT-1, DTDST and DRA was down-regulated when the medium pH was lowered to 6.6 for 24 hours. Caco-2 cells express a number genes encoding for anion exchangers that may participate in transmembrane oxalate flux. While elevations in luminal oxalate concentration, *per se*, may not modulate the expression patterns of these exchangers, luminal [H^+] activity may impact transepithelial oxalate transport by decreasing the abundance of some like SAT-1 and DTDST.

A-67
ABSORPTIVE HYPERCALCIURIA & ADENYLYL CYCLASE

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Absorptive hypercalciuria (AH) is characterized by gut hyperabsorption and low bone formation. Half of the patients have a family history of nephrolithiasis. We identified a gene linked to absorptive hypercalciuria in three pedigrees (LOD score > 12). The same gene cloned from rat testis is a soluble adenylyl cyclase. We have called the human ortholog AH-related adenylyl cyclase (AHRAC). In addition to linkage, we performed association studies to examine genotype-phenotype relationships in adults with AH and children with hypercalciuric hematuria or hypercalciuric calcium stones. AHRAC base changes are seen in the normal and affected populations; some alleles have the same frequency (polymorphisms), some are statistically more frequent in AH (possible mutations) and some are not found in normals (mutation). The cumulative number of base changes in affected individuals is positively correlated with gut calcium absorption and negative correlated with bone mineral density. AHRAC is ubiquitously expressed in humans including small intestine, bone, and kidney. Unlike the rat ortholog, human AHRAC has 3 alternatively spliced transcripts that are differentially expressed in different organs. In gut epithelial cells, osteoblasts and osteoclasts, AHRAC is an intracellular and nuclear protein. When heterologously expressed in sf9 cells, AHRAC exhibits robust adenylyl cyclase activity that is regulated by divalent cations and physiologic isohydric bicarbonate concentration in a complex fashion. AHRAC translates ambient bicarbonate into a cAMP signal and may be an important regulator of gut calcium absorption and bone formation. The function of mutant AHRAC is being defined.

A-68
INCOMPLETE RENAL TUBULAR ACIDOSIS AND BONE MINERAL DENSITY: A POPULATION SURVEY IN AN ENDEMIC AREA OF RENAL TUBULAR ACIDOSIS.

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A high incidence of partial renal acidification defect or incomplete renal tubular acidosis (iRTA) in patients with "primary" osteoporosis has been reported. We, therefore, conducted a community-based survey to determine the impact of iRTA on bone mineral density in Khon Kaen province, Thailand, where a very high incidence of RTA had been reported. Three hundred and sixty-one apparently healthy adults, 146 men and 215 women, were randomly enrolled in this study. Bone mineral density (BMD) of spine and femur were determined in all subjects. The diagnosis of iRTA based on the presence of normal serum electrolytes and failure to decrease first morning urinary pH to less than 5.5 after acid loading test. There were 23 (6.4%) iRTA subjects in the studied population. The age, height, body weight, and calcium intake for iRTA and normal subjects were comparable. The BMD of spine and femur for iRTA subjects were not different from those of normal subjects. No difference in the distributions of BMD over age for both areas between both groups was demonstrated. Multiple regression analysis demonstrated that age, body weight, duration of menopause (for females) and gender (only for femoral neck) were independent variables affected BMD in the studied population. The presence of iRTA was excluded from the regression model. Incomplete distal renal tubular acidosis alone was not associated with lower bone mass. However, it may still be valuable to monitor serum electrolytes and BMD in this group of patients due to the tendency to develop intermittent metabolic acidosis.

A-69
URINARY RISK FACTORS FOR RECURRENT CALCIUM STONE FORMATION IN THAI STONE FORMERS

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To survey the urinary risk factors associated with recurrent calcium stone and the contribution of renal tubular acidosis to the prevalence of recurrent calcium stone formation in Thai recurrent stone formers. There were 86 consecutive recurrent calcium stone formers. Three-day dietary record, serum biochemical parameters, first morning urine pH, and two 24-hour urine collections were obtained from each subject. Urinary risk factors for calcium stone formation were determined from the average of the 2-day urine collection. Normal controls were 34 subjects matched for aged, sex, and weight, and without history of renal stone formation. Hypocitraturia was the most common urinary risk factor found in Thai recurrent idiopathic calcium stone formers followed by hypercalciuria and low urinary volume. Almost one-fourth of the stone formers had multiple risk factors. Hypocitraturia might result from low potassium and low alkaline intake. iRTA was common among recurrent calcium stone formers. Determination of morning urine pH should be a part of the investigations for urinary risk factors to avoid overlooking the diagnosis of iRTA

E. GENETIC DISORDERS AND PAEDIATRIC STONE DISEASE

A-70
NICKEL/DITHIONIDE RAPID-TEST – A DIAGNOSTIC INSTRUMENT WITH HIGH RELEVANCE FOR CYSTINURIA SCREENING

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We designed a search and rapid-test for routine clinical use and verified it analytically and diagnostically. A test tablet is dissolved in 2 ml of fresh or cool stored urine. After 2–3 minutes, a color-reaction develops by foaming up and mingling of the sample and the reagent. The color-complex is correlated with cystine-concentration between 0–2,5 mmol/l (0–600 mg/l) from greenblue to increasing dark brown. In comparison with a printed color gradation board (reference gradient), a semi-quantitative determination of cystine concentration up to 2,5 mmol/l is possible. In the area of Jena, 2395 children in day nurseries between the ages of 1–3 years were screened with this rapid test. The nickel/dithionide-test is easy to administer and is not subject to restrictions of toxic law. It is an economical instrument for diagnosis and metaphylaxis in cystinuria for urologists, pediatricians and general practitioners and it meets the diagnostic requirements of an orientational search and rapid-test. The urine-test is helpful for further family anamnesis and human genetic investigations as well as for prophylaxis from a lithogenetic perspective. Patient self-examination is a big advantage in followup prevention of cystine stones.

A-71
PEDIATRIC UROLITHIASIS : LONG-TERM FOLLOW-UP RESULTS IN 153 CHILDREN

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As a recurrent pathology which could reveal functional as well and morphologic changes in the urinary tract, apart from urogenital abnormalities, metabolic and enviromental factors should be evaluated thoroughly in each children suffering from stone disease. In this present prospective study, 95 children with stone disease are being evaluated regarding the patients? And family history together with serum and urine risk factors. Between 1996–2003, 153 children (113 male, 40 male) referring to our Department with complaints of urolithiasis, with an age range of 0.4–15 years (mean 6.6) have been evaluated. All patients were examined in detail and in addition to standard risk factors (hypocitraturia, hypercalciuria, hyperoxaluria, hyperuricosuria, hypomagnesuria), 24 hours urine volume were also well assessed. Of these children 153 have been followed more than 96 months (mean 60 months) and the course of stone disease in this group has been evaluated in an individual basis from different aspects. Our results indicated that; apart from stone removal procedures; treatment of pediatric urolithiasis requires a thorough metabolic and environmental evaluation of all patients in an individual basis. Children with positive family history should be followed carefully with respect to stone recurrence. Children bearing residual fragments should be forced for an urine volume increase in accordance to body mass index and medical treatment with K-citrate have been found to be preventive enough in these children.

A-72
TISSUE DISTRIBUTION AND MITOCHONDRIAL LOCALIZATION OF GRHPR

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The gene mutated in patients with primary hyperoxaluria type II (PH2), Glyoxylate Reductase/Hydroxypyruvate Reductase (GRHPR), is a mutifunctional oxidoreductase that has glyoxylate reductase (GR), and hydroxypyruvate reductase (HPR) activities. Investigation of the tissue distribution of specific enzymatic activity for GRHPR was conducted on frozen human tissues. Liver was found to be the greatest source of GRHPR (DGDH activity = 0.4 mole/min/mg protein), followed by kidney (25% of liver), and then lung, colon, bladder, and lymphocytes, which all had about 10% of the activity of liver. These data demonstrate that the tissue distribution of GRHPR is more widespread than previously thought and raise questions about the role of GRHPR in glyoxylate metabolism in non-hepatic sites. We next investigated GRHPR intracellular localization. GRHPR (enzymatic activity and immunoreactive protein) was located in purified mitochondria from HepG2 cells, frozen liver, and peripheral lymphocytes. Further analysis of the molecular biology of GRHPR demonstrates the presence of a putative mitochondrial localization sequence. Transfection of COS 1 cells with GRHPR constructs followed by immunoblot analysis of purified mitochondria demonstrates that this sequence targets the protein correctly. We hypothesize that mitochondrial targeted GRHPR is important for detoxification of glyoxylate produced by the mitochondrial hydroxyproline catabolism that occurs in hepatic and renal cells, or by other unidentified mechanisms in non-hepatic cells.

A-73
MODERN MANAGEMENT OF CYSTINE STONES IN PEDIATRIC PATIENTS

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Prevalence of cystinuria in Germany is about 1%. Mostly clinical manifestation of this autosomal recessive disorder is in the childhood. Irrespective to poor results extracorporal shock wave lithotripsy is often preferred because of its non-invasive feasibility. Unfortunately we are not able to avert stone recurrence in cystinuric patients with metaphylaxis due to persistent residual fragments. Mini-Perc seems to be an interesting alternative in these patients. We present the case of a 9 year old caucasian boy with homozygous cystinuria. Both sonography and IVP revealed a kidney stone (size 10 mm) in the right kidney. From 1998 to 2002 the calculi was treated with 13 sessions of ESWL. Nevertheless stone burden remained unchanged. Finally, in 2003, a Mini-Perc rendered the urinary tract of this boy stone-free. The compliance of the patient to the medical treatment recommendations was good. For general recurrence prevention sufficient fluid intake and moderate daily protein intake was obeyed. Drug based prevention with citrate was ensued since 1999 and completed with 1 g/d of ascorbic acid in 2003. Results of metabolic evaluation showed decreasing values for urinary cystine levels from 2.55 mmol/d (08/02) to 1.03 mmol/d (06/03, ''stone-free''). Oxalate excretion rate under ascorbic acid therapy was 0.39 mmol/d. Rendering a kidney stone-free in cystinuric patients Mini- Perc seems to be a minimal-invasive but highly effective method. Retrospectively urine parameters change noticeably after stone removal. Cystine stones in the urinary tract influence the results of metabolic evaluation. Treatment outcome of medical recurrence prevention improves if the patient is completely stone-free. However, effective recurrence prevention needs good metabolic treatment before stone removal.

A-74
DENT'S DISEASE IN END-STAGE RENAL FAILURE AND PREVALENCE OF RENAL STONES IN DIALYSIS PATIENTS: THE VENETO REGION STUDY

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The X linked recessive Dent's disease (DD) is characterized by nephrocalcinosis, urolithiasis, hypercalciuria, LMW proteinuria, and renal failure in variable associations. Males are more severely affected. It is caused by mutations in the chloride channel CLCN5 gene. It has been suggested that DD may be under-diagnosed, occurring more commonly than believed, in less overt forms and apparently without family history. However, a recent study has looked for CLCN5 mutations in 32 adults and children with idiopathic hypercalciuria (a possible pool of missed DD diagnosis), did not find CLCN5 mutations. Another way to look at the problem is to investigate for CLCN5 mutations in another groups of pts who possibly have a high prevalence of mutations, i.e. ESRF pts with previous stones. We looked for CLCN5 mutations in 24 male chronic dialysis pts with a personal history of renal stones singled out from all pts (1806 of which 1116 males) of 14 dialysis units in the Veneto region (North East of Italy). All the coding sequences (exons 2 to 12) and the exon-intron boundaries of CLCN5 were screened by SSCP analysis. No mutation was found. We also found 23 females with ESRF and renal stone history. The prevalence of renal stone formers among all dialysis pts in our Region was 2.6% (2.2 in males, 3.3% in females) much lower than the prevalence observed in older studies. This study shows that Dent's disease is indeed very rare, that it is not missed at diagnosis, and that prevalence of stones in ESRF pts is decreased, suggesting that modern treatment options have significantly improved the prognosis of the urolithiasis.

A-75
UNUSUAL ASSOCIATION OF MEDULLARY SPONGE KIDNEY (MSK) AND CONTRALATERAL CONGENITAL SMALL KIDNEY: HINTS ON THE MOLECULAR BASIS OF THE MSK

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MSK is a malphormative condition associated with a high risk of nephrocalcinosis, renal stones, nephronic tubule dis-functions, and precalyceal collecting duct cysts. It may involve all papillas bilaterally, but may also be unilateral or involve only few papillas. We have observed 4 unrelated cases of renal stone pts with MSK and congenital contra-lateral small kidney. The cases will be described. Secondary causes (obstructing stones, etc) were ruled out. In one subjects renal function was moderately reduced. MSK pathogenesis should explain the involvement of anatomical districts (the precalyceal ducts and the nephron) having different embryologic origin. In embryogenesis, the mesonephric blasteme, through the synthesis of the chemiotactic GDNF, prompts the branching from the Wolf's mesonephric duct of the ureteric bud which approaches and invades the blasteme. The bud expresses on its top the GDNF receptor C-Ret. C-Ret/GDNF binding is crucial for the correct formation of ureters and collecting ducts (of wolfian origin as well), but also for nephrogenesis (and tubular function). We advance the hypothesis that MSK is due to a disturbance in the ''ureteric bud/mesonephric blasteme'' inter-facing, possibly due to mutations/polymorphisms of C-Ret and/or GDNF genes. Molecular studies are now in progress in our lab. This hypothesis would explain concurrent alterations in precalyceal ducts and functional defects in the nephron tubule, and the occasional association here described with size and functional asymmetry between the 2 kidneys, or with horse shoe kidney which was previously described.

A-76
INTERNATIONAL REGISTRY FOR PRIMARY HYPEROXALU RIA AND DENT'S DISEASE

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Primary Hyperoxaluria (PH) and Dent's disease (DD) are inherited disorders that can cause calcium urolithiasis, nephrocalcinosis, and renal failure. Rarity of their occurrence limits the experience of any single physician or medical center, thereby hindering diagnosis and treatment advances. Using a multidisciplinary team of nephrologists, study coordinators, epidemiologists, biostatisticians and web designers, we developed a secure, internet-based data collection system for PH and DD patients worldwide. Any physician or health care provider can register and enter patient data in an IRB- and HIPAA-compliant format. A structured questionnaire allows collection of information at initial registration and annual updates. Patient confidentiality is rigorously maintained. Statistical reports of aggregate data will be provided at regular intervals to all registry contributors, including patient characteristics, modes of presentation, clinical course, renal function, treatments, and management of renal failure, and outcomes of transplantation. The PH and DD Registry will help to define the spectrum of expression of these two rare diseases, identify prognostic markers, describe clinical interventions and their outcomes, and establish well-defined patient cohorts.

A-77
THE INFLUENCE OF G630A HOMOZYGOSITY ON PHENOTYPE IN TYPE I PRIMARY HYPEROXALURIA.

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Type I Primary Hyperoxaluria (PHI) is due to deficiency or mistargeting of hepatic alanine:glyoxylate aminotransferase (AGT) activity resulting in hyperoxaluria, calcium oxalate urolithiasis and renal failure. Phenotype is variable and to date, genotype-phenotype correlations are lacking. Recently, we reported an association between the most common mutant allele (G630A) and the clinical response to pyridoxine (VB6) in PHI. To establish additional differences in disease expression due to the G630A change, we divided patients into cohorts of G630A homozygotes (AA, $n = 6$) and compound heterozygotes (CH, $n = 6$) and compared their clinical characteristics. Genotyping was by Msp I restriction enzyme digestion of exon 4 and/or by direct sequencing. Baseline (pre-VB6) urine oxalate excretion (mg/1.73 m^2/24 hrs) was 148 ± 45 in AA and 261 ± 89 in CH ($p = 0.028$). Residual AGT catalytic activity was similar ($p = 0.317$) in both groups. The age (yrs) at first symptoms was 8.2 ± 6.0 in AA and 16.7 ± 10.5 in CH ($p = 0.117$). The age (yrs) at time of diagnosis was 24 ± 14 in AA and 26 ± 14 in CH ($p = 0.786$). 1/5 (20%) AA and 5/6 (83%) CH patients developed ESRD 21 and 28 ± 9 years, respectively, from the onset of symptoms. In the remaining patients, serum creatinine at last follow-up was 1.0 ± 0.3 mg/dL ($n = 5$) in AA and 1.5 mg/dL ($n = 1$) in CH. The clinical phenotype in AA appears milder a priori VB6 treatment in PHI.

A-78
THE MANAGEMENT OF MILD METABOLIC HYPEROXALURIA (MMHO) IN RECURRENT KIDNEY STONE FORMATION

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Four major clinical entities are responsible for calcium oxalate (CaOx) stones: the genetic (oxalosis), enteric (EHO), dietary (DHO) and mild metabolic (MMHO) hyperoxalurias. To assess the efficacy of various forms of treatment in above groups (excluding genetic hyperoxaluria). All patients underwent a full stone workup and dietary assessment. They were then placed on a ''standard'' sodium and calcium diet as well as a low oxalate intake. Ten days later a repeat 24-hour urine oxalate was measured. Of 76 patients, 28 were then classified as DHO, 43 as MMHO and 5 as EHO. All patients were maintained on low oxalate diet and those with

MMHO or EHO were then treated with either pyridoxine (B_6), $CaCO_3$ (Titralac) or both. Repeat urine chemistry was measured yearly for follow-up of 1–10 years. Stone formation rate (SFR) before and after therapy was documented.

Group	*N*	*Pre Oxal (umol/day)*	*Post Oxal (umol/day)*	*% change*	*p-value*
DHO					
a)diet	28	456(96)	223(67)	51	< 0.001
MMHO					
a)diet	43	446(103)	421(73)	6	NS
b)B_6	26	450(91)	340(146)	24	< 0.001
c)$CaCO_3$	13	486(151)	280(108)	42	< 0.001
d)both	5	473(129)	292(89)	38	NS
EHO					
Any Rx	5	657	523	20	NS

DHO is controlled on diet only, strongly suggesting hyper absorption (max contribution of U_{ox} excretion = 15% of diet). About 50% of patients with MMHO are successfully treated with $CaCO_3$ being superior to B_6. SFR decreased significantly post therapy (data to be presented).

A-79
ETIOLOGY OF PEDIATRIC UROLITHIASIS IN PAKISTAN

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To determine the demographic patterns, etiology and risk factors in paediatric urolithiasis. An interventional study was conducted in 437 children with stones with normal renal functions and no urinary tract anomaly. History, physical examinations and dietary assessment was done. Lab investigation included complete blood picture, serum urea, creatinine, electrolytes, calcium, phosphate, uric acid, protein, magnesium, urine analysis and culture. Twentyfour hours urine for metabolic analysis included calcium, oxalate, phosphate, magnesium, citrate, uric acid, cystine, sodium and potassium. Radiological investigations included ultrasound, x-ray KUB and x-ray IVU in all patients. Surgical management included ESWL, PCNL and open surgery. Stone analysis was done by IR spectrophotometer. A large number of these patients have family history of stone disease. There are number of dietary and urinary risk factors identified in this patient population. The presence of Ammonium acid urate in the core of kidney and bladder stones in children points to a common etiology i.e. malnutrition and diarrheal state.

A-80
PEDIATRIC UROLITHIASIS WITH RENAL FAILURE: SIUT EXPERIENCE

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Pediatric urolithiasis is endemic in Pakistan and constitutes about 13% of all urolithiasis cases. Urolithiasis associated with renal failure is one of the most important causes of pediatric ESRD in Pakistan. We present retrospective analysis of our 400 patients age ranging less than 1 year to 15 years. Of them more than 50% have positive family history of stone disease. Apart from obstruction and infection, poor socioeconomic status (88%), rural residence (75%), neglect and delay in acquiring treatment leads to renal failure in these patients. Majority of these patients have history of abdominal pain (65%), anemia (80%) and haematuria (33%). However they usually present with signs and symptoms of advanced uremia (70%) and sepsis (23%). Ultrasonography of kidney, ureter and bladder has been the main stay of radiological diagnosis and assessment. More than 60% of these patients have bilateral stones and 72% had associated UTI. The initial management of these patients required dialysis mostly peritoneal dialysis in small children. Percutaneous nephrostomy (38%) with or with out dialysis was also the main stay of initial management especially those presenting with pyonephrosis. Some were also placed "Double J" stents to relieve obstruction. Open surgery, PCNL and ureterorenoscopy were done to provide definitive treatment. Stone analysis showed ammonium acid urate (AAU) 44% and 12% struvite stones. About 70% of the patients had good recovery of renal function. These good results were achieved because of provision of free high technological treatment to all our patients under one roof with close cooperation of pediatric urology, nephrology and intensive care teams.

F. URIC ACID STONES

A-81
CLINICAL SIGNIFICANCE OF URIC ACID DIHYDRATE IN URINARY STONES

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Uric acid crystallizes as an anhydrous compound, a dihydrate or a mixture of both. A monohydrate form is very rare. About 20% of uric acid stones contain a significant amount (≤ 20%) uric acid dihydrate. N = 85 patients with pure uric acid calculi were studied. Stone analysis was performed using x-ray diffraction. According to the stone analysis, they were divided in two groups: 1. ≤ 20% uric acid dihydrate, 2. < 20% uric acid dihydrate. In all patients the following parameters were examined: age, sex, number of recurrences, body weight, height, body mass index; blood: creatinine, uric acid, calcium; urine: pH-profiles, volume, calcium, uric acid, citrate, ammonia, and urea. Group 1 (≤ 20% dihydrate) consisted of n = 68, group2 (< 20% dihydrate) of n = 17 patients. Between these groups, there was a significant difference concerning the number of recurrences and the urinary excretion of calcium. Patients with ≤ 20% dihydrate had a mean number of recurrences of 0.23 ± 0.44 and a calcium excretion of 4.65 ± 2.70 mmol/24 h, whereas those with < 20% dihydrate had 1.00 ± 1.29 recurrences and a calcium excretion of 2.97 ± 1.93 mmol/24 h. All the other parameters tested, were not significantly different. Our examinations demonstrated for the first time metabolic data in uric acid patients with a significant amount of dihydrate. The comparison between this group and those patients with < 20% dihydrate showed that the first group is less prone to develop recurrences.

A-82
URIC ACID MONOHYDRATE – A NEW URINARY STONE

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Among 100,000 stones that have been analyzed in our lab since 1972, 15 samples of similar characteristics from 8 patients could not be sufficiently identified by standard laboratory methods, i.e. polarization microscopy, infrared spectroscopy and X-ray diffraction. Therefore an extended spectrum of physical methods had be applied to get insight into true nature of this new component. X-ray structure analysis from powder diffraction data turned out to be most successful by identifying the unknown material as uric acid monohydrate. This substance crystallizes in

the monoclinic space group P 2_1/c. Acid and water molecules are connected by hydrogen bonds forming infinite layers in the crystal. By contrast, for the anhydrous as well as for the dihydrate form of uric acid three-dimensional networks were found. Additional analytical procedures like solid state NMR spectroscopy and scanning electron microscopy confirmed its distinctive nature as compared to the known forms of uric acid and its salts. All investigated stones consisting of the monohydrate contained as additional amorphous component a large amount of long aliphatic chains reminiscent of structure of fatty acids. To our best knowledge, the existence of a monohydrate form of uric acid has not been reported so far. The special conditions of the very rare formation of uric acid monohydrate concrements, esp. the role of aliphatic material, should be clearified by further investigations of the metabolic situation of patients concerned.

A-83
URIC ACID NEPHROLITHIASIS AND INSULIN RESISTANCE

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One invariant pathogenic hallmark of uric acid stones is unduly low urinary pH due to low urinary ammonium. We previously provided three levels of evidence for a link between uric acid stones and insulin resistance. Population studies showed high incidence of glucose intolerance in uric acid stone formers, high incidence of uric acid stones in diabetic stone-formers, and correlated body mass index with urinary acidity. Metabolic studies showed negative correlation between glucose disposal rate and renal ammonia excretion. Laboratory studies showed insulin stimulation of the key ammonium transporter in the renal proximal tubule, the Na/H exchanger NHE3. In the Zucker Diabetic Fatty (ZDF) insulin resistant rat, we found lower urinary pH and ammonium when compared to paired-fed lean controls. The ZDF rats also express lower levels of NHE3 protein but not transcript. In addition to lower NHE3, *in vitro* ammonium production from cortical slices are also decrease at baseline and after insulin stimulation. Renal tissue triglyceride (TG) in ZDF rats is much higher than lean controls and increases with age. High TG correlates with low NHE3 expression. IN cultured renal cells, insulin directly stimulates NHE3 activity via the PI-3–kinase-serumglucocorticoid kinase-1 pathway leading to increase NHE3 activity and surface protein abundance. Incubation of cells with free fatty acid leads to steatosis and insulin resistance. In summary, one mechanism of low urinary pH and uric acid nephrolithiasis is impaired ammonium excretion due to renal insulin resistance possibly caused by lipotoxicity in the proximal tubule.

A-84
CLINICAL CHARACTERISTICS OF MIXED URIC ACID & CALCIUM OXALATE KIDNEY STONES

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Formation of uric acid stone is known to be initiated by homogeneous nucleation, but that of calcium oxalate stone is heterogenous. In order to analyze the pathogenesis of mixed uric acid and calcium oxalate renal stones, we studied the risk factors possibly implicated in the development of mixed kidney stones. From the medical records of 1491 kidney stone patients, we evaluated seventy patients with pure uric acid stone (UA group), 44 patients with mixed uric acid and calcium oxalate stone (UACOX group) and 648 patients with pure calcium oxalate stone (COX group). Prevalence of female in UA group was 4.3%, 18.2% in UACOX group and 24.7% in COX group. To exclude the influences of sexual bias, we analyzed 24-h urinalysis, serum chemistry and stone recurrent rates of male patients in UA group (67 patients), UACOX group (36) and COX group (648). Average age of patients in UA group was 54.4 years old, 52.9 in UACOX group and 46.6 in COX group, respectively. Twenty-four hours urinary amount of citrate in UACOX group was 383.6 mg and it was significantly decreased in comparison with 483.4 mg in UA group or 412.2 mg in COX groups. Amount of urinary calcium in UA group was 145.1 mg & # 12289; 174 mg in UACOX group and 223.6 mg in COX group, respectively. Urinary excretion of oxalate in UA group was 54.2 mg & # 12289; 49.2 mg in UACOX group and 46.2 mg in COX group, respectively. Urinary excretion of uric acid in UA group was 742.2 mg & # 12289; 737.2 mg in UACOX group and 666 mg in COX group, respectively. Amounts of daily urinary calcium, oxalate and uric aid in UACOX group were intermediate between those of UA group and COX group. It seemed to be an important factor that excretion of urinary citrate was decreased in patients with mixed uric acid and calcium oxalate stones.

A-85
EFFECT OF ALKALINIZATION ON URIC ACID EXCRETION IN IDIOPATHIC URIC ACID STONE FORMERS

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Previous studies have suggested a normal uric acid excretion in the majority of uric acid stone formers. We examined the effect of treatment with potassium citrate on uric acid excretion in 8 uric acid stone formers. Patients collected 24 hour urine specimens before and after treatment with the alkalinizing agent and the excretion values compared. The data is given as the mean ± SEM.

	UV	$U_{Cr}V$	$U_{UA}V$	UA/Cr	pH	$U_{Ca}V$	$U_{Na}V$	$U_{Ox}V$
Pre	1590 ± 309	1277 ± 253	347 ± 77	0.29 ± 0.04	5.5 ± 0.06	148 ± 31	145 ± 19	30 ± 5
Post	1877 ± 218	1464 ± 214	606 ± 100*	0.40 ± 0.03 *	6.5 ± 0.12	107 ± 22	153 ± 30	34 ± 4

*$p < 0.05$ pre vs post

Uric acid excretion expressed as the absolute value or as the uric acid to creatinine ratio significantly increased following alklinization of the urine with potassium citrate. None of the other excretion values were affected. Whether this effect of alkalinization is a result of altered renal tubular handling of uric acid or the correction of a collection artifact induced by collecting the urine while acid is not addressed by this study.

G. MEDICAL AND SURGICAL MANAGEMENT OF RENAL STONES

A-86
AMORPHOUS CARBON COATINGS REDUCE THE EXTENT OF ENCRUSTATION OF INDWELLING CATHETER SURFACES

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Blockage and encrustation of urethral catheters by crystalline bacterial biofilms remains a major complication in the care of patients undergoing long-term indwelling bladder catheterisation. Approximately 25 % of all nosocomial infections are related to bladder catheterisation. In this study, we investigated the

influence of amorphous carbon coatings on surfaces of indwelling catheters in respect to its ability to reduce the extent and to affect the mineral composition of the encrustation. For the investigation of crystallisation processes under constant conditions, an in-vitro constant-flow-crystalliser model and a standardised test procedure were developed. 24 commercial indwelling catheters were incubated in synthetic urine for 12 h; urease was added to increase urinary pH. On 18 of the catheters, a carbon layer was deposited on the surfaces by *P*lasma *E*nhanced *C*hemical *V*apour *D*eposition (*PECVD*). Encrusted surfaces were analysed with SEM; the chemical composition of the minerals deposited on the catheter surface was determined. The results of the presented pilot study clearly show that the total amount of stone material which precipitated on the coated test catheters' surfaces can be reduced by up to 50 % compared to untreated catheters.

A-87
NOVEL ANALYSER ALLOWS RAPID DETERMINATION OF URINARY CALCIUM OXALATE STONE FORMATION RISK

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So far, doctors determine from the patient's urine sample a number of biochemical values to determine stone formation risk — a costly method which leads only to a rough estimate of the "true" crystallization risk. Any crystallization risk index which bases on the evaluation of a biochemical analysis principially only includes a small fraction of all urinary components. Mostly, these urinalyses are restricted to the determination of some electrolytes and few low molecular constituents. The present "gold-standard"-indices, the APCaOx-Index and the model value of the relative urinary supersaturation, only take account for 5 and 23 parameters, respectively. However, kinetics of crystal formation are controlled, in particular, by thus disregarded macromolecular substances. The BONN-Risk-Index (BRI) is a proven stone-risk evaluation strategy which overcomes all of the previously mentioned limitations and disadvantages. This parameter is determined by a crystallization test performed in unprepared native urine; all urinary constituents contribute to the result in their native chemical environment. The presented *URINETTE* instrument enables urologists to conduct desk-top BRI-assessments of urine samples within minutes. No physical or chemical treatment prior to analysis is required. This allows the physician instant feedback to evaluate the effectiveness of dietary changes or medication given to the patient. The *URINETTE* will be available by end of 2004.

A-88
FRAGILE! THE CRUX OF FLEXIBLE URETEROSCOPY (AND HOW TO AVOID IT)

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Flexible ureteroscopy is more and more used worldwide for retrograde minimally invasive treatment of upper ureteric and intrarenal pathologies. A major drawback to flexible ureteroscopy is the fragility of the instruments and the oftenprohibitive costs of repair. In spite of new developments, the instruments remain delicate and easily break if some basic rules are not observed. In co-operation with one of the main suppliers of flexible ureteroscopes in the United Kingdom, the authors analysed sales and repair figures, analysed the possible causes of damage to the instruments, and calculated cost figures for maintenance of the instrument as opposed to repair and replacement costs. All damages did not fall under manufacturer's warranty and therefore implied significant costs to the customer. Regular maintenance is shown to have an influence on the durability of the instruments. Most damages are handling induced, prompting the need for laser courses for medical staff and maintenance courses for support staff. After extensive consultation and discussion with the engineering staff of the supplier, the authors, in addition to recommendations found in the literature, discuss and list some easy suggestions as to how to avoid damages.

A-89
IN-VITRO SWL TESTING OF A NEW DOUBLE-J WIRE STENT (ZEBRASTENT.)

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Recently, a new double-J ureteric stent with a stable shaft and flexible ends has been marketed. This concept provides a splinting of the ureter without using much of the lumen for the stent itself. Thus stone fragments have the necessary space to pass easily alongside the stent through the straightened ureter. We tested shock wave resistance of the stents when used for pre-SWL stenting to facilitate fragment passage. Zebrastents. were treated with defined numbers and energy levels of shock waves in a water bath. As typical parts exposed to shock waves, the flexible J-part (for renal stones) and the shaft (for ureter stones) were shot at marked spots. The Lithotriptor was set at typical as well as at maximum treatment settings. The stents were examined by micro-Xray for damages to the metallic core, and micro-photography and electron microscopy for the hydrophilic surface. In addition, each a conventional stent and a wire stent that remained simultaneously in the same patient for 6 weeks were examined for encrustration.
There were no damages to either the core or the coating after SWL. Whereas the conventional JJ-stent of our patient showed a considerable degree of encrustration, there was none on the wire stent. The Zebrastent. does resist shock waves up to maximum levels and appears therefore safe to use under SWL treatment. The hydrophilic coating appears to prevent encrustration to some degree. Studies on the practical clinical use of the stents are underway.

A-90
OPEN STONE SURGERY-A SOON FORGOTTEN ART

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Following the general shift towards minimally invasive surgery, endourology is rapidly expanding. There are progressively more applications for endourological procedures and fewer applications for open procedures. We reviewed our data on surgical procedures over a period of 18 months to assess the role of open surgery in a modern endourology service. Within 18 months, our stone & endourology unit treated a total of 902 stone and endourological patients. This included 649 (72%) SWL, 240 (26.6%) endourology procedures, and 13 (1.4%) open procedures. The latter included 8 nephrectomies, 3 ureteric neo-implantations, and 2 pyeloplasties. Each one nephrectomy, ureterimplantation and pyeloplasty were secondary indications after failed endourological procedures. The others were primary indications for non-functional kidneys (7 nephrectomies), non-recanalizable ureteric strictures (2 neo-implantations), and aberrant renal artery (1 pyeloplasty). With 1.4% of all treatments, our rate of open surgery compares with the literature. As it stands, open surgery is mainly limited to ablative surgery or management of complications. With the advent of laparoscopic nephrectomy and pyeloplasty, the majority of those indications will also shift to minimally invasive methods, thus further reducing the need for open surgery. Nevertheless, some aptitude in open surgery will remain necessary even for an endourologist to manage rare complications.

A-91
PCNL TECHNIQUES FOR TREATMENT OF UPPER URINARY STONE (18 YEARS CLINICAL REPORT)

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To report the 18 years clinical experience of PCNL techniques for treatment of upper urinary stone in our hospital from Nov. 1984 to Nov. 2003, and to evaluate the safety and efficacy of new technique: Mininephrostomy ureterolithotripsy (Mini-PCNL) technique we developed since 1992. Total 2263 patients with Upper urinary stone, include pelvis stone 254, calyceal stone 402, UPJ stone 202, Staghorn stone 1405. This series consists of 1407 male and 970 female with age ranging from 8 to 82 years (mean age 46 years). Three types of PCNL we performed in the 18 years: The stander PCNL, Retrograde PCNLand Mini-PCNL. In early period time (between 1984–1991) the stander PCNL technique are care out in 289 cases and Retrograde PCNL in 22 cases and Minu-PCNL in 41 cases. after 1992, all of procedure was performed with Mini-PCNL.The success rates were 90–96% for Pelvis stone and calyceal stone, 98% for UPJ stone, 80–86% for staghorn stone.Tree types of PCNL no significance difference ,but for complication rate in treatment of staghorn stone, the stander PCNL was 20%, while Mini-PCNL only 2%. (Mininephrostomy with uerterolithotripsy) for the treatment of staghorn stone have a significance effect on reducingh blood loss and increasing stone free rate compared with standar PCNL.

A-92
CLINICAL SIGNIFICANCE OF TEXTURE TYPES IN CALCIUM OXALATE STONES

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Previous studies (Schubert, Brien) suggested a higher metabolic activity in calcium oxalate stone patients with texture types III–IV (i.e. primary crystallization of weddellite) when compared to those with texture types I–II (i.e. primary crystallization of whewellite). We were interested to know whether we could corroborate these findings and in consequence whether patients with texture types III–IV have higher recurrence rates. N = 104 consecutive patients with calcium oxalate stones were studied. Stone analysis was performed using X-ray diffraction. The determination of texture types (IIV) was performed on grain preparations using polarization microscopy. The patients were grouped according to the texture type (texture group 1: types I and II; texture group 2: types III and IV). The following parameters were examined: age, sex, body weight, height, n° of recurrences, blood: creatinine, calcium, uric acid. 24-h-urine: volume, calcium, uric acid, ammonia, citrate, urea. Urine pH was measured as circadian profiles. There were n = 77 patients in texture group 1 and n = 27 in texture group 2. The excretion of calcium was significantly higher, the urine volume (24 h) significantly lower in texture group 2. All the other parameters, however, were not different. Since calcium excretion is significantly higher in patients with primary crystallization of weddellite (texture types III and IV) than in patients with primary crystallization of whewellite (texture types I and II), calcium excretion is apparently the predominant factor determining the composition and texture of calcium oxalates stones. The increased excretion of calcium and the decreased urine volume can be interpreted as a sign of increased metabolic activity in texture group 2. On the other hand, this potentially increased metabolic activity did not result in an increased recurrence rate. This is in accordance with our previous findings that the determination of urine parameters cannot predict the risk for recurrence. The n° of recurrences was similar in both texture groups (1.23 ± 3.23 versus 1.04 ± 1.37). Thus, the analysis of the stone texture also cannot be used as a predictor for the risk of recurrence in a stone forming patient.

A-93
TRAINING MODEL FOR PERCUTANEOUS NEPHROLITHOTOMY

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Percutaneous nephrolithotomy and similar endourological procedures require an advanced level of skills. To facilitate the training of the proper technique, simulators are helpful. Non-biological models, useful to learn the basic steps, do not represent the clinical situation in an ideal way. Recently, we developed a porcine urinary tract model for ureteroscopy. Proceeding from this experience, we developed a further ex vivo model for training of percutaneous endourological procedures. The kidney with the ureter is dissected off the retroperitoneal organ package of freshly slaughtered pigs. It is embedded in silicon. The renal pelvis can be filled with saline to simulate hydronephrosis; stones can be implanted for percutaneous nephrolithotomy. This ex vivo model allows for training of all percutaneous endourological procedures (e.g. percutaneous nephrostomy, percutaneous nephrolithotomy, endopyelotomy). Our ex vivo model for training of percutaneous nephrolithotomy and other endourological procedures is an ideal way to train these techniques. Concerning ''tissue feeling'', the anatomic relations and the great variety of procedures that can be trained, it is superior to non-biological models. Nevertheless, it is easily available and inexpensive.

A-94
EFFECTS OF SHOCK WAVE LITHOTRIPSY ON PLASMA AND URINARY LEVELS OF NITRITE AND ADRENOMEDULLIN

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In this prospective clinical study, we aimed to determine whether Shockwave Lithotripsy (SWL) has any specific effect on plasma as well as urinary nitrite, a stabile metabolite of Nitric Oxide (NO) and adrenomedullin (AM) concentrations, and to investigate whether these variables may be used as a marker for detecting shockwave-induced impairment of renal tubular and glomerular cells. A total of 30 patients with renal pelvic or caliceal stones & # 8804; 2 cm undergoing anesthesia-free SWL without auxiliary measures and a control group of 10 patients without any urologic symptoms were included in this study program. The plasma and urinary concentrations of nitrite and AM were measured before, 24 hours, and 7 days after SWL. Nitrite levels were measured by Griess reaction. Reverse-phase high-performance liquid chromatography (HPLC) was used to determine AM levels. Application of High Energy Shock Waves (HESW) in our study caused a statistically significant increase in plasma levels of both NO and AM which reflected an organized response of the kidney to this type of trauma in an attempt to maintain normal renal hemodynamics. On the other hand again, comparative evaluation of urinary levels of both nitrite and AM levels before as well as 24 h after SWL application revealed a statistically significant increase related in markers. This first clinical study on plasma-urinary nitrite and AM levels in patients undergoing SWL procedure, indicated that plasma and urine levels of both peptides were increased. Our findings in turn suggested that SWL application to kidney may stimulate the NO-cGMP signalling pathway to increase NO production in the kidney. Our findings also indicated that the increased levels of NO and AM secretion during renal

parenchymal ischemia may be protective enough for renal pathologic alterations resulting from SWL induced renal trauma. We suggest that this increase may be a compensatory response to SWL induced injury.

A-95
DOES VERAPAMIL THERAPY HAVE ANY EFFECT ON STONE RECURRENCE AND RESIDUAL FRAGMENTS AFTER SHOCK WAVE LITHOTRIPSY ? : A RANDOMIZED CONTROLLED STUDY

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The beneficial physiological and protective effects of Verapamil (a calcium channel blocking agent) on the parenchymateous organs have revealed to its use as a protective agent in an attempt to limit the functional as well as morphological adverse effects induced by SWL. To evaluate the long-term (> 30 months) efficacy and tolerability of this agent in preventing stone recurrences and the possible growth of residual fragments after shock wave lithotripsy (SWL), a prospective randomized clinical trial has been carried out. Totally 45 patients undergoing SWL for kidney stones have been included into the study program. Age of the patients in all groups ranged from 18–42 years with an average value of 30.6 years. Male/Female ratio was: 1.1 (24 M, 21 F) The patients were independently randomized into three different sub-groups. Comparative evaluation of our results with respect to stone recurrence and regrowth demonstraed a 20% regrowth rate (3/15) in Verapamil group and 15% (2/15), 45% (6/15) in the remaining two groups respectively. Again the stone recurrence rates were 15% (2/15), 0% and 20% (3/15) in three groups. Thus medically treated patients seemed to demonstrate significantly lower recurrence and regowth rates when compared with control ones.The number of stone recurrence throughout the study in the 45 patients was 11% (5/45) Of the 15 patients on high fluid intake 13 patients (85%) remained stable throughout the study. While no true recurrence has been noted in these patients, (0%), stone regowth rate in this group was 15%. Our results indicated that in addition to carefully controlled fluid intake, verapamil therapy have been found to be significantly effective in the medical prevention of stone recurrence as well stone regrowth after a successful SWL. This specific effect may be attributed to its regulatory role on the blood distribution during possible transient ischemia induced by high energy shock waves.

A-96
USE OF A PROBIOITIC TO DECREASE URINARY OXALATE EXCRETION IN ENTERIC HYPEROXALURIA.

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Patients with inflammatory bowel disease have a 10–100 fold increased risk of nephrolithiasis with enteric hyperoxaluria being the major risk factor for these and other patients with fat malabsorptive states. Endogenous components of the intestinal microflora can potentially limit dietary oxalate absorption. A recent study demonstrated that a mixture of lactic acid bacteria degraded oxalate *in vitro* and reduced urinary oxalate excretion when given by mouth. In the current study we evaluated a similar mixture of lactobacilli (Oxadrop®, VSL Pharmaceuticals). Nine patients were studied with chronic fat malabsorption, calcium oxalate stones, and known enteric hyperoxaluria (gastric bypass (3), S/P gastrectomy (2), jejunoileal bypass, primary sclerosing cholangitis and ulcerative colitis in remission). Urinary oxalate fell a mean of 25% ($P < 0.05$) after one month (one dose per day), and urinary oxalate levels were stable during the second month (two doses per day) ($P < 0.05$). During the third month, on 3 doses per day, the mean oxalate excretion increased slightly so that the mean was close to baseline off treatment. Urinary calcium oxalate supersaturation fell throughout the study while on Oxadrop®, largely due to the decrease in oxalate excretion. Therefore manipulation of GI flora can influence urinary oxalate excretion, effectively reducing urinary supersaturation levels. We predict these changes would have a salutary effect on stone formation rates.

A-97
MUSIC DECREASES THE PERCEPTION OF PAIN DURING EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY

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Extracorporeal shock wave lithotripsy (ESWL) is a non-invasive method of treating urinary calculi. Often patients are treated on an outpatient basis. The treatment is painful. The ESWL machines generate an unpleasant banging noise as the shock waves are generated. We assess the efficacy of administration of music in reducing patients pain perception. We randomly selected 54 patients into three groups. 17 had no music, 19 were played ambient music, 18 were played music via headphones. The patients were asked to complete a visual analogue pain score (VAPS) at 4 predefined stages during the treatment. Once at a standard setting, this was used as a control. Then three more times at different times during their treatment. The power setting was recorded for each pain score. The pain scores were compared for each patient with those obtained with the standard stimulus. The derived pain scores for each group were then compared between music and no music groups, headphones and no music groups, and headphones and ambient music groups. There were significantly lower VAPS for patients with music played via loudspeakers ($t < 0.05$) or headphones($t < 0.05$). This is particularly significant at lower power settings. There was no significant difference between the headphones and ambient music groups. Music played via headphones or loudspeakers during ESWL and is a safe and useful adjunct to pharmaceutical analgesics.

A-98
PELVI-CALYCEAL HEIGHT, A PREDICTOR OF SUCCESS WHEN TREATING LOWER POLE RENAL STONES WITH EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY?

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Extra corporeal shock wave lithotripsy (ESWL) is the treatment of choice for the majority of renal stones. Stone size, composition and collecting system anatomy are contributing factors in the success of a treatment. ESWL has the lowest success rate in complete clearance of stones located in the lower pole. We assess the usefulness of pelvi-calyceal height in predicting success rates with these stones. We evaluated 52 patients with solitary lower pole calculi of less than 20 mm treated with ESWL. Pelvi-calyceal height was measured on intravenous urogram and stone location and size were determined by plain radiograph. The number of treatments, number of shocks and the relative intensity of shock waves were recorded. Success was defined as complete stone clearance. Twenty-eight patients (54%) had successful treatment while the remaining 24 (46%) had incomplete stone clearance (including two patients in whom treatment had no effect). At a pelvicalyceal height less than or equal to 2.0 cm there was a 75% success rate. Less than or equal to 1.5 cm there was an 85% success rate. At equal to or less than 1.0 cm there was a 92% success rate. Pelvi-calyceal height is a useful predictor of success when treating lower pole renal stones with ESWL. The greater the pelvi-calyceal height the less likely ESWL is to be successful in completely clearing lower pole renal calculi.

A-99
EFFECT OF LASER LITHOTRIPSY BY MEANS OF FREQUENCY-DOUBLED DUAL-PULSE ND: YAG LASER (FREDDY) – AN *IN VITRO* STUDY WITH NATURAL URINARY CALCULI

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Laser lithotripsy is indicated when flexible endoscopy is used, especially in the case of flexible ureterorenoscopy for the treatment of lower caliceal concrements and as a therapeutic alternative for ballistic and ultrasound lithotripsy with rigid endoscopy. Lasers of various wavelengths are available for laser lithotripsy. Besides alexandrite and holmium lasers, most recently also Frequency-Doubled Dual-pulse ND: YAG Laser (Freddy) has become available. An *in vitro* experiment was performed on 288 natural urinary calculi of varying composition in order to evaluate the disintegration performance of Freddy. The results confirm the good disintegration performance of Freddy for the eradication of stones containing calcium oxalate dihydrate. The disintegration performance is lower for COM, CP and S. UA can be disintegrated even less. C exhibits practically no disintegration. Mixed stone types disintegrate more easily than stones composed of only one type of mineral. Moreover, the *in vitro* experiments provide evidence that the angle of administration is significant for the disintegration performance. Further experimental studies are needed to clarify the extent to which the tangential application of the laser probe can improve the disintegration performance.

A-100
MINIMAL INVASIVE PCNL (MINI-PERC) IN PATIENTS WITH RENAL PELVIC AND CALYCEAL STONES – EXPERIENCE IN 93 CASES

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Stones of the renal pelvis can be treated either by extracorporeal shock wave lithotripsy (SWL) or percutaneous nephrolithotomy (PCNL). As a low-risk procedure with a longer treatment period, SWL often leads to persistent residual stone fragments, whereas conventional PCNL achieves a higher stone-free rate and allows a shorter treatment period albeit with a somewhat higher surgical risk. To reduce the invasiveness of conventional PCNL, the application of a miniaturised instrument for PCNL (MPCNL) was evaluated. For MPCNL a rigid nephroscope with a calibre of 12 F was developed and used in 93 patients. After puncture of the kidney under ultrasound control and single-step dilatation a 15 F Amplatz sheath was placed. Data on the stone size and location, stone-free rate, blood transfusions, operating time and complications were recorded. In 90 patients, the part of the kidney afflicted by the stone was successfully punctured. On average re-treatment rate was 0.7. The mean stone size was 2.7 cm^2. The average operating time was 90.7 minutes. In every case, the absence of residual stones was confirmed radiologically and nephroscopically. Haemorrhages requiring a blood transfusion did not occur. A febrile pyelonephritis occurred as a postoperative complication in 3 patients (= 3.2%). MPCNL represents an alternative to SWL for renal calculi with a size from 1 to 2 cm located in the renal pelvis and calices, especially the lower calix. The advantages are the short treatment time, the high stone-free rate and the accessibility of lower pole stones which are less amenable to SWL. MPCNL is not suitable for large concrements since the limited sheath diameter would increase the operating time. Due to this limitation, MPCNL represents an extension of the indication for conventional PCNL that it can in no way replace.

A-101
A SINGLE CENTRE, RETROSPECTIVE COMPARISON OF TREATMENT OUTCOMES OF URINARY CALCULI USING PIEZOELECTRIC, ELECTROHYDRAULIC AND ELECTROMAGNETIC SHOCK WAVE LITHOTRIPTORS

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We compared the treatment efficacy for patients attending a single centre for shockwave lithotripsy (SWL) by the same team using either Wolf Piezolith 2300 (piezoelectric, PZ), Dornier MPL 9000 (electrohydraulic, EH) or Dornier Compact Delta (electromagnetic, EM) in the period Jan 1992 to June 2002. 3163 (1449 PZ, 780 EH and 934 EM) solitary, radio-opaque urinary stones of size ≤ 15 millimetres in adult patients, receiving primary SWL, were identified. Stone free was defined as absence of radiological evidence of stone. Treatment outcomes were assessed by stone free rate at 3 months after one treatment session (SF3m), re-treatment rate (reTx) and auxiliary procedure rate (AUX). Univariate and multivariate statistical analyses were preformed for different variables that may have an impact on the treatment outcomes, including the type of lithotriptors. Comparison of treatment outcomes for the 3 machines was then done. Patient characteristics were similar for the 3 groups. There was significantly less ureteric stone in PZ group. Using the multiple logistic regression model, the adjusted odd ratio of SF3m for PZ and EH (using EM as the referent category) were 1.38 (95% CI = 1.15–1.65) and 1.72 (95% CI = 1.39–2.11) respectively. Patients treated using the EH were significantly less likely to require re-treatment (AOR = 0.57, 95% CI = 0.48–0.69) than the other 2 machines. No significant difference for AUX for the 3 machines was noticed. Dornier MPL 9000 had the best treatment outcomes in term of both SF3m and ReTx among the 3 lithotriptors. New technology does not necessarily result in better outcomes!

A-102
URETEROSCOPIC MANAGEMENT OF RENAL CALCULI: DO URETERAL ACCESS SHEATHS IMPROVE STONE-FREE RATES?

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Routine use of the new generation of ureteral access sheaths (UAS) has greatly facilitated upper tract access during flexible ureteroscopy. However, reports of stone-free rates while using UAS have been contradictory. We therefore evaluated our experience with the ureteroscopic management of renal calculi, specifically focusing on stone free rates with and without the use of a ureteral access sheath. A retrospective review of ureteroscopically managed renal stones between 1997 and 2002 was performed. Data from these procedures were entered into a dedicated database and queried for stone size, location, method of treatment, and overall efficacy. The use of adjunctive procedures, such as UAS insertion or stone re-positioning, is reported as well. Overall stone free rates were stratified by stone location and the use of the UAS. Stone free status was confirmed by either tomograms or non-contrasted renal CT. A total of 181 patients (103 males and 78 females) underwent 270 procedures for symptomatic renal calculi. Flexible ureteroscopy was used in 91% of the cases. UAS were used in 67% of the cases. The lower renal pole was the most common presenting location for kidney stones and required stone displacement with a nitinol basket 34% of the time. The majority of stones were effectively fragmented with a holmium laser, resulting in a 75% overall stone free rate. The stone free rates in the upper pole were 68% vs. 52%, in the mid pole were 84% vs. 79%, in the lower pole were 80% vs. 73%, and in the renal pelvis were 83% vs. 67% with and without the use of an

UAS, respectively. Overall, the stone free rate was 79% vs. 67% in the 2 different groups. In our experience, the ureteral access sheath is associated with significantly improved stone-free rates in all portions of the kidney. This finding can be explained by simplified repetitive access to the collecting system and improved irrigant flow with resultant washing out of small fragments.

A-103
APPROPRIATE MEDICAL TREATMENT AFTER PERCUTANEOUS NEPHROLITHOTOMY CAN CONTROL ACTIVE STONE DISEASE IN THE PRESENCE OF RESIDUAL CALCULI

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Second look nephroscopy is often performed after percutaneous nephrolithotomy (PNL) in an attempt to document a stone-free status, based on established clinical implications of residual stone fragments. However, the second look procedure involves inherent morbidity and increased costs. We evaluated the utility of aggressive medical management and its impact on residual fragments that might remain following PNL. We retrospectively evaluated 43 patients who had undergone PNL and metabolic evaluation and were placed on selective medical management of their stone disease. New stone formation was assessed by spontaneous stone passage in the absence of residual stone fragments, surgical removal of newly formed stones, stone passage without change in the number of residual fragments, or appearance of new stones or increase in size of stone or fragments noted on the abdominal radiograph. Patients were placed into 4 groups following PNL: stone-free or residual fragments, who remained on or discontinued medical therapy. Patients in all groups were similar with regards to age, sex and length of follow-up. Selective medical therapy significantly decreased stone recurrence in the stone-free and residual fragment groups (see table), as determined by the Wilcoxon rank sum test (* = $p < 0.05$). Moreover, remission rates were significantly higher in the medically treated patients. Our findings suggest that comprehensive metabolic evaluation and aggressive medical management can control active stone formation and growth in patients with or without residual stone fragments after PNL, thereby moderating the need for routine second-look procedures. Additional studies are in progress to investigate whether medical management is a cost-effective alternative to second-look nephroscopy.

A-104
SIMULTANEOUS COMBINED USE OF FLEXIBLE URETEROSCOPY AND PERCUTANEOUS NEPHROLITHOTOMY REDUCED THE NUMBER OF ACCESS TRACTS IN THE MANAGEMENT OF COMPLEX RENAL CALCLULI

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Complex and branched renal calculi occupying multiple calices often require multiple access tracts during percutaneous nephrolithotomy (PNL). We therefore evaluated the use of combination flexible ureteroscopy (URS) and PNL to decrease the number of percutaneous access tracts to manage complex renal calculi, under a single anesthetic. Patients who had multiple and branched renal stones of large volume scheduled for PNL were included in the study. Following general anesthesia and retrograde pyelography, a ureteral access sheath was used to facilitate passage of 7.5 F flexible uretero-renoscope into the collecting system. Stones in peripheral calyces that would have required a second or third nephrostomy access were fragmented with a holmium laser. Alternatively, a 2.4 F nitinol basket was used to transfer the calyceal stones into renal pelvis for easier PNL access. The patient was then placed into prone position for placement of a single tract percutaneous access tract. In 2 cases where there was an established percutaneous tract, the procedure was done in a synchronous manner. A combination of ultrasonic, pneumatic and Holmium laser contact lithotripsy was used to completely fragment the large volume portion of the stone, as well as the fragments that had been repositioned into the renal pelvis. Six patients (3 female and 3 male) with a mean age of 52 years (range, 34–67) underwent the combined approach. All patients had 2 or more stones in separate locations in the collecting system. The average stone volume was 410 mm^2. All patients had only one percutaneous access tract. The mean operative time was 148 minutes. There were no intraoperative complications. One patient had a small residual stone, which required a second ureteroscopic intervention. The combined use of flexible ureteroscopy and percutaneous nephrolithotomy effectively reduced number of percutaneous access tracts which would be otherwise required for the management of complex and branched renal calculi. This maneuver reduces operative time and patient morbidity, without having a significant impact on stone-free rates.

A-105
URETEROSCOPIC MANAGEMENT OF CALCULI IN ANOMALOUS KIDNEYS

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Shock wave lithotripsy (SWL) and percutaneous neprolithotripsy (PNL) are considered the standard of care for managing renal calculi in anomalous kidneys. However, the development of holmium laser lithotripsy, smaller actively deflectable flexible ureteroscopes, and nitinol baskets/graspers have allowed successful retrograde management of renal calculi in orthotopically positioned kidneys. We reviewed our experience with ureteroscopy (URS) in treating renal calculi in anomalous kidneys to evaluate the efficacy of this approach. 8 patients with renal calculi in anomalous kidneys undergoing URS were identified. Demographic information, preoperative stone burden, operative information (ureteroscope size, lithotrite utilized, instruments utilized, length of surgery, complications, stenting), follow-up imaging, and complications were obtained from the medical records. This information was analyzed to determine the most frequently used instruments and stone free rates. Our cohort consisted of four horseshoe kidneys (HSK) patients, 4 pelvic kidneys (PK) patients, and 6 males with a mean age of 50.6 years. Average pre-operative stone burden of 11 treated calculi was 1.4 cm with 5 located in the renal pelvis, 2 in the upper pole, and 4 in the lower pole. A 7.5 French flexible ureteroscope, holmium laser lithotripsy, and nitinol baskets/graspers were utilized in all patients. Six patients had complete clearance of the stone on post-operative imaging (75% HSK, 75% PK) with symptomatic relief and no additional interventions. One patient had a 6 mm residual fragment in a PK with resolution of symptoms being managed medically. The other patient had a 2 mm interpolar fragment in a HSK with ureteral stent placement by his local urologist despite normal split renal function and excretion on lasix renal scan. Flexible URS with holmium laser lithotripsy and utilization of nitinol baskets and graspers provides a reasonable alternative to SWL in the management of renal calculi in anomalous kidneys. In addition, URS can be considered prior to PNL in PKs.

A-106
INNOVATIONS IN SHOCK WAVE LITHOTRIPSY TECHNOLOGY

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Since its introduction in early 1980s, shock wave lithotripsy (SWL) has been used widely in clinic for the treatment of kidney

and upper urinary stones. Although a variety of methods have been developed for shock wave generation, coupling, and focusing, the core of SWL technology has not changed significantly. Recently, we have performed a series of investigations, aiming at innovations in SWL technology. Our strategy is to first better understand the mechanisms by which stone comminution and tissue injury are produced in SWL using various experimental and theoretical techniques. Based on this knowledge, we then developed novel techniques that can be used to optimize the effect of cavitation in SWL via modification of the waveform profile, pressure distribution, and pulse sequence of lithotriptergenerated shock waves. These new techniques have been upgraded on a Dornier HM-3 lithotripter, the gold standard in SWL. Both *in vitro* phantom and *in vivo* animal experiments have been carried out which demonstrate that the performance and safety of the upgraded HM-3 lithotripter is superior to the original HM-3 lithotripter. Finally, strategies to improve stone comminution efficiency while reducing tissue injury in SWL will be discussed.

A-107
SURGICAL TRENDS IN UROLITHIASIS

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Over the last 2 decades, minimally invasive surgical procedures have dominated the treatment of upper tract stones. However, endoscopic techniques and instrumentation have improved, and SWL indications have become more refined. Using several data sources, we examined changes in the number and distribution of surgical procedures for upper tract stones, including shock wave lithotripsy (SWL), ureteroscopy (URS), percutaneous nephrostolithotomy (PCNL) and open surgery. We analyzed data derived from 2 national datasets– the Center for Health Care Policy and Evaluation (a database of commercially insured individuals from 1994–2000) and The Centers for Medicare and Medicaid Services (CMS, 1992–1998)–to determine trends in the surgical management of stone disease. In the Medicare population with a diagnosis of urolithiasis, the rates of treatment for each of the minimally invasive treatment modalities (SWL, URS and PCNL) remained stable from 1992–1998. SWL rates varied from 10,942 to 11,738 per 100,000 population, URS ranged from 8372 to 8839 per 100,000 and PCNL varied from 665 to 882 per 100,000 population. In contrast, rates of open surgical treatment declined from 920 per 100,000 population in 1992 to 333 per 100,000 in 1998, representing a 64% reduction. Accordingly, the distribution of surgical procedures has changed little over the years of study for SWL, URS and PCNL; SWL comprised 51–54% of procedures from 1992–1998, URS comprised 40–41% and PCNL comprised 3–4%. Open surgery, however, dropped from 4.3% of procedures in 1992 to only 1.6% of procedures in 1998. A similar distribution of surgical procedures was identified among commercially insured individuals. SWL comprised 49% of procedures in 1994, increasing to 54% in 2000. URS and PCNL remained stable at 40–43% for URS and 5–6% for PCNL. Open surgery comprised 2% of procedure in 1994, dropping to less than 1% by 2000. SWL remains the most commonly performed surgical procedure for nephrolithiasis, followed closely by URS. PCNL has consistently comprised less than 10% of procedures. Open surgery, however, has shown a steady decline, such that it currently comprises well under 1% of surgical procedures for stones.

A-108
ENDOPYELOTOMY FOR THE TREATMENT OF PELVIURETERIC JUNCTION OBSTRUCTION

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Endourological techniques were proposed to overcome the disadvantages of an invasive procedure; large skin incision, postoperative pain and prolonged convalescence, inherent in traditional open surgery for the treatment of pelviureteric junction obstruction. We have reviewed our recent practice using these techniques and present our results. An audit of our endopyelotomy practice from 1999 to 2002 was carried out. The endopyelotomies were performed by urologists working in conjunction with interventional radiologists, at a single unit. The information was obtained from patient notes and radiological reports, including nuclear medicine and magnetic resonance imaging. A total of 18 patients, 11 males and 7 females were included. The mean age of presentation was 29 yrs 11 months (range of 7–56 yrs of age). The commonest presenting symptom was loin pain (15/18). 28% (5/18) of patients had previous open pyeloplasty. 22% (4/18) of endopyelotomies also combined percutaneous nephrolithotomy. Clayman stents were universally used at our hospital (94%). The mean period of follow up was 20.5 months. A successful outcome was defined as symptom resolution at first outpatient follow up post endopyelotomy. A success rate of 78% was achieved. 11/18 patients had a Magnetic Resonant Angiogram to assess the presence of aberrant renal vasculature. The presence of an aberrant renal artery correlates to a success rate of 80%. The absence of an aberrant vessel correlates to a success rate of 83%. The comparable success rate in the presence or absence of abnormal renal vessels at the pelviureteric junction implies that the presence of an aberrant vessel did not affect operative outcome. We found no overall strong predictor of operative outcome. Our results demonstrate a satisfactory success rate in comparison to other series.

A-109
MDCT (MULTIDETECTOR COMPUTER TOMOGRAPHY) - THE ESSENTIAL NAVIGATOR

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Planning endourological procedures requires knowledge of the anatomy of renal vessels and collecting systems. This can be obtained from conventional CT/IVU studies but in complex cases and repeat procedures it is essential to have more precise anatomical detail. MDCT allows multiple overlapping thin axial scans to be obtained through the body in very short periods of time (< 15 sec). This technique coupled with the use of IV contrast can be used to evaluate the renal vasculature, cortex and collecting systems. We present 6 cases in which MDCT images obtained prior to endourological procedures changed the technique or approach significantly. Cases include difficult PCNL (percutaneous nephrolithotomy), where identification of the relationship of stones to the pelvicalyceal system changed the puncture site or enabled a safer puncture site to be chosen. Other cases will demonstrate a '3D roadmap' to assist flexible ureterorenoscopy and identification of aberrant vessels prior to endopyelotomy. MDCT is a quick and accurate method of demonstrating 3D renal anatomy. It is an essential tool when planning endourological procedures in difficult kidneys.

A-110
IS STONE RADIODENSITY PREDICITIVE OF ESWL SUCCESS FOR STONES $\leq$ 2 CM WITHIN THE KIDNEY?

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It has been suggested that stone radiodensity, as determined either by plain radiography or computed tomography, may be an independent predictor of SWL success. We examined the outcome of ESWL for solitary stones less than or equal to 2 cm located

within the renal pelvis, based on its radiodensity (relative to the radiodensity of the 12th rib) on a plain radiograph of the kidneys, ureter, and bladder (KUB). Over a two-year period (1/98–12/99), 211 patients with solitary renal pelvic stones measuring less than or equal to 2 cm were treated on a Dornier Doli 50 lithotriptor under general anesthesia. On the preoperative KUB, the radiodensity of the stone was determined to be either less than, equal to, or greater than, the radiodensity of the ipsilateral 12th rib. Stone-free rates (SFR) were determined at 3 months by KUB. Patients requiring retreatment or auxiliary procedures were considered failures of ESWL. For stones < 10 mm within the renal pelvis, the SFRs were similar regardless of stone radiodensity. For stones between 11 and 20 mm, the SFR was 60% if the stone had a radiodensity $>$ 12th rib compared to a SFR of 71% if the stone radiodensity was $<$ 12th rib. However, these differences in SFRs were not statistically significant. On the Doli machine, stone radiodensity alone (as determined by a KUB) has limited ability to distinguish lithotripsy treatment outcome for stones ≤ 1 cm within the renal pelvis. However, for stones measuring between 1 and 2 cm, increased radiodensity is likely a predictor of worse outcome with ESWL.

A-111
IMPACT OF TREATMENT RATE ON THE EFFICACY OF ESWL FOR SOLITARY RENAL STONES BETWEEN 1 AND 2 CM

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We examined the effect of treatment rate on the efficacy of ESWL for solitary stones, 10 to 20 mm in size, within the kidney. Between May 2002 and August 2003, 94 patients with solitary, radioopaque, kidney stones between 10 and 20 mm in size, underwent ESWL on a Dornier Doli 50 lithotriptor under general anesthesia. Adequate follow-up data was available in 77 patients (82%). Of these 77 patients, 39 were treated at a rate between 70 and 80 shocks per minute (slow rate, SR). The other 38 patients were treated at 120 shocks per minute (fast rate, FR). Stone-free rates (SFR) were determined at approximately 1 month by KUB. Overall, 28 of 39 (72%) of the SR group and 20 of 38 (55%) of the FR group were found to be stone free on follow-up ($p < 0.05$, Chi Square analysis). When the renal stones were stratified as lower pole vs. non-lower pole, the SFR followed a similar pattern. For lower pole stones treated at the SR, 16 of 20 (80%) were stone free, whereas 7 of 11 (64%) that were treated at the FR were stone free ($p < 0.001$). For stones located elsewhere in the kidney, 13 of 19 (68%) and 13 of 27 (48%) were stone free in the SR and FR groups, respectively ($p < 0.05$). The mean stone size and power indices were not statistically significant between the SR and FR groups. Our study suggests that for solitary, medium sized stones within the kidney, a SR for ESWL results in better treatment outcomes compared to a FR for ESWL. This finding needs to be confirmed in a prospective, randomized clinical trial.

A-112
EVALUATION OF STONE RISK FACTORS IN THE PATIENTS OF UROLITHASIS WITH ESSENTIAL HYPERTENSION AND WITHOUT HYPERTENSION

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The pathogenetic link between hypertension and stone disease is not understood. To investigate the association, we evaluated the stone risk factors in the stone patients with essential hypertension and without hypertension. We have studied 88 consecutive patients with primary calcium nephrolithiasis, of whom 20 had essential hypertension. Hypertension was defined when diastolic blood pressure was more than 95 mmHg or being on drug therapy for hypertension. The patients had one fasting blood sample and a 24 h urine collection. Fasting blood samples were ananlysed for calcium, phosphorus, uric acid, creatinine, and PTH. Urine was analysed for sodium, calcium, phosphorus, uric, oxalate, citrate and total volume. Serum levels of calcium was decreased in the hypertensive group (9.025 mg/dl vs 9.362 md/dl). The other serum risk factors were not different in the two groups. Mean urine levels of sodium (257.9 meq/day vs 227.9 meq/day) and calcium (235.5 mg/day vs 222.9 mg/day) were higher in the hypertensive group compare to normotensive group. But those were not statistically significant. The other urinary risk factors were not different. The pathogenetic link between hypertension and stone disease might be in the calcium metabolism.

A-113
THE USEFULLNESS OF THE UNENHANCED HELICAL COMPUTERIZED TOMOGRAPHY IN PATIENTS WITH URINARY CALCULI

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This study was performed to ascertain the usefullness of the unenhanced computerized tomography in patients with urinary calculi. 72 patients with actue flank pain, suspected of having urinary calculi, underwent an unenhanced CT, followed by intravenous urography(IVU) within 24 hours. The enhanced CT and IVU were compaired for the presence and location of the urinary calculi and secondary signs of ureteral obstruction. 65 of the 72 patients had 74 urinary calculi. Unenhanced CT detected 72 urinary calculi with the sensitivity and specificity of 97.3% and 100% respectively, with 2.7% false negative rate. IVU detected 49 urinary calculi with the sensitivity and specificity of 63.5% and 80% repectively, with 36.5% false negative rate. For the secondary signs of ureteral obstruction, CT diagnosed 71.2% with hydronephrosis, 63.6% with hydroureterosis, 15.2% with strands of perinephric fat and 12.1% with strands of periureteric fat and IVU diagnosed 76.1% with hydronephrosis, 60.9% with hydroureterosis and 56.5% with delayed ureteral opacification. Besides the urinary calculi, CT revealed gall stones and renal cysts. The cost of the CT was about four times higher than that of IVU. In conclusion, Unenhance CT is more accurate, safe and rapid technique for the assessment of urinary stones.

A-114
PREDICTING FACTORS OF STONE CLEARANCE IN LOWER CALYX BY SHOCK WAVE LITHOTRIPSY (SWL)

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To identify different variables that may influence the outcome of ESWL for the treatment of lower calyceal stones to help in selecting patients that were likely to benefit from the treatment. Materials and Methods: Between November 2001 and June 2003, 130 patients and 134 renal units with lower calyceal stones were treated with Shock Wave Lithotripsy (SWL) on Dornier HM4 and DL-50 Lithotripters. The size, number and area of calculi, length and width of the lower calyx and infundibulopelvic angle were measured on intravenous urography (IVU). This study revealed that calyceal anatomy has a significant role in determining the stone-free rate following satisfactory fragmentation of stone with ESWL. Thus using these radiographic parameters ESWL can be selected as a treatment modality for a predictably favourable outcome in individual cases.

A-115
MONOTHERAPY EXTRACORPOREAL SHOCKWAVE LITHOTRIPSY (ESWL) FOR THE TREATMENT OF PARTIAL STAGHORN STONES: A SINGLE CENTRE EXPERIENCE

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We evaluate the efficacy of extracorporeal shockwave lithotripsy (ESWL) for partial staghorn calculi. Material and Methods: Between January 1990 and December 2002, 13428 patients of urinary tract stone disease were treated by ESWL. Of the cases, 814 (6.1%) were partial staghorn stones. A partial staghorn stone was defined as one that filled renal pelvis plus one or two calyces on X-ray IVU. Patients were treated on Dornier HM4 and DL 50 lithotriptors in a day care setup. Each treatment consisted of 3000 shockwaves on 20 to 22 kv on Dornier HM4 and power of 40–60 on DL50 machine. A subsequent session was performed after 2–4 weeks. Treatment end points were a stone free kidney or fragments less than 3 mm in size. Patients were followed up in stone clinic after every 2 weeks time with plain x-ray abdomen. Ultrasonography was performed to exclude obstruction in events of pain and fever. The status free of stone was decided on plain x-ray abdomen and ultrasonography. This retrospective analysis suggests that ESWL monotherapy of partial staghorn stones has suboptimal stone free rate, however stones with non dilated pelvicalyceal system and stones with single calyceal involvement can be treated by ESWL with reasonable outcome.

H. NUTRITION, DIETARY RISK FACTORS AND WATER INTAKE

A-116
EFFECT OF SUPPLEMENTATION WITH FISH OIL CONCENTRATE ON URINARY OXALATE EXCRETION IN HEALTHY INDIVIDUALS

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High concentrations of eicosapentaenoic (EPA) and docosahexaenic acid (DHA) present in fish and fish oil are suggested to reduce urinary calcium and oxalate excretion in stone formers. The aim of the study in healthy individuals was to evaluate the physiological effects of supplementation with fish oil concentrate under standardized conditions. Fifteen healthy subjects, 7 women and 8 men (mean age: 28.1 ± 4.5 years) participated in a study lasting 35 days. The subjects were studied initially while consuming a standard diet for 5 days (control phase). During the following phases I (standard diet, 5 days), II (free diet, 20 days) and III (standard diet, 5 days) of intervention, the participants were administered the fish oil concentrate thrice daily, corresponding to approximately 900 mg/d EPA and 600 mg/d DHA. During ingestion of the standard diets, daily 24-hour urines were collected from each subject. Urinary parameters on the last day of each standard diet were compared. The long-term supplementation with fish oil concentrate to healthy individuals is effective in reducing urinary oxalic acid excretion and the risk of calcium oxalate crystallization. As mechanism for the physiological effect a decreased cellular oxalic acid exchange is discussed, which is attributed to an altered fatty acid pattern of membrane phospholipids and concomitant alterations in protein kinase activity and band 3 phosphorylation. Dietary fish oil supplementation may benefit calcium oxalate stone formers.

A-117
OXALATE CONTENT IN CEREALS AND CEREAL PRODUCTS

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Detailed knowledge of food oxalate content is of essential importance for dietary treatment of recurrent calcium oxalate urolithiasis. The consumption of foodstuffs rich in oxalic acid can induce hyperoxaluria already in healthy individuals without disturbances in oxalate metabolism. The oxalate content of cereals and cereal products, which play an important role in daily nutrition, has not yet been analyzed. The soluble and insoluble oxalate content of about 50 cereals, cereal products and other mill products was analyzed by a recently developed selective and sensitive method for the determination of oxalate in foods using high-pressure liquid chromatography (HPLC)-enzyme-reactor. This method combines enzymatic conversion and chromatographic separation with amperometrical detection of oxalate. The higher oxalate content in whole-grain than refined-grain cereals suggests that oxalic acid is primarily located in the outer layers of grains. Cereals and cereal products contribute to the daily oxalate intake to a considerable extent. Vegetarian diets may contain high amounts of oxalate when whole-grain wheat and other mill products are ingested. Recommendations for prevention of recurrence in calcium oxalate stone disease have to take into account the oxalate content of these foodstuffs.

A-118
EFFICACY OF DIETARY TREATMENT OF CALCIUM OXALATE STONE PATIENTS WITH ILEAL RESECTION

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The aim of the present case-control trial was to evaluate the efficacy of dietary treatment of calcium oxalate stone patients with ileal resection. The case group was limited to 12 recurrent calcium oxalate stone formers, 10 men and 2 women, with ileal resection. The control group consisted of 12 age-, sex- and BMI-matched patients with idiopathic calcium oxalate urolithiasis. The patients received a standardized balanced mixed diet according to the recommendations for calcium oxalate stone patients for a period of 7 days. The 24-hour urine collection was performed after 7 days on the usual diet and after 7 days on the standard diet. The ingestion of the balanced standardized diet resulted in a significant reduction in the risk of calcium oxalate stone formation by 47% in patients with ileal resection and by 39% in the control group, due to the improvement of urinary risk profiles compared with the usual diet. Nevertheless, calcium oxalate stone patients with ileal resection showed a lower urinary calcium ($p < 0.001$), magnesium ($p < 0.001$) and citrate excretion ($p = 0.001$) and a higher oxalate excretion ($p < 0.001$) than patients with idiopathic calcium oxalate urolithiasis. Accordingly, the stoneforming potential on the standard diet was higher by 56% in patients with ileal resection than in the control group ($p = 0.045$). Oxalate and calcium hyperabsorption were diagnosed in 91% and 0% of patients with ileal resection and in 36% and 67% of the control group, respectively. A nutritionally balanced diet according to the recommendations for calcium oxalate stone formers may favorably influence urinary risk profile and the stone-forming potential in calcium oxalate stone patients with ileum resection.

A-119
ALKALI LAODING AFTER EATING DURIAN

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Durian contains a considerate amount of potassium (520 mg/100 g) and some citrate (285 mg/100 g) and magnesium (32 mg/100 g) but little calcium (5 mg/100 g). Ten normal subjects

(6 males) volunteered to eat durian twice daily (adlibitum), at breakfast and dinner, for four days. The 24-h urine was collected before and on days 3 (D3) and 4 (D4) during the durian-eating period for analysis of pH, potassium, magnesium, calcium and citrate. Significant increases were observed in urinary pH (D3 & D4, $p < 0.001$), potassium (D3, $p < 0.001$; D4, $p < 0.0001$) and citrate (D3 & D4, $P < 0.002$). While urinary magnesium was unchanged, urinary calcium decreased significantly by D4 ($p < 0.01$). These preliminary results suggest that eating durian may provide an alkali load similar to a potassium citrate drug, making it an alternative management for hypocitraturia and uric acid nephrolithiasis.

A-120
GUT COLONISATION OF OXALATE-DEGRADING BACTERIA IN SOUTH AFRICAN BLACKS AND WHITES

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The objective of the present study was to investigate the colonization of the gastrointestinal oxalate-degrading bacteria in South African black and white subjects, in order to establish whether these bacteria may play a role in the low incidence of stone formation in the black race group. Participants ($n = 10$ for each group) provided a 24-hr urine sample and a stool sample on their free selected diets. Urines were analysed using standard biochemical techniques. Stools were analysed by selective media plates. Two-day food diaries and semi-quantitative food questionnaires were completed. Black subjects were found to have significantly higher counts of oxalate-degrading bacteria relative to whites ($p = 0.01$). Oxalate-degrading bacteria isolated from blacks utilized significantly more oxalate relative to healthy whites ($p = 0.001$). Black subjects consumed a diet significantly higher in oxalate and significantly lower in calcium ($p = 0.01$) and magnesium ($p = 0.01$) compared to white subjects. Despite their hyperoxaluric diet there was no difference in urinary oxalate ($p = 0.95$) between healthy black and white subjects. The greater gastrointestinal occurrence of oxalate-degrading bacteria in blacks is a highly significant finding which could be an important contributing factor to the low frequency of stone formation in this race group.

A-121
INFLUENCE OF HIGH OXALATE DIET ON INTESTINALE OXALATE ABSORPTION AND EXCRETION

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Hyperoxaluria is a risk factor for renal stones and the urine load of oxalate plays a central role in calcium oxalate stone formation, even in normocalciuric patients. In normal individuals, the majority of urinary oxalate is derived from the endogenous metabolism of glycine, glyoxylate and ascorbic acid, 10 to 20% is derived form oral ingestion. The small intestine is the major site of oxalate absorption with 7.9% of dietary oxalate being absorbed under normal conditions with 800 mg Calcium per day [1]. An increased intestinal absorption of oxalate may lead to hyperoxaluria with a significantly enhanced risk of urinary stone formation. Method: 25 healthy volunteers participated in this study. The volunteers carried out the [$^{13}C_2$]oxalate absorption test once under standardized conditions [2] with low oxalate (63 mg oxalate/d) and once with high oxalate (600 mg oxalate from spinach). For each [$^{13}C_2$]oxalate absorption test, the volunteers had to adhere to an identical diet for two days and collected their 24 h urines. In the morning of the second day, a capsule containing [$^{13}C_2$]oxalate was ingested. Results: The mean oxalate excretion of all volunteers under low oxalate diet was 0.292 ± 0.106 mmol/d and it increased under 600 mg oxalate to 0.485 ± 0.098 mmol/d. The mean intestinal oxalate absorption of all volunteers (with low oxalate) was $7.8 \pm 3.9\%$. Due to the diet enrichment with oxalate rich food, the oxalate absorption was almost twice as high ($13.7 \pm 6.3\%$). Discussion: The oxalate excretion increased under the oxalate rich diet by approximately 66% and the absorption by approximately 76%. The oxalate rich diet resulted not only in an increase of the oxalate excretion in the urine, but also in an increase of the intestinal oxalate absorption under constant calcium intake. These short time increases caused by the high intake of oxalate are not dramatic for healthy persons, but for calcium oxalate patients the crystallization risk is considerably enhanced.

A-122
PREVENTION OF MICROGRAVITY-INDUCED RENAL STONE RISK BY POTASSIUM-MAGNESIUM-CITRATE (KMGCITRATE)

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Increased risk of renal stone formation is known to occur during spaceflight. Hypercalciuria, secondary to increased bone resorption, contributes to the increased risk as well as other nutritional and environmental contributions during spaceflight. While prevention of bone loss and increased fluid intake might avert increased risk for renal stones, no such countermeasure is currently in use. We have proposed that provision of alkali therapy as a dietary supplement would be an effective countermeasure against microgravity-induced renal stone risk. This hypothesis is currently being tested for KMgCitrate in a double-blind, placebo-controlled randomized trial in 20 normal subjects. We are using an Earth-based model of skeletal unloading entailing 5 weeks of strict bed rest. Fifteen subjects have successfully completed the study (8 on KMgCitrate and 7 on matching placebo). We have performed an interim analysis, comparing the urinary values observed during the last week of bed rest to the baseline (ambulatory) evaluation, while maintaining the blindness. As expected, KMgCitrate significantly increased urinary pH and the net GI absorption of alkali. Although urinary calcium increased by about 55 mg/d in both groups, the relative saturation of calcium oxalate was significantly lower in the KMgCitrate group with no difference for brushite. Undissociated uric acid solubility was also increased by KMgCitrate. If confirmed at study completion in all 20 subjects, these preliminary findings support the use of KMgCitrate as an effective countermeasure against microgravity-induced renal stone risk.

A-123
URINARY RESPONSES TO A CONTROLLED METABOLIC DIET

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Calcium oxalate stone formation is due to an interaction between environmental and genetic factors. Metabolic studies are undertaken for the study of both of these domains. These frequently involve placing patients on controlled diets. The appropriate length of lead-in time on these diets has not been adequately defined. We undertook this study to address this issue. Eleven healthy, non-stone forming adults (5 men, 6 women, mean age 26.9 years) collected daily 24- hour urine collections while consuming a self-selected diet (SS) for 4 days and a controlled, metabolic diet (CM) for 10 consecutive days. The CMD consisted of 20 % protein, 30% fat, and 50% carbohydrate and contained 250 mg oxalate/2500 kcal and 1002 mg calcium/2500 kcal. The following urinary analytes were measured and indexed to urinary creatinine

excretion: calcium, citrate, magnesium, oxalate, phosphate, potassium, sodium, urea nitrogen, and uric acid. There are statistically significant differences in oxalate, magnesium, potassium, sodium, and urea nitrogen excretion when comparing the SS diet to the CM diet. The coefficients of variability for calcium ($p < 0.01$) and citrate ($p < 0.01$) excretion on the CM diet was significantly less than on the SS diet. This also approached statistical significance for oxalate excretion. Rapid stabilization of urinary excretion of all metabolites occurs on the CM diet: equilibration on day 1 for oxalate, calcium, citrate, magnesium, potassium, uric acid, phosphate; equilibration by day 2 for sodium, urea nitrogen. Although undefined environmental or genetic factors influence the absorption and excretion of the major factors associated with calculogenesis, intra-individual variability in non-stone formers is low on a controlled diet, inter-individual variability is present on a controlled diet, possibly due to genetic factors, and urinary stabilization is rapid. Lead-in times for metabolic studies using controlled diets can be limited in non-stone formers. Further similar investigations on stone formers need to be undertaken.

A-124
REDUCED BONE MINERAL DENSITY IN IDIOPATHIC CALCIUM NEPHROLITHIASIS

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A reduced bone mineral density (BMD) in idiopathic calcium nephrolithiasis has well known from almost 20 years. In order to evaluate bone involvement in idiopathic calcium nephrolithiasic patients we have determined bone mineral density as well as its relationship with urinary excretion of calcium, citrate and parameters involved in the dietary habits. We consecutively enrolled 51 patients with idiopathic calcium nephrolithiasis (21 males and 30 females, mean age 45 +/– 12 and 48 +/– 12 respectively) while they ate their customer diet. Daily urinary excretion of calcium, citrate, nutrient intake parameters (urea, sodium, phosphate) and urinary pH on morning urine sample were calculated. Bone mineral density on the lumbar spine and neck was mesaured using dual energy x-ray absorpiometry (Lunar DPX) and was expressed as T score according WHO classification. Statistical analysis were evaluated with linear regression. Our results confirm that osteopenia and osteoporosis are common in idiopathic calcium nephrolitiasis. Dietary habits seem to influence bone mineral density, confirming their role in the pathogenesi of nephrolithiasis. Osteopenia and osteoporosis occur in nephrolithiasic patients with or without hypercalciuria. A correlation between bone mineral density and urinary excretion of citrate could support the protective role of citrate not only in the nephrolithiasis, but also in the prevention of loss bone mass.

A-125
THE EFFECT OF DIETARY ADVICE ON THE RECURRENCE OF RENAL STONES

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To investigate further the recurrence rate in relation to the compliance to dietary advice we reviewed our clinical records of patients who were followed up in our stone clinic after a stone episode 6 to 15 years ago. At the first follow up visit after the passage or treatment of the stone patient's data were recorded and serum and 24-hour urine samples were taken for metabolic evaluation. All patients were offered conservative measures comprised a high fluid intake (> 1.5 lt) and avoidance of excessive dietary protein. The subsequent stone history was evaluated in a follow-up study. In all 245 patients were followed successfully. Out of them, 139 patients (57%) experienced stone recurrence after a mean of 5.3 +/– 4.2 years. A total of 195 patients (80%) stated that they were able to increase their fluid intake in the follow up period, whereas only 108 complied to dietary measures. Surprisingly only 25% of our patients stated to be on the prescribed low protein diet whereas most of them (40%) were on a low calcium diet owing to the advice of general practitioners and medias. The comparison of recurrent and not recurrent patients showed that recurrence was not influenced by selfestimated fluid intake and dietary advice. Compliance to dietary advice seems to be crucial in order to reproduce the results obtained in clinical controlled trials, but unfortunately it is time consuming and requires excessive economic resources for the general urologic practice.

A-126
DIETARY ACID LOAD IN CALCIUM RENAL STONE FORMERS

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The aim of this study was to investigate the influence of diet on urinary risk factors for renal stone formation. The present series comprises 187 renal calcium renal stone formers (RSFs) and 170 controls (Cs) chosen at random from the records of a general practitioner.Each partecipant was subjected to a investigation including 24-hour dietary record and 24-hour urine. Nutrients, calories and dietary potential renal acid load of were calculated using a computerised procedure.The mean daily potential renal acid load was higher in RSFs than in Cs (21.0 +/– 22.9 vs 15.6 +/– 18.3 mEq/day, $p = 0.016$). Only in RSFs a negative correlation (– 0.18) was found between daily PRAL and daily urinary citrate ($p < 0.01$). The regression analysis confirmed the negative correlation between PRAL and urinary citrate in RSFs ($p = 0.006$), whereas in Cs a less significant negative correlation between PRAL and urinary magnesium ($p = 0.021$), potassium ($p = 0.013$) and pH ($p = 0.027$) was shown. Fruits, fruit juices, vegetables and alkali-rich low phosphorus beverages have the lowest PRAL, whereas fish, meats and cheeses the highest. Using this model we demonstrated that urinary citrate excretion of RSFs is dependant from dietary acid load. The absence of this correlation in Cs could be explained by a genetical predisposition of RSFs to the effect of dietary acid load on citrate metabolism.

A-127
DIETETIC EVALUATION OF URINARY STONE PATIENTS – A NEW CONCEPT

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The paper reports the use of an Oracle based dietetic evaluation program for assessing the dietetic intake of stone formation. We had earlier created a DOS based computer program for assessing the dietetic ingredients in the stone patients. But since the DOS based programs have become outmoded, it became necessary to create a Windows based dietary evaluation program. This program was created and all the nutrients contained in the foodstuff were incorporated in to the program. Similarly the foodstuffs that are incorporated in each meal items were also organized in the program. After the program was organized the intake of food as told by the patient in layman's language was entered in the computer and the computer was organized to calculate the nutrients namely calcium, carbohydrates, carotene, chloride, citrate, copper, energy, fat, fibre, iron, magnesium, minerals, moisture, niacin, oxalic acid, phosphorus, potassium, protein, riboflavin, sodium, sulphur, thiamine, uric acid, vitamin C and water. 200 patients were studied using the new program and the intake of nutrients was quantified. The oxalate intake among stone patients

ranged from 10 to 615 mg/day. The calcium intake among stone patients ranged from 50 to 2060 mg/day. The uric acid intake among stone patients ranged from 5 to 400 mg/day. The citrate intake among stone patients ranged from 10 to 700 mg/day. The intake and output of calcium showed linear correlation only with intake ranges above 2000 mg/day. The uric acid, oxalate and citric acid intake correlated directly with the output values. With add on facility, the program was found effective in the follow up study. It was found that the Oracle based program is very user friendly, scientific in calculation and fast in reproduction of results.

A-128
DIET AND RENAL LEAKS IN THE PATHOGENESIS OF CALCIUM NEPHROLITHIASIS – AN INTEGRATED MODEL

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We examined the relationship between dietary composition, metabolic activity and the renal handling of stone-forming ions in 120 idiopathic Ca and/or UA stone-formers. Fasting blood and urine samples, two 24 h urines and a diet history were taken from each patient on a free home diet. The data show that 25% had a renal leak of Ca, 12% a renal leak of PO_4 and 9% a renal leak of UA. A total of 47% had hypercalciuria, 29% hypocitraturia, 28% mild hyperoxaluria, 30% hyperuricosuria, 27% hypernatriuria, 13% hypomagnesiuria, 11% a 24-h urinary pH < 5.5, 23% a urinary pH > 6.5 and 27% a urine volume < 1.4 litre/day. The risk of forming stones (P_{SF}) was high in 75% of the patients and normal in 21% because they had changed to a high fluid intake. Dietary intake was high in protein in 33% of patients, sodium (27%), oxalate (25%) and calcium (21%) and intake was low in fluid (17%), potassium (29%) and magnesium (17%). High sodium intake $\rightarrow \uparrow$ fasting Na/Cr ratio $\downarrow$ notional Tm_{Ca}/GFR (renal leak of calcium) $\rightarrow \downarrow$ plasma Ca $\rightarrow \uparrow$ PTH $\rightarrow \downarrow$ TmP/GFR (renal leak of phosphate) and $\uparrow$ intestinal absorption of Ca $\rightarrow$ hypercalciuria. High protein intake $\rightarrow \uparrow$ fasting urinary urea/Cr $\rightarrow \uparrow$ FE UA(%) (renal leak of uric acid) and coupled with the parallel high purine intake $\rightarrow$ hyperuricosuria. Low potassium intake $\rightarrow \downarrow$ urinary potassium and $\downarrow$ fasting urinary pH which together $\rightarrow$ hypocitraturia. High dietary oxalate $\rightarrow$ mild hyperoxaluria and low dietary magnesium and low fluid intake $\rightarrow$ hypomagnesiuria and low urine volume respectively. We conclude that dietary and metabolic factors are linked to cause an increase in the risk of calcium stones.

A-129
DIETARY FACTORS AND THE RISK OF INCIDENT KIDNEY STONES IN MEN: NEW INSIGHTS AFTER 14 YEARS OF FOLLOW-UP

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Evidence suggests that the pathophysiology of nephrolithiasis may change with increasing age. In addition, recent metabolic trials suggest that the ingestion of large amounts of vitamin C results in increased urinary oxalate excretion. In the last ten years, as the participants in the Health Professionals Follow-up Study have aged, the number of incident kidney stones in this cohort has nearly tripled. This allows us the opportunity to examine the effects of age on dietary risk factors for nephrolithiasis, and provides the statistical power to reevaluate the previously null association between dietary vitamin C and the risk of stone formation. We conducted a prospective study of the relation between the intake of dietary factors and the risk of symptomatic kidney stones in 45,619 men without a prior history of nephrolithiasis. Nutrient and vitamin consumption was ascertained every four years by means of a semiquantitative food-frequency questionnaire. During 477,732 person years of follow-up, 1473 cases of kidney stones were documented. The results suggests the importance of individual dietary factors on the risk of developing kidney stones.

A-130
BODY SIZE AND THE RISK OF KIDNEY STONES

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Increased body size has been associated with an increased risk of incident kidney stone formation. Larger body size may result in increased urinary excretion of oxalate and uric acid, risk factors for calcium stones. To clarify which measures of body size best predict the risk of stone formation, we prospectively studied 45,619 men without a prior history of nephrolithiasis. Estimates of body size were obtained by height and updated measures of weight and waist and hip circumference. Body-mass index (BMI), lean body mass (LBM), and body surface area (BSA) were calculated using standard formulas. We adjusted our analysis for age, thiazide diuretic use, the intake of supplemental calcium, and dietary fluid, alcohol, calcium, sodium, potassium, magnesium, vitamin C, and animal protein. During 477,732 person-years of follow-up, 1473 cases of kidney stones were documented. Increasing body size was associated with kidney stone formation. The relative risk of kidney stones in men over 91 kg compared to men less than 64 kg was 1.47 (95% confidence interval 1.00–2.17). The relative risk in men with a BSA of 2.3 m^2 or more compared to men with a BSA of less than 1.7 m^2 was 1.78 (1.05–3.02). The risk in men with a BMI less than 21 kg/m^2 compared to men with BMI between 21 and 23 kg/m^2 was 0.61 (0.42–0.89); however further increases in BMI were not significantly predictive of stone formation. Increases in LBM were not associated with increased risk. Waist size was associated with kidney stone formation but hip size was not. Men with a waist circumference of greater than 109 cm compared to those with a waist less than 86 cm had a relative risk of 1.79 (1.12–2.87). Waist size was significantly predictive of risk even after adjusting for body weight. Waist to hip ratio was not associated with risk. Increased central body fat, rather than increased lean body mass, may be largely responsible for the association between body size and kidney stone formation. Further research is needed to clarify this issue.

A-131
THE INFLUENCE OF ALCOHOL CONSUMPTION OF THE RISK OF KIDNEY STONES

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There are some reports that discuss the relation between alcoholic beverages and kidney stone, however it is still in dispute. We studied the effects of alcoholic beverages on change in urine composition in rat. Male Wister rates were fed nutritionally adequate liquid diet for 4 or 8 weeks in which ethanol provided 5% of liquid. Pair-fed littermates consumed the same diet that an isocaloric amount of carbohydrate was substituted for ethanol. 24-hour urine collection was performed before feeding ethanol, and after 4 or 8 weeks. The analysis of each 24-hour urine specimen include urine volume, osmolarity, pH, and excretion of the following ionic components: calcium, phosphate, uric acid, urea nitrogen, sodium, potassium, magnesium, citrate and oxalic acid. Urine volume was low and osomlarity was high in rats, which fed ethanol. Urinary citrate was significantly lower in rats with ethanol than control rats. There were no statistical differences in other metabolic substances between rats with ethanol and control rats. It is suggested that the risk of stone formation increase with the consumption of alcohol, which conducts low urinary volume, high urinary osomlarity, and hypocitraturia.

I. EPIDEMIOLOGY AND ECONOMICS

A-132
A COMPREHENSIVE PROGRAM FOR THE MULTI-CENTRE SCREENING OF PATIENTS WITH UROLITHIASIS

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One of the most useful tools for the screening of stone patients throughout the world would be an agreed, standardised system for recording data. This would allow the comparison of data from different parts of the world and at the same time create the possibility of establishing a unique worldwide database for the statistical analysis of the factors known to be relevant to the formation of stones. This paper describes a possible model that has been tried and tested over a 7-year period at University College London. The database is set up in Excel and includes on each patient demographic information on age, sex, age-at-onset of stone-formation, occupation, data on medical history, urinary tract infection, surgical history, drug treatment(s), family history of stones and other medical disorders, stone episode history, quantitative stone composition, blood and spot urine analysis for metabolic evaluation, a 7-day Diet Diary completed by the patients on their free home diet, two 24-h urine analyses collected on days 6 and 7 of the Diet Diary period, automatic calculation of the biochemical risk of forming various types of stone and a summary of the patient diagnosis. The database also includes the automatic calculation of various renal parameters of tubular reabsorption and excretion, creatinine clearance, body mass index and surface area, and a calculated prediction of 24-h urine pH based on dietary composition. Finally, the program contains an automatic print-out of a summary of the patient data for inclusion in Case Notes, a set of graphs predicting the best way to approach the treatment of the patient and a set of Target Diagrams for the patients to try and motivate them to adhere to their particular form of prophylaxis.

A-133
PREDICTORS OF OUTCOMES AFTER RADICAL PROSTATECTOMY: A LOCAL PROSPECTIVE

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Detectable serum PSA develops in approximately third of the patients after radical prostatectomy. This study looks at whether pre-operative PSA and the histology findings could predict biochemical failure (BF) after radical Prostatectomy. Radical prostatectomies performed between 1993 and 1995 were retrospectively reviewed. Data was collected from a dedicated database. Missing or conflicting data was checked and updated. BF was defined as PSA > 0.4 and rising. Logistic regression was used to assess association between patients' characteristics and biochemical failure. 115 patients were identified, mean age 65 ± 5.7 years with median preoperative PSA 11. The median length of follow up was 7 years. The median survival was 9.3 years. Over the follow up period 47 (41%) patients had a BF. The cancer being prostate and specimen confined was protective against BF. There was no association between age and biochemical failure. Pre-operative PSA was associated with increased risk [OR 2.4 95% CI (1.1, 5.3), $p = 0.034$] as was seminal vesicle and vascular or perineural invasion [OR 5.7 95% CI (2.0, 15.9), $p = 0.001$ and OR 2.2 95% CI (1.00, 4.7), $p = 0.048$ respectively]. An increase in Gleason score was associated with a 7% increase risk of BF [OR 1.7 95% CI (1.2, 2.3), $p = 0.001$]. The findings of this study agree with international experience which showed that PSA and histology readings are good predictors of BF, but prostate confinement was the only significant predictor of long term BF.

A-134
CLINICAL IMPLICATIONS OF ABUNDANT CALCIUM PHOSPHATE (CAP) IN ROUTINELY ANALYZED STONES

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CaP is often found admixed with calcium oxalate (CaOx) in stones, and sometimes as the major stone component. Stone formers (SF) whose main stone phase is CaP may differ clinically from those with predominantly CaOx stones. We calculated average % CaP (CaP%) in all stones of 1201 SF, and classified them as CaOx ($n = 1011$) or CaP ($n = 190$) SF. We divided CaP SF into apatite (AP) or brushite (BR) SF. We compared clinical and laboratory data in these patients. CaP% in stones has risen threefold over 3 decades, and is higher in women. AP stones are more common in women, BR in men. Lithotripsy (ESWL) rates adjusted for number of stones and duration of disease were higher in CaP SF (0.6 vs 1.9 and 0.7 vs 1.8, CaOx vs CaP, male and female, $p < 0.001$) and in BR SF (2.9 vs 1.0 and 3.1 vs 1.4, BR vs AP, male and female, $p < 0.001$). CaP% rose with the number of ESWL/patient. Urine pH and CaP supersaturation rose in proportion to CaP%. Relapse rates of CaP and CaOx SF on treatment did not differ. Stone CaP% has risen for three decades. CaP SF, especially those with BR stones, have more ESWL treatments than CaOx SF, not explained by stone number or duration. CaP and CaOx SF respond equally well to preventive therapy.

A-135
CHANGE IN DEMOGRAPHY OF STONE DISEASE OVER FOUR DECADES

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In this paper the incidence of stone disease in Kerala has been compared from 1960 to 2000. The incidence rate has been taken from the records of the Medical College Hospital and the SAT Hospital Trivandrum. The incidence of stone disease has increased over the years (2.53 times from 1960s to 2000s). The increase in incidence of paediatric urolithiasis has been far more than the increase in incidence in adult stone disease (3.1 times). The incidence of female urinary stone has also been very much more than the increase in the incidence of male stone disease (0.8% different). The percentage of patients with upper urinary trust stones has been increasing over the years (68 to 79%). The percentage of patients with bladder stone has been decreasing over the years in the adults. In children bladder stone have been very low in incidence even in the 1960's. It continues to be low even now. The incidence of some metabolic abnormalities like hypercalciuria (13 to 7%) and hyperuricemia (13 to 10.5%) has come down, whereas some like hyperoxaluria (53 to 70.5%), hyperuricosuria (38 to 67.4%), hypocitraturia (29 to 44%), hypomagnesuria (9 to 23%) and hyper calcaemia (5 to 5.6%) has been increasing over the years.. The incidence of stone disease was most in the 20–30 years age group in the 1960's and the same was followed in all the subsequent decades (37% and 37.9%). The increased percentage of recognized biochemical abnormalities might be attributed to the increased investigative modalities for the stone disease in the stone clinic set up, the change in dietary habits and economic improvement particularly related to emigration of many people to the Gulf countries.

A-136
GENETIC PREDISPOSITION IN UROLITHIASIS

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This paper presents the findings of the biochemical study of patients with positive family history of stone disease. This is part

of an ongoing study meant to find out the possible genetic abnormalities of familial urolithiasis. The study population consisted of 200 patients with proved urolithiasis and who were having a positive family history. The positive family history was classified as Group 1 - First order single (one person in the immediate family - father, mother, siblings or children), Group 2 - First order multiple (more than one member in the above group), Group 3 -Second order single (one person in the blood relatives in family grandparents, grand children, uncles, aunts, cousins etc) and Group 4 - Second order multiple (more than one member in the above group). The selected patients underwent a detailed history, clinical examination and biochemical analysis. The findings were compared with a control stone population of 200 without positive family history. Blood and urine samples of some affected family members were also included in the study group. Efforts are on to study the chromosomal pattern and genome. It was found that 21% of patients had a positive family history of stone disease. 8% belonged to Group 1, 5% to group 2, 5% to group 3 and 3% to group 4. On comparing the biochemical parameters between the stone patients with positive family history and those without, it was observed that the risk factors of stone disease namely urine uric acid and urine oxalate were higher in the patients with positive family history. The urinary citrate level was lower in the positive family history group. Among the four groups with positive family history, the risk factors were more in the immediate family groups 1 and 2, compared to the blood relative groups 3 and 4. No distinct differences were forthcoming between the groups with single involvement and multiple family involvements. Further studies are expected to find out the possible chromosomal anomalies responsible for familial urolithiasis.

A-137
ECONOMIC IMPACT OF UROLITHIASIS

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The prevalence of stone disease is approximately 10%. Considering the cost of emergency treatment, surgery, medical evaluation and prophylaxis, the financial burden of stone disease is substantial. We examined the economic impact of urolithiasis in the U.S. from the payer point of view. We analyzed cost data derived from a variety of national datasets, including Ingenix, a database of claims from 75 large employers (1999), National Ambulatory Medical Care Surgery (NAMCS, 1992–2000), National Hospital Ambulatory Care Medical Survey (NHAMCS, 1994–2000), Health Care Utilization Project (HCUP, 1994–2000), Marketscan, a database of 100 health plans serving the Fortune 500 companies (1999) and Medical Expenditure Panel Survey (MEPS, 1996–1998), to determine trends in the total amount and distribution of medical expenditures in the last decade. From 1994 to 2000, the total cost of urolithiasis, excluding the cost of outpatient prescription drugs, increased by 50% from $1.37 billion to $2.07. The proportion of total expenditures attributed to outpatient services increased from 43% in 1994 to 53% in 2000. Likewise, among Medicare beneficiaries age 65 and older, total expenditures increased by 36%, from $613 million in 1992 to $834 million in 1998, with outpatient services assuming a greater relative proportion of the total (31% to 38%, respectively). For privately insured individuals, the average annual expenditure for those with a medical claim related to urolithiasis was $7656 in 1999, which exceeded the average annual expenditure of those without a urolithiasis claim by $4472. The total medical expenditure for those with a urolithiasis claim was highest among the 45–54 year age group, which likely reflects the peak incidence of stone disease in this group. Of the $7656 annual expenditure for the group, medical care comprised 85% and prescription drugs 15% of the total. Among individuals filing a claim for a diagnosis of urolithiasis, 30% of individuals missed workdays in association with their claim, with an average 2.5 days of missed work. The total annual cost of urolithiasis is over $2 billion and rising. A shift in expenditures from inpatient to outpatient treatment reflects the less invasive approaches to surgical management of stones and fewer inpatient admissions for stone management. The increasing expenditures may reflect an increase in the population or an increase in the prevalence of stone disease.

A-138
FACTORS IN THE CAUSATION OF URINARY TRACT STONES IN PATIENTS WITH ENTEROCYSTOPLASTIES

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It is well-known that patients who have undergone bladder and/or ureteral reconstruction involving intestinal tissue are at increased risk of forming stones in the lower urinary tract. In this paper, we examine the possible causes of urolithiasis in a group of such patients. Fifteen patients (6 male and 9 female, aged 15–50), who had undergone various enterocystoplasty operations using intestinal tissue and who had subsequently formed lower urinary stones, were screened at a Stone Clinic for the potential metabolic and nutritional factors that might have led to their stone-formation. Three had spina bifida. Eleven had had multiple recurrences of stones (between 2 and 5 episodes) and four had so far only experienced 1 episode. All gave a history of recurring urinary tract infection and, of those in whom stone analysis was performed, 86% formed infection stones consisting of various mixtures of CaP and MAP; 14% formed sterile stones consisting of a mixture of CaP and CaOx. All had raised 24-hour urinary pH values (average 6.93), 5 were hypercalciuric ($>$ 6 mmol/day) all with a renal leak of calcium, 5 had mild hyperoxaluria ($>$ 0.45 mmol/day) including one with enteric hyperoxaluria, and all were hypocitraturic ($<$ 2 mmol/day, but mostly $<$ 0.7 mmol/day). All had high indices (PSF) of the risk of forming CaP-containing stones and 12 had an additional high risk of forming CaOx-containing stones. Dietary excess in terms of animal protein, calcium, oxalate and sodium were contributory factors in 6 patients. We conclude that, although urinary tract infection remains the main cause of stones in patients with enterocystoplasty, dietary and metabolic factors may also play a role in the risk of urolithiasis.

A-139
A TALE OF TWO DATABASES – PUBLIC VERSUS PRIVATE SECTOR STONE-FORMERS

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For the past 6 years two databases of stone patients have been established at the Middlesex Hospital in London – one for National Health Service (NHS) patients and one for Private Patients (PP). In this paper, the data sets are compared in order to identify whether or not there are any differences between the two groups and, if so, to determine if such differences help our understanding of why stones form. The NHS group consisted of 1444 patients and the PP group of 324 patients with a history of recent stone-formation. The patients were screened for the known demographic, metabolic, nutritional and biochemical factors involved in stone-formation using our standard LITHOSCREEN procedure. As expected, the PP group contained more individuals in the higher socio-economic groups than the NHS group. Analysis showed that the PP group consumed a diet that was higher in most food items, but especially animal protein and purine, than the NHS group. The PP group passed urine that was more acidic and contained more uric acid, calcium and oxalate than that of the NHS group. As a result the PP group had a higher biochemical risk (P_{SF}) for forming UA- and CaOx containing stones and formed more UA and CaOx stones and fewer phosphatic stones than the NHS group. We conclude that the results are

consistent with the theory that diet and lifestyle play a major role in the generation of the risk of forming stones through their effect on urine composition. The results also emphasise the importance of a high animal protein diet in the genesis of calcium and uric acid stones.

A-140
AN ANALYSIS OF 1862 KIDNEY STONES FROM SOUTH AFRICA

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There have been no recent reports on kidney stone composition in South Africa. Previous studies showed similar compositions to Western countries. In the present study the composition of 1862 calculi collected during the period 1980–2003 were analysed. Stone compositions were determined semi–quantitatively by x–ray powder diffraction and the results were analysed using Microsoft Access. The major component of the stones was calcium oxalate (CaOx), which was present in 74% of all calculi. Of the total, 72% were pure CaOx, and CaOx monohydrate was twice as prevalent as CaOx dihydrate in both sexes. Phosphate and urate were the next most common stone components, occurring in 19% and 17% of all calculi, respectively. Apatite and uric acid were the most frequent constituents of the phosphate and urate stones, respectively. Uric acid and CaOx co–precipitated in 22% of the urate stones, which is likely due to epitaxial growth of uric acid on CaOx. A high proportion of the stones (71%) were from male patients, and the proportion containing urate was four times higher amongst these patients. Pure struvite stones (2% of the total calculi) were more common amongst females. Analyses confirmed previous reports that the composition of kidney stones in South Africa is similar to that of Western countries. Factors influencing the precipitation of urate and struvite may be important discriminators between the two genders with regard to stone formation in South Africa.

A-141
SEX AND RACIAL DIFFERENCES IN THE PRESENTATION OF SYMPTOMATIC UROLITHIASIS

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We determined sex differences in the acute presentation of symptomatic kidney stone disease among the Hispanic population and compared that to the African American and Caucasian populations. A chart review was performed on all patients seen in the Emergency Department or Urgent Care Center for symptomatic kidney or ureteral stone disease over a 5 year period in a county hospital serving a large Hispanic population in Denver, Colorado. A total of 486 patients were identified by ICD9 code for a primary diagnosis of kidney or ureteral stone and demographical information was obtained including race, gender, age, location of stone, stone size, and type of urological intervention. Of the 486 patients, 265 (55%) were Hispanic, 180 (37%) were Caucasian, and 41 (8%) were African American. The male:female ratio of the incidence of symptomatic kidney stone disease was 2.05, 1.17, and 1.11 for the Caucasian, Hispanic, and African American patients, respectively. The percentage of ureteral stones in males was approximately 68% and similar among the 3 populations. In the symptomatic women, however, the percentage of ureteral stones was 73%, 56%, and 53% for Hispanic, Caucasian, and African American patients, respectively. The rate of urological intervention was similar among Caucasian males and females and Hispanic females (approximately 33%), but significantly lower among Hispanic males (18%). The relative male to female incidence of symptomatic ureteral stones among the Hispanic population is nearly one to one, whereas this ratio in the Caucasian populations approaches the previously reported two to one. In comparison to Hispanic men, Hispanic women undergo significantly more urological interventions for symptomatic kidney stone disease.

A-142
ECONOMICS OF STONES IN PORTUGAL

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ESWL has changed the approach, risks, costs and complications of stone treatment. ESWL encouraged urologists and patients to pass over metaphilaxis although, nowadays this is seen as necessary and cost effective. To analyse specific data from the epidemiological survey of 43032 subjects carried out in 1994 by the author in Portugal; a cohort of 8600 randomised subjects from this study was followed-up between 1994 and 1998. Also data from patients treated by ESWL over 1½ years at the Institute of Urology, Lisbon was collected to estimate the costs. The cost of treating a renal cholic episode in Portugal and the annual costs of stone disease can be seen from this data. The figures give us an idea of the tremendous expense involved and the importance of metaphylaxis.

A-143
COMPOSITION OF RENAL AND BLADDER CALCULI IN PEDIATRIC STONE FORMERS

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To analyse the composition of renal and bladder calculi in pediatric stone formers and its role in etiological assessment. Two hundred and ten renal and 44 bladder calculi were analysed by InfraRed Spectroscopy. Separate analysis was done for core and surface in all stones where possible. Mixed stones and stones containing urates were also analysed by chemical methods. Small calculi were fractured and analysis undertaken by SEM using EDS analysis. Stone analysis is important in determining the etiology of stone disease and SEM is a useful tool to assess composition. The presence of Ammonium Acid Urate in kidney and bladder calculi suggests a common cause in our population i.e. endemic calculi due to poor nutrition, diarrhea and dehydration. Increasing age gives pattern similar to developed world i.e. CaOx stones.

A-144
EPIDEMIOLOGY AND RISK FACTORS IN PEDIATRIC AMMONIUM ACID URATE LITHIASIS

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To evaluate epidemiology, dietary and metabolic risk factors for formation of Ammonium Acid Urate (AAU) stones in children as compared to non stone forming siblings. Epidemiology, dietary and 24 hour urinary risk factors were evaluated in 58 children who had pure ammonium acid urate stones and 30 siblings. Diet was evaluated by a food frequency questionnaire and blood chemistry included calcium, phosphate, uric acid, urea, creatinine, electrolytes, magnesium and albumin. Twenty-four hour urine included the above and citrate, oxalate, ammonium and protein. Dietary and metabolic risk factors in these ammonium acid urate (AAU) stone formers shows malnutrition and dehydration, resultant hyperuricosuria, increased ammonia cause preferential precipitation of AAU in pH ranging above 6.0.

J. ANIMAL UROLITHIASIS

A-145
EXAGGERATED OXALOGENESIS AFTER INTRAVENOUS LOADING WITH GLYCOLIC ACID IN VITAMIN B6-DEFICIENT RATS

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Administration of various oxalate precursors, including glyoxylate and glycolate, has been reported to increase endogenous oxalate production in normal rats. Urinary oxalate plays an important role in calcium oxalate stone formation, and hyperoxaluria is noted in vitamin B6-deficient rats. Therefore, we measured urinary oxalate, glycolate, and citrate after an intravenous load of glycolic acid in vitamin B6-deficient rats to study their potential interactions in oxalate metabolism. Male Wistar rats weighing 180–200 g were divided into two groups of 6 rats each. The animals were fed a control diet or a vitamin B6-deficient diet for 3 weeks, and were intravenously administered 10 mg of glycolic acid. Urine samples were collected by bladder puncture just prior to and at hourly intervals until 5 hours after glycolic acid loading. Urinary oxalate, glycolate, and citrate were measured by capillary electrophoresis. Urinary oxalate and glycolate excretion peaked within 1–2 hours after glycolic acid loading in both groups. Urinary oxalate and glycolate excretion were higher in vitamin B6-deficient rats compared with control rats and remained high up to 5 hours. Urinary oxalate accounted for 2.03% and 14.40% of the administered dose of glycolic acid in control and vitamin B6-deficient rats, respectively, while urinary glycolate accounted for 3.60% and 12.53% of the dose in control and vitamin B6-deficient rats, respectively. Urinary citrate excretion was significantly lower in vitamin B6-deficient rats than in control rats. This study demonstrated that a glycolic acid load increases urinary oxalate excretion, and that vitamin B6 deficiency causes an exaggerated increase of endogenous oxalate production and urinary oxalate excretion associated with hyperglycolic aciduria and hypocitraturia.

A-146
VITAMIN B6 DEFICIENCY AUGMENTS ENDOGENOUS OXALOGENESIS AFTER INTRAVENOUS L-HYDROXYPROLINE LOADING IN RATS

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Glycolate and glyoxylate appear to be effective substances for promoting oxalogenesis in experimental animals. Hydroxyproline induced calcium oxalate stones in rats (Thomas 1971) and exaggerated urinary oxalate in B6-deficient rats (Ribaya 1981). We measured urinary oxalate after load of L-hydroxyproline to normal and B6 deficient rats. Male Wistar rats (2 groups of 6 each) were fed a control diet or a B6-deficient diet for 3 weeks. Under anesthesia, the rats were intravenously given 100 mg of L-hydroxyproline (10 min). Hourly urine specimens were collected before and until 5 hours. Urinary oxalate and glycolate were measured by capillary electrophoresis. *Results:* Urinary oxalate and glycolate peaked at 1–2 hours after load in controls. In B6-deficient rats, urinary oxalate was higher than in controls and remained high up to 5 hours, while urinary glycolate did not increase. Urinary oxalate accounted for 0.27% and 2.01% (mol/mol) of the hydroxyproline dose in controls and B6-deficient rats, respectively. Urinary glycolate accounted for 0.32% (mol/mol) of the dose in controls. A major source of glyoxylate is the oxidation of glycolate in peroxisomes, and glyoxylate is also synthesized from 4-hydroxyproline- 2-ketoglutarate in the mitochondria. The increase of urinary oxalate and glycolate after L-hydroxyproline dose (630 mg) was reportedly 0.24% and 0.06%, respectively. We also confirmed that L-hydroxyproline increases oxalate with glycolate in controls. B6 deficiency exaggerated the endogenous production of oxalate from hydroxyproline, but not glycolate, suggesting that glyoxylate reductase may be inhibited by B6 deficiency. Animal protein contains hydroxyproline as high as 4%. The risk of increased oxalate must be remembered after meat intake, especially, by persons lacking of vitamin B6 (malnutrition, long-term antibiotic therapy, bowel disease, etc.)

A-147
VITAMIN B6 DEFICIENCY INCREASES ENDOGENOUS OXALATE PRODUCTION FROM XYLITOL AFTER INTRAVENOUS LOADING IN RATS

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Glyoxylate and glycolate are important precursors of oxalate that promote oxalogenesis after administration to animals. An exaggerated increase of urinary oxalate excretion has been reported in vitamin B6-deficient rats (Hannett 1976). Therefore, we measured the response of urinary oxalate, glycolate, and citrate to an intravenous dose of xylitol in normal and vitamin B6-deficient rats, in order to study the potential role of these substances in oxalate metabolism. This study demonstrated that administration of xylitol increases urinary oxalate excretion in vitamin B6-deficient rats. Therefore, the risk of increased oxalate excretion must be kept in mind after intake of a xylitol-rich diet as well as after parenteral administration, especially in persons with vitamin B6 deficiency.

A-148
COLONIC OXALATE FLUX IS INCREASED IN A RAT MODEL OF ENTERIC HYPEROXALURIA AND STONES

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It is known that patients with small bowel resection (SBR) may develop hyperoxaluria, and are at increased risk for calcium oxalate (CaOx) stone formation. The colon is the site of increased oxalate (Ox) absorption, but the mechanisms of the increase are not known. We have developed a model of enteric hyperoxaluria and CaOx stone formation in rats with SBR coupled with high Ox (0.57%), low calcium (0.02%) diet. Six male Sprague-Dawley rats were randomized to undergo 50% SBR or transection (sham operated). Animals were fed the high Ox diet for 2 weeks after surgery. Urinary Ox was significantly higher in resected rats compared to sham-operated ($p < 0.0001$). Animals were sacrificed, and Ox transport measured in Ussing chambers. After SBR, ileum, proximal and distal colon mucosa-to-serosa Ox flux and net Ox flux increased ($p < 0.05$). SITS, an inhibitor of anion transporters, decreased net Ox flux. RNA expression was measured of downregulated adenoma (DRA), a known Ox transporter. DRA expression was increased significantly in rats with SBR ($p < 0.05$). Active transport of Ox appears to increase in rats after SBR.

A-149
DOWN-REGULATION OF COX-2 BY OXALATE ADMINISTRATION IN RAT KIDNEY

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Oxalate exposure has recently been demonstrated to elicit lipid signaling cascades including COX-2, an inducible enzyme that converts arachidonic acid to prostaglandins. Since prostaglandins reportedly prevent the adhesion of crystals on renal epithelial

cells, oxalate-induced COX-2 expression might serve as a defense mechanism against crystal retention in the renal tubules. The present study examined the changes in COX-2 expression in kidneys of rat model of nephrolithiasis. Male Sprague-Dawley rats received an intraperitoneal injection of sodium oxalate (10 mg/100 g B.W.). Rats were killed 2, 4, 6 hours after oxalate injection, and COX-2 mRNA expression in the kidney was assessed quantitatively using real time polymerase chain reaction. Kidneys were also processed for immunihistochemical staining for COX-2. Oxalate administration induced time-dependent decreases in COX-2 mRNA in rat kidneys. Immunohistochemistry revealed that the expression of COX-2 in control rats was localized in distal tubules. These sparse basal expressions were further decreased following oxalate administration. Oxalateinduced down-regulation of COX-2 may predispose rats to crystal retention in renal tubules.

A-150
EPIDEMIOLOGY OF CANINE UROLITHIASIS (181,386 UROLITHS) – 1981 TO 2002

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Urolithiasis is a common disorder of dogs. Over the past 20 years, the composition of uroliths retrieved and analyzed from dogs has changed. In 1982, the prevalence of struvite was 68% and calcium oxalate was 9% (Figure). In 1992, the prevalence of struvite was 56% and calcium oxalate was 28%. In 2002, the prevalence of struvite was 42% and calcium oxalate was 39%. These changes occurred without significant change in the prevalence of other mineral types. For example, purines remain 5 to 8% of canine uroliths. The reciprocal relationship between the prevalence of struvite and calcium oxalate may, in part, be related to development and use of medical protocols to dissolve canine struvite uroliths. Struvite uroliths in dogs are usually associated with a urease-producing microbe. Dissolution protocols employ appropriate antimicrobial therapy and use of a low protein, magnesium-restricted, phosphorous-restricted, acidifying, and diuresing diet. However, this does not completely explain the changes in struvite and calcium oxalate prevalence because the prevalence of other mineral types has not changed proportionately. Thus, it is apparent that the increase in calcium oxalate and decrease in struvite is associated with other factors.

A-151
SAFETY AND TOXICITY OF INTRAVENOUSLY ADMINISTERED NA2EDTA IN HEALTHY DOGS

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Ethylene diamine tetra-acetic acid (EDTA) has been evaluated previously for chemolysis of calcium oxalate uroliths *in vitro* and *in vivo*. EDTA is more effective at dissolving calcium oxalate uroliths *in* vivo when compared with citrate and phytate. It is often administered intravenously for chelation therapy. Infusion of EDTA through nephrostomy tubes in dogs was associated with urothelial damage; however, these changes have not been reported when administered intravenously. The purpose of this study was to evaluate the safety and toxicity of IV administered EDTA to healthy dogs. Six healthy, reproductively altered, female Beagle dogs, aged 4–6 years, were used. Dogs received one IV infusion over one hour for 5 days of either saline (50 ml) or Na_2EDTA (25 or 50 ml of 0.015, 0.03, or 0.06 mM). Cystoscopy was performed before and after the 5 day period. Dogs were euthanized after the last infusion and a complete necropsy was performed. Ionized calcium decreased in a dose-dependent fashion with the lowest concentration of 1.12 mM/L (reference: 1.2–1.45 mM/L) in the dog receiving 0.03 mM EDTA at 50 ml/hr. Serum total magnesium concentration decreased slightly, but not significantly in all groups over the 5 day period. Dogs did not exhibit any adverse effect including lower urinary tract signs. Although cystoscopic changes were found in dogs receiving 0.03 and 0.06 mM EDTA, gross and histologic changes were not present in the urinary system. IV EDTA can be administered safely at 0.03 mM at 2 times maintenance rate or at 0.06 mM at maintenance rate to dogs. Further studies are underway to evaluate IV administered EDTA for chemolysis of ESWL-generated calcium oxalate fragments of nephroliths.

A-152
EPIDEMIOLOGY OF FELINE UROLITHIASIS (46,755 UROLITHS) – 1982 TO 2002

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Urolithiasis is a common disease of cats. In 1982, 88% of uroliths retrieved and analyzed from cats were composed of struvite and 3% were composed of calcium oxalate. In contrast, in 2002 40% of uroliths were composed of struvite and 50% were composed of calcium oxalate. Over 95% of nephroliths occurring in cats are composed of calcium salts. Why the change in mineral composition? Effective strategies for dissolution and prevention of struvite uroliths have been developed for cats. Feline struvite uroliths form unassociated with bacterial urinary tract infections, which is different from dogs and human beings. Dissolution and prevention has been accomplished by feeding acidifying, phosphorous-restricted, and magnesium-restricted diets. Part of the explanation for the increase in calcium oxalate uroliths, therefore, may be related to effective treatment of struvite uroliths. However, other factors are apparently involved. Age-related changes in urinary mineral excretion and urinary saturation occur. Young cats apparently excrete urine, which is lower in saturation for calcium oxalate than older cats. Additionally, male cats are more often affected with calcium oxalate uroliths than female cats. There is also a breed predisposition observed in cats with Burmese, Persian, and Himalayan breeds being over-represented.

A-153
FELINE XANTHINE UROLITHIASIS: A NEWLY RECOGNIZED CAUSE OF URINARY TRACT DISEASE

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Review of 46,755 feline uroliths submitted to the Minnesota Urolith Center revealed 64 to be composed of xanthine. Almost all were composed of pure xanthine, although a few contained small quantities of uric acid. None of the cats had been treated with the xanthine oxidase inhibitor, allopurinol. Sixtyone xanthine uroliths were obtained from the lower urinary tract while xanthine uroliths from 3 cats came from the upper urinary tract. Xanthine uroliths occurred in 30 neutered and 8 non-neutered males and 25 neutered females (the gender of one cat was not specified). Mean age of cats at time of diagnosis of xanthine uroliths was 2.8 ± 2.3 years (range = 4 months to 10 years). Urinary uric acid excretion was similar between 8 xanthine urolithforming cats and healthy cats (2.09 ± 0.8 mg/kg/d vs 1.46 ± 0.56 mg/kg/d);

however, urinary xanthine excretion (2.46 ± 1.17 mg/kg/d) and urinary hypoxanthine excretion 0.65 ± 0.17 mg/kg/d) were higher (neither are detectable in urine from healthy cats). No medical dissolution protocol for feline xanthine uroliths exists. Prevention involves feeding a proteinrestricted, alkalinizing diet. Without preventative measures, xanthine uroliths often recur within 3 to 12 months following removal. In 10 cats consuming the protein-restricted alkalinizing diet and followed for at least 2 years, only 1 has had recurrence. The precise metabolic abnormality has not been identified in cats; however, a familial or congenital defect in xanthine oxidase activity is likely.

A-154
INFLUENCE OF ALKALINIZATION AND ACIDIFICATION ON URINE SATURATION WITH CALCIUM OXALATE AND STRUVITE AND BONE MINERAL DENSITY IN HEALTHY CATS

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The study involved feeding 3 groups of 4 cats (2 males and 2 females) diets differing in only their acidifying or alkalinizing properties. The acidifying diet was formulated to induce a urine pH of 6.0–6.4, the basal diet was formulated to induce a urine pH of 6.4–6.8, and the alkalinizing diet was formulated to induce a urine pH of 6.8–7.2. Diets were fed for 12 months and cats were randomly assigned to their diet group. Body composition was estimated using dual energy x-ray absorptiometry (DEXA) at the beginning of the study and at 6 and 12 months. Forty-eight hour urine samples were collected from cats at 6 and 12 months. Cats maintained stable body weight. Urine pH values were significantly different between diet groups with the lowest urine pH values observed with the acidifying diet (6.2 ± 0.0.1) and the highest values with the alkalinizing diet (7.1 ± 0.3); the basal diet was associated with urine pH values between the other diets (6.8 ± 0.2). Differences were not observed in other variables except urinary ammonia excretion, which was significantly higher in cats consuming the basal diet. There was a significant difference in urine saturation with calcium oxalate between the 3 diets with the highest saturation occurring in cats consuming the acidifying diet and the lowest saturation occurring in cats consuming the alkalinizing diet. Urine saturation with struvite was not different between groups. Diet was not observed to significantly influence bone mineral content or density. From this study, it is possible to induce urine undersaturation with calcium oxalate using diet. This is influenced by urinary calcium, oxalate, magnesium, and phosphorous excretion, and by inducing an alkaline urine pH. Second, feeding an alkalinizing diet was not associated with increased urine saturation with struvite; therefore, urine pH is only one influence on this risk. Third, bone demineralization was not apparent in these healthy cats.

A-155
APPLICATION OF EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY (ESWL) FOR CANINE NEPHROLITHS

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Although development of ESWL has relied heavily on investigation in the dog model, clinical veterinary experience has been limited, due to the lack of access to lithotriptors. A 90% success rate is reported by one center using a Dornier HM3 lithotriptor. Using the Storz Modulith SL20, we have completed 26 ESWL treatments on 21 nephroliths in 15 dogs. All dogs were small (3.1–9.5 kg) purebred dogs aged 3?1 yrs (mean 8.8), with historical signs iincluding UTI, pain, hematuria, vomiting and cystoliths. For ESWL, dogs were anesthetized and nephroliths visualized by fluoroscopy. SW were applied at 16? kv for 900–3900 SW (mean 2469). Immediate post-treatment findings included gross hematuria (6), pain(4), hepatic enzyme elevation (4), retroperitoneal fluid(3), diarrhea (1) and ureteral dilation(1). Second treatments were required for 5 nepholiths and 2 ureteral fragments. Pre and post-treatment GFR (7?0 days) were measured in 8 dogs. GFR declined posttreatment in 7/8 dogs, but rebounded on follow-up GFR in 5/5. In 9 of 14 dogs (64%) nephroliths were successfully fragmented and clinical signs resolved. Additional ESWL was recommended for further fragmentation in 3 dogs (21%), but not pursued. Two dogs developed progressive renal failure after incomplete ESWL; 1 was dramatically painful. All 3 failing treatment had large, bilateral nephroliths and chronic pyelonephritis. In summary, ESWL is a valuable treatment modality in the management of nephroliths in dogs. A higher SW dose may be required when compared to results using the HM3 lithotriptor.

A-156
DRY?? EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY (ESWL) FOR CANINE AND FELINE URETEROLITHS

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Although development of ESWL has relied heavily on investigation in animal models, clinical veterinary experience has limited due to the lack of access. Although an excellent success rate is reported for canine nephroliths using an HM-3 lithotriptor, the technique was less effective for canine and feline ureteroliths. Using the Storz Modulith(SL20, we have treated 9 canine ureteroliths and 2 feline ureteroliths. All dogs were small (2.7?.5 kg) purebred dogs aged 3?6 yr yrs (mean 7.4), with historical signs including UTI (3), pain (1), vomiting (3) and previous uroliths (3). Two ureteroliths were fragments from previous ESWL of nephroliths. Both cats (DSH) had chronic renal failure. Animals were anesthetized and ureteroliths visualized by fluoroscopy. In dogs, SW were applied at 16?9 kv for 900–35000 SW (mean 2616). Microscopic hematuria (2) and pain (1) were observed post-treatment. In 8/9 dogs (89%) ureteroliths were successfully fragmented and passed into the lower tract after one treatment. Partial fragmentation and progressive ureteral obstruction was observed in 1 dog (the smallest); retreatment was not attempted. In 1 cat, minimal change in ureterolith appearance was observed after conservative treatment (2850 SW at 12–16 kv), but azotemia was reduced. Successful fragmentation was obtained in the other cat after 2 treatments (4900, 3700 SW at 17–19 kv). Ureteral obstruction worsened between treatments, however. In our hospital, ESWL appears to be a valuable treatment modality for ureteroliths in dogs. A higher SW dose and multiple treatments may be required in cats.

A-157
ROLE OF SCORPARIA DULCIS IN PREVENTING RENAL DAMAGE PRODUCED BY SODIUM OXALATE

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Scoparia dulcis an Ayrvedic plant, whose extract is administered as an antistone agent was tested in the animal models to study the protective role in calculogenised rats. Male albino rats of 150–200gm body weight were selected for the study. 1 gm of sodium oxalate was dissolved in 1 litre of water and fed the rat. One group of rat was fed on oral scoparia dulcis extract also. The rats were sacrificed at the end of two months using anaesthetic ether. Tissues collected from the rats were fixed in 10% formalin and cut into bits and dehydrated using ascending grade of ethyl alcohol, embedded in paraffin wax and sliced using a rotary microtome. The slices were then placed on glass slide cleared off paraffin wax and stained using haematoxylin and eosin. After mounting with DPX, the slides were studied microscopically. The administration

of sodium oxalate produced significant tissue destructive changes in the rat kidney tubules and cortex namely hydrocalycosis, interstitial oedema, and epithelial injury. The administration of Scoparia dulcis along with the sodium oxalate resulted in clearing of renal injuries like hydrocalycosis, interstitial oedema, and epithelial injury produced by the high oxalate diet. It is concluded that Scoparia dulcis is effective in controlling the renal injuries produced by the abnormal oxalate metabolism.

A-158
SULFATE/OXALATE EXCHANGER (SAT-1, SLC26A1) MRNA EXPRESSION IN KIDNEYS OF HYPEROXALURIC RATS.

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Net urinary oxalate secretion is thought to be mediated primarily by the basolateral sulfate/oxalate exchanger (Sat-1, Slc26a1) of proximal tubules. We examined Sat-1 mRNA expression by Northern Blot analysis in hyperoxaluric rats.
Rats treated with 0.75% ethylene glycol (EG) for 2 weeks or a single injection of sodium oxalate (NaOx) were used for the study. A DIG labeled cDNA probe was used for the Northern Blot analysis.

Group	Sex	Castrate	Implant	Ox Load	Density	*% change
1	M	No	No	0.75% EG	7.356	
2	F	No	No	0.75% EG	0.328	−89.1% vs G1
3	M	Yes	No	0.75% EG	6.302	
4	F	Yes	No	0.75% EG	5.603	0.2% vs G3
5	M	Yes	Testosterone	0.75% EG	5.816	
6	M	Yes	Estradiol	0.75% EG	4.434	−28.1% vs G5
7	F	Yes	Testosterone	0.75% EG	5.519	
8	F	Yes	Estradiol	0.75% EG	3.134	−22.2% vs G7
9	M	No	Estradiol	0.75% EG	7.711	
10	F	No	Testosterone	0.75% EG	5.897	−17.6% vs G9
11	M	No	No	1 mg NaOx	8.191	
12	F	No	No	1 mg NaOx	4.694	−41.0% vs G11
13	M	No	No	3 mg NaOx	8.039	
14	F	No	No	3 mg NaOx	5.946	−15.9% vs G13

*after standardization with β-Actin

Our results suggest that the increased renal oxalate excretion noted in hyperoxaluric male or testosterone treated rats may be secondary to an increased expression of the kidney Sat-1 exchanger.

A-159
THE EFFECT OF INDOMETHAZINE TREATMENT ON THE INJURIOUS EFFECTS OF HYPEROXALURIA ON TUBULAR EPITHELIUM IN RABBIT MODEL

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To evaluate the possible preventive effect of agent (indomethazine) on the well established harmful effects of hyperoxaluria on renal tubular epithelium an experimental study in rabbit model has been carried out. Fourty white New Zealand rabbits, each weighing 3–5 kg. were included in the study program. The animals were divided into three main groups. Animals were sacrificed from after 1 week and 4 weeks and bilateral flank incision was performed and the kidneys were removed for tissue evaluation. The presence and the degree of crystal formation along with the histopathologic alterations and the percent of apoptotic changes have been evaluated in the renal tissue specimens obtained. Apoptotic changes in renal tubular cells were examined by using TUNEL method. Animals undergoing renal ischemia during hyperoxaluric diet demonstrated evident alterations in tubular morphology along with the markedly increased apoptotic changes. Inflammatory changes were also evident in this group with high tubular crystallization index. On the other hand however, animals receiving indomethacine medication did reveal limited morphologic changes with less pronounced inflammatory changes. Again mean tubular cell apoptotic index and the degree of crystal formation were significantly lower in this group when compared with previous one. In the light of our findings and the literature data as well, we may say that both oxalate and CaOx crystals are injurious to renal epithelial cells.These findings in turn again supported the hypothesis that apoptotic changes do occur during hyperoxaluric phase and these alterations may result from tubular ischemia causing lipid peroxydation as well as inflammatory processes .Although pathophysiologic remains to be determined, limitation of both crystal deposition and apoptotic changes might be well instituted by antiinflammatory agents and by this way the formation and prophylaxis of urinary stones could be well achieved.

ABSTRACT INDEX

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