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I. Clinical Urolithiasis

1 Urolithiasis in a Large Kindred Deficient in Adenine Phosphoribosyltransferase (APRT)

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The poorly soluble purine 2,8-dihydroxyadenine is excreted in urine when there is a homozygous deficiency of APRT. Calculus formation, nephrotoxicity and renal failure have been described mainly in children. Dihydroxyadenine calculi are falsely positive for uric acid with standard colourimetric procedures, and are typically radiolucent. The defect has been inherited as an autosomal recessive trait in European families and those heterozygotes studied have not excreted adenine, 8-hydroxyadenine or 2,8-dihydroxyadenine. However, four patients have been reported from Japan with 2,8-dihydroxyadenine calculi in association with a partial deficiency of APRT. This inconsistency suggests that 2,8-dihydroxyadenine urolithiasis may be genetically heterogeneous.

We have studied APRT in 18 subjects from 3 generations of a Newfoundland family, including 12 of 13 members in the second generation. Both maternal and paternal sides of the family came to Newfoundland from England. Inheritance was autosomal recessive. Two of the 12 were homozygotes with virtual absence of erythrocyte APRT activity. One presented at age 42 with 2,8-dihydroxyadenine urolithiasis established by infrared and mass spectrographic analysis (N Engl J Med 305:1570, 1981). Adenine compounds comprised 16% of total urine purines, in spite of a daily dose of 300 mg (5 mg/kg) of allopurinol. The second homozygote was a 24-year-old woman who has no past or present evidence of urolithiasis. She also had normal renal function and excreted substantial amounts of adenine, 8-hydroxyadenine and 2,8-dihydroxyadenine. None of the 10 heterozygotes have any past or present evidence of urolithiasis or impairment of renal function.

For the 10 heterozygotes, the mean value for APRT enzyme activity in erythrocyte hemolysates was 28% of controls, substantially less than the expected 50%. A radioimmunoassay method for assay of APRT gave similar results. Using cultured lymphoblasts in 5 heterozygotes, the average level of APRT specific activity of 46% was close to the expected 50%.

Studies of linkage between haptoglobin (α-subunit) and APRT suggest a minimum map distance of 27.5 (SE = +22, −15) centimorgans between these two chromosome 16 loci.

Urolithiasis related to homozygous APRT deficiency may present in middle age, and some young adults may have no detectable evidence of urolithiasis. Inheritance of APRT deficiency in this family was clearly autosomal recessive and no urolithiasis has occurred in any heterozygote.

2 Studies on Urine Composition in Patients with Calcium Oxalate Stone Disease

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During recent years several forms of medical therapy have been introduced in order to prevent recurrent stone formation. Different regimens affect urine composition in different ways, and it is therefore of importance to have a routine program for biochemical evaluation of these patients.

Aims. The purpose of this investigation was to compare urine composition in stone formers and normal subjects, and thereby provide a biochemical basis for selective prophylactic treatment in calcium oxalate (CaOx) stone formers.

Method. Twenty-four-hour urine composition was analyzed with respect to calcium, oxalate, magnesium, citrate, urate and inhibition of calcium oxalate crystal growth rate. Urine collections were obtained from 483 male and 226 female calcium stone formers, 100 normal men and 40 normal women, All urine was collected on an out-patient basis with normal dietary and drinking habits.

Results. Stone formers had an increased excretion of both calcium and oxalate, whereas magnesium and urate did not separate stone formers from normal subjects. A large number of both male and female patients had a low citrate excretion. The inhibition of CaOx-crystal growth was lower in male but not in female patients. Urinary excretion of calcium, oxalate, citrate and magnesium were used to formulate a metabolic risk index and simple estimates of the ion-activity product of CaOx. Such indices were subsequently used in the evaluation and follow-up of these patients.

Conclusions. By means of an analytical program of this design, it is possible to evaluate the individual stone former from a biochemical point of view. The effect of different urine abnormalities on the risk of forming a urine supersaturated with CaOx can be approximately expressed by the suggested indices.

3 Intestinal Flora and Oxalate Excretion in Patients with Enteric Hyperoxaluria

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The major cause of hyperoxaluria today is associated with the short bowel syndrome or GI malabsorptive diseases and is coined "enteric hyperoxaluria". It is most pronounced following intestinal resections due to Crohn's disease or following jejunoileal bypass (JIB). It has been shown to be due to increased absorption of oxalate, especially in the colon. However, the mechanism is not fully understood.

Little is also known about the composition of the intestinal flora in patients with enteric hyperoxaluria.

Aim. The aim of the present study was to investigate if the hyperoxaluria to some extent is related to changes in the intestinal flora.

Method. Eleven patients, who had undergone JIB because of obesity > 2 years before the investigation, were studied under surgical ward conditions for 5 days. The patients were maintained on a constant diet. During days 3, 4 and 5 clindamycin (Dalacina) 1.8 g/24 h were given parenterally in three divided doses. Urine and faeces samples were collected during days 2 and 5.

Results. Day 2: All patients had hyperoxaluria with a mean of 0.94 ± 0.11 mmol/24 h (± SEM; normal < 0.45 mmol/24 h).

No significant disturbances in the colonic microflora in comparison to healthy controls were found.

Day 5: The degree of hyperoxaluria did not change (0.92 ± 0.07) during clindamycin administration in spite of a significant decrease in the number of anaerobic bacteria (*Bacteroides, Fusobacteria* and *Veillonella*).

Conclusion. Our patients with enteric hyperoxaluria had a normal colonic microflora. The degree of hyperoxaluria does not seem to be related to changes in this intestinal flora.

4 Studies on the Endogenous Production of Oxalate in Man

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The interest in oxalate metabolism has increased because of its importance in urinary stone formation. Oxalate is a metabolic end product in man and is excreted in urine.

Aim. One purpose of this investigation was to study urinary oxalate excretion as an indicator of the endogenous oxalate production in a model in which exogenous oxalate sources were excluded. Another was to study the effect of a short-time parenteral load of two important oxalate precursors, namely glycine and ascorbic acid on the urinary oxalate excretion.

Material. We studied six patients with malnutrition who were given total parenteral nutrition (TPN) in preparation for major surgery. In one patient the urinary oxalate excretion was determined for 20 consecutive days during TPN. In five patients the study of urinary oxalate excretion was done for 12 consecutive days of TPN. On days 4 and 5, a glycine solution (10 and 20 g) was added to the TPN. Ascorbic acid was added to the TPN solution on days 8, 9 and 10 (1 and 3 g).

Results. The mean urinary excretion of oxalate was 0.33 mmol/24 h in six patients during TPN. The urinary excretion of oxalate was considered to be equal to the endogenous oxalate production. A 2-day load of the oxalate precursor of glycine given to five patients did not influence the oxalate excretion in spite of increased serum glycine concentrations. A 3-day load of the oxalate precursor ascorbic acid increased the oxalate excretion in all patients. In one patient TPN was prolonged for 20 days without any change in the amount of oxalate excretion.

Conclusion. Studies on urinary oxalate excretion during TPN in man might give a good picture of the endogenous oxalate synthesis. Through variations in the composition of the TPN further studies on oxalate metabolism in man is possible.

This study also indicates that short-term increases in S-glycine concentration do not change endogenous oxalate production and confirms earlier findings of the importance of ascorbic acid as an oxalate precursor.

5 The "Stone Clinic Effect" in Patients with Idiopathic Calcium Urolithiasis

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The "stone clinic effect" refers to the effect of encouraging a high intake of fluid and avoiding dietary excesses on stone growth and stone formation in patients with urolithiasis. To determine the extent of this effect, we reviewed the clinical courses of 108 patients with idiopathic calcium urolithiasis and indeterminate metabolic activity. Sixty-three patients (58.3%) showed no evidence of stone growth or new stone formation (metabolic inactivity) after a mean follow-up of 62.6 months. Twelve of the 17 patients with hypercalciuria (70.6%), 7 of the 15 patients with hyperuricosuria (46.7%), and 35 of the 54 patients with neither hypercalciuria or hyperuricosuria (64.8%) showed no evidence of metabolic activity at follow-up. Comparison of initial and follow-up 24-h urine volumes demonstrated a significant increase in urine volume ($P < 0.001$) in patients who were metabolically inactive at follow-up. No increase in urine volume was detected in patients who were metabolically active at follow-up. We recommend that specific drug therapy should not be commenced in patients with idiopathic calcium urolithiasis until the "stone clinic effect" has been evaluated.

6 The Urinary Excretion of Citrate in Normal Persons and Patients with Idiopathic Calcium Urolithiasis

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Urinary citrate forms soluble complexes with calcium, thus reducing relative supersaturation of calcium-containing crystals, and acts as an inhibitor of crystal growth of hydroxyapatite and calcium oxalate. However, the importance of a low-urinary citrate concentration for diagnosis, therapy or prognosis of patients with idiopathic calcium urolithiasis (ICU) remains undefined. In order to determine the relevance of urinary citrate measurements in management of patients with ICU, we studied a group of normal persons, to define normal levels of urinary citrate excretion, and a group of patients with ICU, in an ambulatory setting.

There were 83 persons in the normal group (43 women, 40 men), and there were 120 consecutive patients with ICU, ages 20–70, with uninfected urine, normal renal function, and on no medication which might interfere with citrate excretion. The urinary excretion of citrate in normal males was not significantly different from that in normal females ($P > 0.05$). There was a significant correlation of urinary citrate excretion with age in normal persons ($P < 0.001$). No correlation of urinary citrate excretion with age was demonstrated in stone formers. Hypocitraturia was demonstrated in 29.2% of patients with idiopathic calcium urolithiasis. Twenty-two of 35 hypocitraturic patients had multiple urinary citrate measurements. In 15 of these 22 patients at least one normal urinary citrate measurement was obtained. Twenty-four of 35 hypocitraturic patients had a proven intact urinary acidification mechanism. Only one patient had a urinary excretion of citrate less than 100 mg per 24 h. Three patients had both hypercalciuria and hypocitraturia. No relationship could be demonstrated between the 24-h urinary excretion of citrate and the severity of stone disease prior to presen-

tation in our Stone Clinic, or the frequency of stone growth or new stone formation in our patient population at follow-up. Further prospective study is required to establish the value of urinary citrate determinations collected in patients on an uncontrolled diet in an out-patient setting.

7 Urolithiasis in Hereditary Renal Hypouricemia

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Hereditary renal hypouricemia due to an isolated renal tubular transport defect is a rare disorder, first reported in 1972. Seven such families have been evaluated in our laboratory since 1974. The primary abnormality in this syndrome is a renal tubular transport defect, either at the presecretory reabsorption site or at both pre- and postsecretory reabsorption sites, manifest in a markedly elevated renal urate clearance and hypouricemia. In addition, mild hyperuricosuria and hypercalciuria of the absorptive type were found to be frequent among such patients. The hyperuricosuria is attributed to diversion of intestinal urate elimination to urinary urate excretion, as a result of the hypouricemia, whereas the reason for the hypercalciuria is not yet clarified.

All affected subjects (13 males and 8 females), were Jews of non-Ashkenazi origin, mainly from Iraq. Genetic evaluation revealed a recessive autosomal mode of inheritance.

Three of the seven propositi studied had urinary stones. In two male patients (ages 37 and 39) the stones were composed of uric acid; in one 37-year-old female, the stone was not available for analysis. A similar high incidence of stones was reported among the propositi of families with renal hypouricemia studied by other investigators. The high incidence of urolithiasis in the patients with renal hypouricemia is attributed to hyperuricosuria and hypercalciuria which are frequent associates in this syndrome. The absence of stones in a substantial proportion of the patients studied probably reflect their young, age (of the 21 affected subjects, 7 were children 2–13 years old, 8 were 22–42 years old and 6 were 48–65 years old) and/or the absence of other important determinants in the causation of stone formation.

8 Response to a Physiologic Dose of Pyridoxine in Primary Hyperoxaluria (PH) Type 1

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During a $2\,^1/_2$-year treatment period with hydrochlorothiazide and inorganic phosphates, a 13-year-old girl with primary hyperoxaluria Type 1 (increased urine glycolate) also received 4 courses of pyridoxine, 2 mg/day, either as multivitamin tablet (2 courses) or pure pyridoxine (2 courses). Urinary oxalate exceeded 120 mg/day before pyridoxine, fell promptly to <60 mg/day during each treatment period and rose to control levels 4–12 weeks after stopping pyridoxine. Stone formation ceased and renal function remained stable during the treatment period. A similar response to pyridoxine was seen in an asymptomatic sibling with primary hyperoxaluria but not in two unrelated sisters with a different form of primary hyperoxaluria (normal urine glycolate).

A response to such a small dose of pyridoxine has not been previously reported in primary hyperoxaluria. Large doses of pyridoxine have been reported to reduce urine oxalate in some patients with primary hyperoxaluria but frequently there has been no significant effect. Our experiments indicate that the amount of pyridoxine present in the normal diet may cause a marked reduction in urine oxalate and thus could mask the response to administered pyridoxine. This plus the lack of response to pyridoxine in primary hyperoxaluria with normal glycolate excretion may partially explain the marked variability in the reported responses to exogenous pyridoxine.

9 Hyperoxaluria and Calcium Oxalate Nephrolithiasis Associated with Hyperabsorption of Dietary Calcium, Phosphorus and Magnesium: Successful Treatment with Hydrochlorothiazide

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This report describes studies performed over an 8-year period in a 13-year-old girl with hyperoxaluria and calcium oxalate nephrolithiasis who did not have primary hyperoxaluria or any of the recognized causes of secondary hyperoxaluria. The patient also had increased urinary excretion of calcium and magnesium and hyperabsorption of dietary calcium, phosphorus and magnesium. It is suggested that the hyperoxaluria resulted from hyperabsorption of dietary oxalate secondary to hyperabsorption of dietary calcium. Hyperabsorption of dietary magnesium and increased urinary magnesium excretion have not previously been reported in this context. Stone formation ceased and urinary oxalate excretion gradually fell to normal during long-term thiazide therapy. This is the first report of normalization of urine oxalate excretion during thiazide therapy in a patient with frank hyperoxaluria.

10 Urinary Composition in Nephrolithiasis Patients Eating Their Usual Diets: Differences Between Patients With and Without Tubular Ectasia

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Urinary composition was measured in 42 normal men, 124 male patients with calcium oxalate stones and 24 male patients with uric acid stones who were not hospitalized and who were eating their regular diets. Patients with calcium oxalate stones were subdivided into four groups on the basis of the presence or absence of hypercalciuria and of tubular ectasia. There was no significant difference in mean age, weight or height of the four groups of patients with calcium oxalate stones.

In normocalciuric patients who did not have tubular ectasia (n = 26), urinary volume, creatinine, calcium, magnesium, phosphorus, uric acid, citrate and sodium did not differ significantly from normal, but zinc excretion was increased ($P < 0.02$). In normocalciuric patients with tubular ectasia (n = 41), only urinary calcium excretion was increased ($P < 0.02$).

In hypercalciuric patients who did not have tubular ectasia (i.e. true idiopathic hypercalciuria) (n = 26), urinary volume, creatinine, calcium, magnesium, phosphorus, sodium and zinc excretion were all significantly increased. In hypercalciuric patients who did have tubular ectasia (n = 33), only urinary volume, calcium and phosphorus were increased. The patients with uric acid stones had increased urinary volume, phosphorus, uric acid and zinc.

Known facts concerning the relationship between protein and zinc metabolism suggest that the increased urinary zinc excretion in the patients with uric acid stones and those with calcium oxalate stones who do not have tubular ectasia is due to increased dietary animal protein. Increased urinary, phosphorus, and magnesium could also be explained on this basis, but other possibilities must be considered. Thus our findings support the hypothesis that increased dietary animal protein is an important risk factor for the development of calcium oxalate stones but only in patients who do not have tubular ectasia.

Our intensive search for tubular ectasia in patients with calcium oxalate stones has led to a series of observations which strongly suggest that these patients form a large and distinct subgroup of the total stone population. In these patients the factors which cause stone formation may be quite different from those which are opera-

tive in patients who do not have tubular ectasia. Failure to identify these patients in clinical stone research may lead to erroneous conclusions.

11 Temporal Changes in Urinary Risk Factors Following Renal Colic

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It has been shown that the incidence of mild hyperoxaluria decreases by approximately 50% when the urines of a group of stone formers are remeasured several months after an episode of stone colic. To investigate further the variations in urinary risk factors with time a series of patients was studied, first, while in hospital with renal colic and then at 1, 2, 3, 4, 8 and 12 weeks following discharge. Twenty-four-hour and fasting urine specimens were collected and analysed for oxalate, calcium, pH, alcian blue precipitable polyanions, uric acid, creatinine and volume.

The sequential changes in urinary risk factors that occur following acute stone colic are presented and possible explanations for the observed changes offered. The optimal time for patient assessment after an episode of colic will be discussed.

12 The Origin of Metabolic Abnormalities in Primary Calcium Stone Disease – Natural or Unnatural Selection

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A number of metabolic abnormalities have been described in patients with primary calcium stone disease, including hyperabsorption of calcium, decreased tubular reabsorption of both phosphate and calcium, and increased plasma concentrations of $1,25(OH)_2$ vitamin D, parathyroid hormone and calcitonin. Although there is considerable dispute about their pathogenesis, there is little disagreement that they only occur in a proportion of patients, they do not necessarily occur together and that there is wide variation in their prevalence from centre to centre. Moreover, no single hypothesis has so far been able to explain these metabolic abnormalities on a common pathological mechanism.

The prevalence of metabolic abnormalities in over 200 male patients with carefully documented primary calcium stone disease will be described. Evidence will be put forward to show that these abnormalities represent the extreme limits of the normal range and in themselves do not represent a disease process. Their prevalence in the population of calcium stone formers is determined through a natural selection process by virtue of their effect on the urinary chemical risk factors responsible for calcium stone formation.

13 Anatomical Localization of Urinary Risk Factors of Calcium Oxalate Stone Formation

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A major limitation of urinary studies in stone formation is that the voided specimen represents the final product of urine formation. It is not known whether there is a difference in the relative concentrations of the various inhibitors of crystallisation between upper- and lower-tract urine.

To characterise these compounds, urine was collected from the upper tract via ureteric catheterisation or aspiration and from the lower tract via cystoscope or catheter in a group of patients. Specimens were analysed for oxalate, calcium alcian blue precipitable polyanions (i.e. glycosaminoglycans, ribonucleic acid, and Tamm Horsfall mucoprotein), uric acid, magnesium, citrate, phosphate, sodium, potassium and creatinine. The results show that there are differences in the concentration of certain of the urinary risk factors between the upper and lower urinary tracts. The implications of these findings will be reviewed.

14 Slow-Growing Urea-Splitting Corynebacterium in Infection Stone Disease

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Aim of the study. Several patients with bladder calculi, encrusted cystitis, and infected kidney stones with negative routine urine cultures were carefully studied. From the clinical point of view, examination of the urinary sediment showed magnesium ammonium phosphate crystals in all these cases. Therefore, we suspected a hidden urea-splitting organism that should be investigated.

Methods. The clinical examination includes routine study of the sediment of a urine sample obtained inmediately after voiding. After centrifugation, the sediment is observed by the urologist under a contrast-phase microscope for leucocytes, bacteria and crystals. Urinary pH is determined by test strip. Special bacteriologic attention was given to those cases and urine cultures in Cled-agar and blood-agar were performed.

Results. In 7 cases in which magnesium ammonium phosphate crystals were observed in the sediment of an alkaline, highly ammonic urine specimen in spite of negative routine 24-h urine cultures, small colonies of a slow-growing bacteria (48–72 h) appeared after incubation at 37° ranging from 80,000 to more than 100,000 per ml. The organisms were small gram-positive rods, with no mobility and very strong urea-splitting action. Their production of urease was more rapid and stronger than that of *Proteus mirabilis.* It was recognized as a *Corynebacterium* species extremely resistant to antibiotics, showing in that respect some features similar to the members of the JK group.

Conclusions. The identification of a urea-splitting *Corynebacterium* is important from the practical and theoretical points of view, since through negative routine cultures some authors have rejected the notion we accept, i.e., that without urea-splitting infection no magnesium ammonium phosphate can crystallize in the urine. Behind such seemingly negative cultures may lie a urea-splitting and slow-growing *Corynebacterium.*

15 The Connection Between Amino Acids, Urinary Enzymes and Stone Formation

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The average activity values of glutamic-oxalacetic transaminase (GOT) and glutamic-pyruric transaminase (GPT) in normal urines were found to be higher by a factor of 3 than those of stone formers (34:11 IU, respectively). There was no significant difference between stone formers' urine with respect to the activity values of γ-glutamil transpeptidase, lactate dehydrogenase and amino peptidase.

GOT and GPT convert aspartic acid and alanine to glutamic acid. An in vitro study showed that glutamic acid is a potent inhibitor for calcium oxalate precipitation. Moreover, the ratio of [glutamic acid/(aspartic acid + alanine)] in the urine of stone formers is significantly lower (<0.57) than in the urine of normals (>0.84)

High activity values of GOT and GPT in the urine have high correlation with the values of the inhibition test of Sarig et al. A total of 75 patients with calcium oxalate urolithiasis were tested for their overall inhibitory potential and for their enzyme activity in the urine before and after the start of medication (phosphates, thiazides, and allopurinol). In some of the patients treated a significant increase in GOT and GPT activities and an improvement in the inhibition potential of the urine were found. However, such

correspondence was not found in all cases. In all 75 patients a negligible change was detected in the other enzymes tested.

The results brought about these assumptions: (1) the existence of a genetic deficiency of the GOT and GPT activity in the stone former; (2) damage in the excretion or in filtration process of these enzymes from the kidney to the urine; (3) the presence of specific blockers (inhibitors) to GOT and GPT which subsequently prevents the production of glutamic acid. In each case the glutamic acid production in situ would be decreased, thus diminishing the beneficial inhibition of CaOx precipitation.

16 A New Rapid Spectrophotometric Method for Detection of Xanthinuria

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The purpose of this study was to develop a rapid technique for detecting this disorder based on the absorption maxima of xanthine (X), hypoxanthine (HX) and uric acid (UA) in urine from normal and xanthinuric subjects. Urine aliquots were titrated to pH 9.3 to ensure solubility of X, HX and UA. To 2.0 ml of borate buffer, 0.1 M pH 9.3, 100 µl of urine was added and read against a matched quartz cuvette containing 100 µl of distilled water in place of the urine. The samples were read on a Zeiss PM QII spectrophotometer at 250, 270 and 292 nm. The absorption ratios 250/292 and 270/292 were then calculated for each urine. The results are summarized in Tables 1 and 2.

Table 1. Absorption ratio for urines of normal subjects: 270/292 and 250/292 nm

Subject	270/292 nm	250/292 nm
B. B.	0.790	1.330
J. C.	0.778	1.355
M. B.	0.734	1.073
N. F.	0.800	1.560
S. F.	0.676	1.170
N. A.	0.774	1.280
R. W.	0.818	1.480
J. O.	0.728	1.060
H. C.	0.812	1.220
Range	0.676–0.818	1.06–1.56
Mean ± SD	0.768 ± 0.047	1.28 ± 0.17

Table 2. Absorption ratio for urines of xanthinuric subjects: 270/292 and 250/292 nm

Subject	270/292 nm	250/292 nm
M. M.	2.41	2.98
F. M.	4.04	4.61
Maj. M.	2.95	3.76

There was a highly significant difference between the values obtained for the three xanthinuric patients and those of the normal subjects at both ratios ($P < 0.001$). This entire procedure can be carried out in as little as 5 min. This method could be used as a rapid screening technique for detecting xanthinuria. The only equipment required is an ultraviolet spectrophotometer. However, after initial diagnosis by this method, confirmation by determining plasma and urinary X, HX and UA values would be advisable.

17 Familial Xanthinuria in a Large Kindred: Purine Metabolites in Plasma and Urine of Xanthinurics, Siblings And Normal Subjects

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Detailed analysis of urine and plasma for xanthine (X), hypoxanthine (HX) and uric acid (UA) was carried out on samples from three xanthinurics, four siblings, their parents and normal subjects, both adults and children. The renal clearance of these compounds was also determined in xanthinuria. The effect of a low-purine diet (< 20 mg purine/day) for 3 days on the urinary excretion of X, HX and UA by xanthinuric subjects was investigated. Their urinary oxalate was also determined. Urinary excretion of X and HX in xanthinuria was determined by the method of Chalmers and Watts[1]. A modification of this procedure was used when urine of normal subjects was analyzed. Plasma concentrations of X and HX were determined on plasma ultrafiltrate, ^{14}C-labelled compounds being used to monitor recovery. In normal subjects uric acid in the ultrafiltrate was first removed with uricase before determining X and HX spectrophotometerically. Plasma urate in xanthinuria was also determined on the ultrafiltrate. Methods for urinary oxalate and urate are as previously described[2,3]. The plasma and urinary oxypurine values are summarized in Table 1.

Table 1. X, HX and UA values in xanthinurics, siblings and normal subjects

Subject	Age	Sex	Urine µmols/24 h			Plasma µmols/100 ml		
			X	HX	UA	X	HX	UA
M. M.	8	M	1,151	194	24.6	0.685	0.545	0.29
C. M.	14	F	84.2		1,935	0.517		16.60
F. M.	7	M	1,133	416	15.0	1.160	0.784	0.089
Maj. M.	5	F	619	155	29.0	0.920	0.283	0.372
			(X + HX)		UA	(X + HX)		UA
Siblings								
S. M.	11	F	59.8		1,494	0.446		10.23
D. M.	13	M	53.6		3,208	0.476		21.70
J. M.	6	M	55.9		1,017	0.708		16.30
Parents								
JS. M.	43	M	99.8		6,017	0.892		27.70
E. M.	41	F	86.9		2,512	0.601		23,60
Normal adults (mean ± SD)			98.8 ± 34.06		3,047 ± 871	0.41 ± 0.06		21.6 ± 7.6
Children (age 4–10) (mean ± SD)			57.4 ± 37.40		1,044 ± 425	–		–

The renal clearance of (X + HX) by xanthinurics M. M., F. M. and Maj. M. was 76,55 and 45 ml/min while that for UA was 7.5, 11.6 and 4.6 ml/min, respectively. On a low-purine diet the urinary excretion of (X + HX) by these subjects decreased by 40 to 52%, while urinary urate increased 3 to 5.7 times. The decrease in urinary (X + HX) on a low-purine diet contrasts sharply with the findings of Ayvazian and Skupps[4], and the increase in urinary urate under similar treatment has not been previously reported. The plasma urate levels of the xanthinurics (Table 1) are considerably lower than that found in earlier studies[5]. Both parents and one sibling (J. M.) have raised plasma (X + HX) values and may be indicative of carriers of a recessive gene for this metabolic defect: This is the first reported finding of this disorder in Ireland.

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18 Calculus Renal Failure

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Pakistan has a high incidence of calculus disease. For a number of reasons the incidence of renal failure due to stones (calculus renal failure) is exceptionally high.

The present study, based on 238 patients over a period of 10 years, has given us an understanding of management problems. We were able to classify 230 patients with calculus renal failure into four types. This classification is based on the degree of calculus obstruction and renal functional mass at a given moment. However, individual patients, over a period of time, can move into other types. Renography and ultrosonography in recent years have proved most valuable. Our classification is as follows:

Type I (109 patients): These patients experience sudden, almost total obstruction in the upper urinary tract, leading to anuria or oliguria. Early relief of the obstruction produces excellent results. This group of patients is labelled "calculus anuria" in the literature.

Type II (32 patients): In these patients one kidney suffers from chronic renal damage due to stones. Obstruction of the "good" kidney by a stone does not produce anuria or oliguria. However, the patient gradually slips into chronic renal failure. Early removal of the stone from the "good" kidney is indicated.

Type III (87 patients): These patients have large non-obstructing calculi in both kidneys (or the only functioning one), leading to renal damage over a period of time. In our opinion stones must be removed in these cases.

Type IV (2 patients): In these patients with bilateral non-obstructing calculi, no cause for anuria could be ascertained. We feel these cases have anuria due to severe infection.

19 Infection Concrements Induced by Ureaplasma Urealyticum

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Infection with a urease-producing microorganism is a prerequisite for the formation of infection concrements, i.e., concrements composed of struvite and/or carbonate apatite. *Ureaplasma urealyticum* is a urease-producing microorganism commonly occurring in the lower urinary tract of acults. In a recent study a relationship between *U. urealyticum* and infection concrements in the upper urinary tract was demonstrated in man (Lancet 1:526–527, 1983).

Aims of the study. The purpose of this study was to investigate the crystallization caused by *U. urealyticum* in vitro and its concrement-forming capacity in vivo in rats. The results were to be compared with that of urease- and nonurease-producing bacteria.

Materials and methods. In vitro crystallization was studied as encrustation on solid glass rods suspended in synthetic urine at 37 °C for 20 h. After inoculation of the synthetic urine with *U. urealyticum, Proteus mirabilis* or urease-negative *Escherichia coli* the amounts of encrusted calcium, magnesium, and phosphate were analyzed and the pH followed. In certain experiments acetohydroxamic acid (AHA), a potent urease inhibitor, was added.

In the animal experiments *U. urealyticum* was inoculated into the bladder of adult male rats. As control inoculates, *Ureaplasma* broth without microorganisms, heat-inactivated *U. urealyticum, P. mirabilis*, urease-negative *Mycoplasma hominis*, and *E. coli* were used. After 2, 4, and 6 weeks the animals were killed and the incidence of concrement formation was recorded. The composition of the stones was analyzed chemically.

Results. Inoculation with *U. urealyticum* and *P. mirabilis* resulted in encrustation of struvite and calcium phosphates on the rods and an alkalinization of the urine, while inoculation with *M. hominis* and *E. coli* caused no crystallization and the pH remained constant. When AHA was added to the vessels inoculated with *U. urealyticum* or *P. mirabilis* the alkalinization was prevented and no encrustation was seen.

Of 37 rats inoculated with *U. urealyticum*, 31 developed bladder concrements compared to 31 of 51 rats inoculated with *P. mirabilis*. Rats inoculated with *Ureaplasma* broth without microorganisms, with heat-inactivated *U. urealyticum*, with *M. hominis* or *E. coli* developed bladder concrements only in a few cases. All stones were composed of pure struvite.

Conclusions. U. urealyticum caused crystallization of struvite and calcium phosphates in synthetic urine in vitro and formation of bladder concrements after inoculation in vivo in rats. The effects appear to be associated with the urease activity of the microorganism.

20 Ureaplasma Urealyticum and Renal Stones

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Urease-producing bacteria such as *Proteus mirabilis* can be detected in the urinary tract of most patients with infection stones (struvite and carbonate apatite). Such bacteria can, however, not always be demonstrated in urine samples or from the stone in these patients. *Ureaplasma urealyticum* is a urease-producing microorganism commonly occurring in the lower urinary tract of both males and females. It is not demonstrable by conventional bacterial-culture techniques and is resistent to penicillins, cephalosporins and sulfonamides. *U. urealyticum* has been shown to produce bladder stones in experimental animals and crystallization of strutvite and calcium phosphates in synthetic urine. Its role for the development of urinary tract concrements in humans has recently been demonstrated (Lancet 1:526–527, 1983). To elucidate this question further an extended clinical study has been performed.

Materials and methods. Fifty patients, 26 males and 24 females with a mean age of 52 years (range 23–73 years), were studied who had been consecutively operated on for renal stone(s) during a 10-month period in 1982–1983. The stones were removed with conventional surgical methods in all except two patients in whom the stones were extracted percutaneously.

Midstream-voided urine specimens were sampled the day before operation. When the renal pelvis was visualized at surgery it was punctured and urine aspirated. After removal of the stone(s) one-half was sent for chemical analysis and the other half for culture. Stone cultures were performed according to Nemoy and Stamey. All urinary samples, the first and the final stone washing, and the washings after stone crushing were cultured for *U. urealyticum* and bacteria under aerobic and anaerobic conditions.

Results. Twenty-seven patients had stones of a metabolic origin. In three bacteria could be cultured from the upper urinary tract, and in one patient *U. urealyticum* was present in the first stone washing. Of the 23 patients with infection concrements, urease-producing bacteria could be detected in the upper urinary tract of 16. In 7 patients *U. urealyticum* was recovered from the upper urinary tract, more often from the stone than from pelvic urine. In four patients *U. urealyticum* was the only urease-producing microorganism cultured from any level of the urinary tract. Of the 7 patients with infection concrements and growth of *U. urealyticum* in the upper urinary tracts, had undergone previous renal stone surgery.

Conclusions. The findings appear to link the presence of *U. urealyticum* in the upper urinary tract to the presence of infection stones. Cultures from urine and the stone should therefore be performed not only with conventional bacterial culture techniques but also with techniques specific for *U. urealyticum*. The presence of *U. urealyticum* could explain the presence of infection stones in patients with negative bacterial cultures.

21 Calcium Oxalate (CaOx) Urine Saturation in Calcium Stone Formers (CSF): Hypercalciuria Versus Hyperoxaluria

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The importance of calcium excretion (UCaV mg/day) versus oxalate excretion (UOxV mg/day) as main determinants of urine CaOx supersaturation in CSF is still open to discussion. We have evaluated CaOx urine relative supersaturation (RS, Marshall nomograms) in relation to UCaV and UOxV in 75 CSF and 10 healthy controls (C) kept on 1 g Ca, 100–150 mg Ox diet. According to UCaV (upper 2 SD normal level: 280, 320), 34 patients were normocalciuric (NC, 205 ± 60) and 41 (55%) hypercalciuric (IH, 388 ± 84). IH had higher RS (1.13 ± 0.19) than NC (0.96 ± 0.15, $P < 0.001$) and C (0.92 ± 0.2, $P < 0.01$), but the frequency of urine supersaturation (RS1) was similar in NC (16/34) and in IH (32/41, P NS); UOxV was higher in IH (47.5 ± 19.5) than in NC (37 ± 15, $P < 0.02$) and C (31 ± 12, $P < 0.01$). UCaV and UOxV were slightly correlated in all CSF ($R = 0.26$, $P < 0.05$). According to UOxV (upper 2 SD normal level: 55) 52 patients (69%) were normooxaluric (NOx, 33 ± 9) and 23 (31%) were hyperoxaluric (HOx, 65.5 ± 12.7). HOx had higher RS (1.23 ± 0.1) than NOx (0.99 ± 0.13, $P < 0.001$), frequency of urine supersaturation (23/23 vs 26/52, $P < 0.05$) and UCaV (362 ± 127 vs 291 ± 129, $P < 0,05$). There was a progressive increase in RS levels and frequency of supersaturation according to four possibilities: normocalciuria-normo-oxaluria (28 patients): 0.94 ± 0.13 and 10/28; hypercalciuria-normo-oxaluria (24 patients): 1.05 ± 0.09 ($P < 0.001$) and 16/24; hyperoxaluria-normocalciuria (6 patients): 1.18 ± 0.03 ($P < 0.001$) and 6/6; hyperoxaluria-hyperoxaluria (17 patients): 1.27 ± 0.07 ($P < 0.001$) and 17/17. RS was stronger correlated in all CSF with UOxV ($R = 0.9$, $P < 0.001$) than with UCaV ($R = 0.45$, $P < 0.001$).

Conclusions: Although both IH and HOx increase urine RS, there is a better correlation with UOxV than UCaV. HOx is more predictably associated with urine supersaturation than IH. Even though less frequent than IH, HOx is an important cause of urine supersaturation in CSF.

22 Prospective Ambulatory Metabolic Study of Idiopathic Calcium Nephrolithiasis in Venezuela

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Urinary lithiasis in Venezuela represents an important public health problem that has not been evaluated prospectively in terms of the metabolic causes. We evaluated 95 consecutive patients with idiopathic, recurrent calcium urolithiasis, using a comprehensive clinical and laboratory protocol in two stages. The first was during the usual patient's diet (at least 3 weeks after the renal colic or surgical intervention to extract the stone), in which we measured 24-h urinary calcium and uric acid excretion, serum parathyroid hormone levels (using a highly specific antibody against the midportion and carboxyl terminus of the molecule), creatinine clearance, urinary acidification test, serum uric acid, total serum calcium (atomic absorption photometry), ionized calcium and serum, and urine phosphorus in order to calculate the tubular reabsortion of phosphorus. In the second stage, carried out after 1 week on a low-calcium diet (approximately 400 mg/day), a calcium absorption test was performed after collecting a 2-h fasting urinary specimen. An acute oral calcium load of 1,000 mg was given as a mixture of calcium gluconogalactogluconate (Neo-calglucon) and milk. Fractional excretion of calcium and cyclic AMP was determined in the fasting urine sample and 2 h after the oral calcium load.

The patients studied were 51 males (52.7%) and 44 females (46.7%). The mean age during the study was 37.6 ± 12.3 years (mean ± SD), and the mean age of the first episode was 31.9 ± 12.2 years. The mean number of stone episodes was 12.6 ± 4.3 per patient.

The results demonstrated that 48 subjects (50.5%) had high urinary calcium excretion values (5.78 ± 0.27 mg/kg body weight) and 48 patients (49.5%) had normal urinary calcium excretion (2.27 ± 0.12 mg/kg).

The 48 patients with hypercalciuria were furhter subclassified according to the calcium absortion test and the parathyroid hormone levels in 14 patients with absorptive hypercalciuria (14.7%), 30 patients with reabsorptive hyercalciuria (31.6%), and 4 patients with resorptive hypercalciuria (4.2%), all of them with primary hyperparathyroidism. Two subjects with the reabsorptive pattern had a distal tubular acidosis. In 12 patients there was a combination of hypercalciuria and hyperuricosuria, 8 of these subjects had reabsorptive hypercalciuria, and 4 an absorptive pattern.

Of the 47 patients without hypercalciuria, 20 subjects (21%) exhibited hyperuricosuria (mean 24-h uric acid excretion of 894.4 ± 49 mg). Of the stones studied by crystallographic analysis, 75% of these patients showed a high composition of calcium oxalate. In 27 patients (28.5%) no metabolic abnormality could be demonstrated.

In summary, a prospective ambulatory protocol was used in our calcium stone population in order to establish the metabolic causes and different etiological factors inducing calcium urolithiasis and subsequently to start a logical and rational therapeutic approach.

23 Hyperuricosuric Calcium Stone Formers: Effect of Fast and Calcium Hyperabsorption

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We have recently reported that following 13-h overnight fast, there are no essential differences in serum urate, urine urate excretion [Ur./creatinine (Cr.), mg/mg], or FE_{urate} between male Ca stone-formers (SF) ($n = 45$) and normal subjects (NS) ($n = 15$). This suggests that the basic renal handling of urate does not differ between the two populations. However, we also reported that these same SF are particularly vulnerable to episodic increases in urate excretion in association with a calciuresis and natriuresis following an acute 1-g oral Ca load (4-h urine collections) which is not found in NS.[1] The present study examines urate levels under similar conditions as described above in these same SF divided into either hyperuricosurics (HU) (24-h non-fasting urate excretion >800 mg) or normouricosurics (NU). The mean (± SE) 24-h urate excretion in HU was 906 ± 15 mg, and for NU, 533 ± 38 ($P < 0.001$). We also examined the significance of Ca hyperabsorption (without an accompanying fasting hypercalciuria) enhancing urate excretion.

Fasting Data	HU ($n = 16$)	NU ($n = 27$)	NS ($n = 15$)
Serum urate (mg/dl)	6.2 ± 0.2	6.0 ± 0.2	6.2 ± 0.3
Ur./Cr. (mg/mg)	0.36 ± 0.03	0.33 ± 0.02	0.35 ± 0.04
FE_{urate} (%)	6.1 ± 0.5	5.8 ± 0.4	6.2 ± 0.9
Ca/Cr. (mg/mg)	0.11 ± 0.01	0.11 ± 0.01	0.06 ± 0.01[b]
Na/Cr. (mEq/mg)	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Increment Post Ca Load[a]			
ΔUr./Cr. (mg/mg)	0.10 ± 0.03	0.14 ± 0.03	0.03 ± 0.02[b]
ΔCa/Cr. (mg/mg)	0.18 ± 0.01	0.16 ± 0.02	0.07 ± 0.01[b]
ΔNa/Cr. (mEq/mg)	0.08 ± 0.02	0.07 ± 0.01	0.02 ± 0.01[b]

Values shown are mean ± SEM. [a]From pre-load 2-h fasting urine collecting value. [b]Significantly different from HU and NU, $P < 0.001$

[1] 59th Annual Meeting of the Western Sect. Am. Urological Assoc., Vancouver, Canada, July 24–28, 1983

These data indicated that HU and NU stoneformers do not differ significantly in their urate parameters following fast, nor in response to an acute Ca load, although both HU and NU differ from normal subjects following the Ca load. We observed that 38% of our HU were fasting normocalciuric (FNC) hyperabsorbers, in contrast to 19% of the NU (based on the Ca load test). However, the % SF with fasting hypercalciuria (FHC) was similar in HU (48%) and NU (50%), thus accounting for a similar fasting Ca/Cr. ratio. In examining the effect of Ca hyperabsorption on urate changes after the Ca load, we compared urate changes in FNC hyperabsorbers with FNC normal absorbers of our stone-forming subgroups:

Increment Post Ca Load	FNC Hyperabsorbers (n = 12)	FNC Normal Absorbers (n = 11)
ΔUr./Cr. (mg/mg)	0.19 ± 0.04	0.11 ± 0.04
Δ Ca/Cr. (mg/mg)	0.22 ± 0.02	0.11 ± 0.01[a]
ΔNa/Cr. (mEq/mg)	0.08 ± 0.02	0.06 ± 0.01

[a] Significantly different from FNC hyperabsorbers, $P < 0.001$

Although the urate changes were not statistically significant different between the two subgroups of stoneformers (hyperabsorbers vs normal absorbers), it is seen that mean changes in urate differed considerably. In prior 24-h urine collections, 55% of the FNC hyperabsorbers demonstrated hyperuricosuria as compared to 17% of the FNC normal absorbers. These various studies suggest that the renal handling of urate does not differ significantly between HU and Nu Ca stoneformers, but that dietary factors, including excessive purine intake as described by Doe et al. (J Chronic Dis 29:793, 1976), and also electrolytes including Ca and Na (as we have previously suggested) may contribute to the incidence of hyperuricosuria, the FNC hyperabsorbers being particularly vulnerable.

24 Urolithiasis Outpatient Clinic – Demonstration of the Organization and First Results

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In 1982, an outpatient clinic for urolithiasis was built up in our department. The reasons for recurrent stone formation were looked for. In addition, stone formers were followed up after surgery for urinary calculi.

Evaluation of stone formers on an outpatient basis is done during 3–4 visits. Patients bring their urine for urine-analysis which is sampled under normal conditions with respect to food and fluid intake.

The step-by-step laboratory evaluation includes all parameters of urinanalysis and serum, from urine-pH to parathyroid hormone.

Up to now 80 patients have been followed up: 30 patients presented with staghorn calculi, 3 with cystinuria. Patients were found to have tubulary defects similar to distal tubular acidosis, accompanied by different types of electrolyte loss.

We are able to define typical risks and can give reasonable care to these patients to prevent recurrence. Allopurinol, hydrochlorothiazide, ethacrynic acid, and suppression of urinary tract infection proved to be the treatment most often used in the metaphylaxis of urolithiasis.

Most patients presented after one or more recurrences and wanted to be followed up. The cooperation of patients during evaluation and thereafter has been excellent. Organization and practicability of this outpatient clinic are demonstrated. The oral calcium-load test and ammonium chloride-load test proved to be manageable even on an outpatient basis. First results show that outpatient evaluation of stone formers give reproducible results under conditions of everyday life.

25 Calcium-Oxalate Stone Formers – Five Years Later

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In 1977, 60 patients with a first calcium oxalate stone episode and 30 untreated recurrent calcium-oxalate stone formers (RSF) (at least one episode within the last five years) were seen in the outpatient department and included in a prospective study. All patients had a complete metabolic check-up. In 21 (70%) of the 30 RSF a metabolic derangement was found (hypercalciuria, hyperuricosuria, hyperparathyroidism, etc.), whereas only 10 (17%) of the 60 single stone formers (SSF showed an abnormality. Of the SSF 30 were treated with an unspecific metaphylaxis (high fluid intake and dietary advice). The other 30 patients were given no metaphylaxis. The 30 RSF were treated by a combined regimen of high fluid intake, dietary advice and thiazide, magnesium or allopurinol medication. The aim of the study was to show the efficiency of a specific metaphylaxis in RSF and the recurrence rate of treated and untreated SSF. RSF and 30 SSF with unspecific therapy ahd check-ups at 6-month intervals. the other 30 SSF had no check-ups up to the year 1982. At that time all patients were called in for a final examination. Twenty-six of the 30 RSF, all 30 of the SSF with therapy, and 26 of the SSF without therapy were reexamined. Of the RSF 21 had stopped medication on their own after 2–3 years. Only 5 still had specific therapy in 1982. Of the 26 RSF, 8 (30%) had one or two stone episodes within these 5 years and the others had no recurrences. Of the 30 SSF with unspecific therapy, 2 had one spontaneous stone passage, as did 1 of the 26 SSF without therapy. *Conclusions* (1) As metabolic abnormalities are low (17%) in SSF only a minimal check-up is necessary. (2) Unspecific metaphylaxis (high fluid intake, dietary advice) is enough in SSF, because the recurrence rate is low (4%), even in untreated patients. (3) in RSF an exact metabolic check-up is necessary because in 70% an abnormality is found. (4) Specific medication (thiazides, magnesium, etc.) over a 5-year period is only possible in a small percentage of RSF, because most patients (about 80%) stop therapy after 2–3 years. Nevertheless, only 8 (30%) of the 26 RSF and stone episodes during the control period.

26 Vitamin D Metabolism in Hypercalciuric Patients

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Aims of the study. In many hypercalciuric patients simple clinical methods are unable to determine the causes of metabolic disorders. Expensive and protracted examinations are used like the calcium load test according to PAK. However, these methods involve some diagnostic problems. We tested to find out whether the determination of 25-OH and 1,25$(OH_2$-D, which are important factors of calcium metabolism, are helpful to classify hypercalciuria.

Methods employed. 25-OH- and 1,25$(OH)_2$-D were determined by competitive protein-binding assay. Calcitonin and PTH were measured by RIA. Determination of calcium was done by flame photometry, ionized calcium by a ion-selective elctrode. To measure phosphate levels we used a commercial test kit.

Summary of results obtained. Both types of absorptive and renal hypercalciuria showed wide-spread concentrations of 1,25$(OH_2$-D from subnormal to increased levels. Similar findings demonstrated the examination of 25-OH-D levels. In 75% of the patients with resorptive hypercalciuria, we found increased 1,25$(OH)_2$-D values: 25-OH-D was decreased in these cases, PTH enhanced, and calcitonin normal.

Conclusions. Our study demonstrates that the determination of vitamin D levels is not very helpful to classify hypercalciuria according to PAK. It is especially not possible to separate absorptive type I from renal hypercalciuria by this method. Both types show a wide range of 1,25$(OH)_2$-D levels. the pathophysiological findings re-

presenting different types of hypercalciuria (according to PAK) (e.g., secondary hyperparathyroidism in renal hypercalciuria) could not be found by our examinations. The importance of $1,25(OH)_2$-D for the pathogenesis of the different types of hypocalciuria is discussed.

27 Urinary Tract Infections in Relation to Renal Stone Disease, Pyelonephritis and Uremia

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In order to investigate the pattern of urinary tract infections among patients with renal stone disease, pyelonephritis and uremia, a retrospective survey was made of all urine cultures that had been performed in the Department of Microbiology, University Hospital, Uppsala, during 1975–1981. These data were matched with the diagnosis register at the University Hospital for the same period, whereby the pattern of urinary tract infections in patients with a certain diagnosis could be obtained.

Results. Out of a total of 238,000 urine cultures, 74,500 were positive in 33,000 patients during 1975–1981. The average recurrence rate was 2.25, but 19,200 patients had only experienced positive cultures on *one* occasion. Of the cultures 63,400 (85%) contained only one type of bacteria. Two-thirds of the patients never changed strain of bacteria, whereas up to eight different strains could exist in a single patient in the other third of patients.

E. coli was the most common strain and was contained in 59% of the cultures, *Enterobacter* in 21%, *Proteus* in 12%, *Staph. albus* in 11%, *Kelbsiella* in 11% and *Pseudomonas* in <1%. Altogether 5,000 patients had experienced infections containing *Proteus*, with an average recurrence rate of 1.7.

A total of 400 stone patients had experienced urinary tract infections (30%). A high frequency of *Proteus* (25%) with a high recurrence rate and of *Staph. albus* (32%) were some obvious features. In 694 patients with pyelonephritis, high frequencies of *E. coli* (82%) and *Klebsiella* (27%) were found; 256 uremic patients had experienced urinary infections, where *Enterobacter* (49%), *Klebsiella* (38%) and *Staph. albus* (30%) were particularly common. The pattern of urinary infections in patients with coexisting renal stones, pyelonephritis and uremia is being investigated.

Conclusions. Patients with renal stone disease have a different pattern of urinary tract infections and a high recurrence rate of infections compared with an unselected population of patients with urinary infections.

28 Stone Analysis and Urinary Tract Infections in Renal Stone Patients

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The aim of the present study was to investigate the frequency of urinary tract infections (UTI) in patients that had been admitted to the clinic for renal stone disease. Stone analysis, frequency and level of surgery for stones in relation to infections were also included in the survey.

Materials and methods. A total of 1,350 patients were admitted during the years 1975–1981, of whom 780 were operated on for stones and 400 had experienced urinary tract infections. The operations included nephrolithotomy (N) or ureterolithotomy (U). Stone analysis was made by infrared spectrometry in about 500 stones. Magnesium content of stones was analyzed in 240 stones.

Results. In patients without urinary infections, two-thirds of the operations were U and one-third N. Of the stones 86% were calcium-oxalate or calcium-oxalate-phosphate stones (Ox), 12% calcium-phosphate stones (P) and 1% magnesium-ammonium phosphate stones (MAP). The magnesium content was 0.18 ± 0.28 mmol/g.

In the patients with UTI, *E. coli* was the most common finding, but often during or after the patients had been admitted to the clinic. *Proteus* was found in 25% and particularly prior to admisson and with a high recurrence rate (5.5).

In patients with UTI, in particular those caused by *Proteus*, there were more N operations, if *Proteus* two-thirds were N. The stones were 10–28% MAP and 18–53% P, with the highest values in cases with *Proteus*, where the frequency of Ox was 20%. The average magnesium content of stones was 0.74 ± 0.7 mmol/g ($P < 0.0001$), with the highest values in stones from patients with Proteus, 1.35 ± 0.93 mmol/g.

Conclusions. Patients admitted for renal stones with urinary tract infections had a different pattern of bacteria than other patients with urinary tract infections. Stone patients with UTI also had a different stone content and level of surgical procedures than stone patients without urinary-tract infections.

29 Bacterial Flora of Urinary Stones

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Since 1982 we have examined microbiologically the surface of 26 urinary stones, which were removed by operation and inoculated in a culture medium. The results were compared with the preoperative urine culture.

In 13 cases the preoperative urine has been sterile, and in 12 we also found no bacteria in the stones. In one case *Proteus mirabilis* was found in the calculus.

In 5 cases the infection was handled specifically with antibiotics, but it was not possible to find any bacteria during the operation in any of these cases. To date we have found relevant bacteria preoperatively in cases, and antibiotic therapy was administered during the operation. In all of these cases bacteria were found in the stone during the operation. In 6 cases the bacteria were identical, in 2 two different germs were found in the stone, while in the urine there was only one germ. In 8 cases we found urease-forming germs, which is about two-thirds of the patients.

In the group of the patients showing infections, the percentage of carbonate apatite was 43%, and therefore this part was markedly higher compared with the patients without infection (26% carbonate apatite). Among the latter, the percentage of calcium oxalate clearly predominated with 62%.

30 Results of the Examination of Test Sticks for Selfcontrol of the Specific Gravity of Urine by the Patient

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Determination of the density of urine is a simple measurement made to control urine dilution. As a method of self-control by the patients, a urometer has so far been used. With this method the density change in all dissolved particles is registered.

With a new test stick (Miles Company) the ionic strength of the urine is measured on the reaction principle. Dependent on the ionic activity of the urine, hydrogen ions are set free from the copolymers of the test stick. The hydrogen ions influence indicator coloring; the color shades are directly proportional to the density. The stick was tested and the correlation found to be 0.84. Separate analyses were made for density values below and above 1.015. In addition, from 24 h urine samples ($n = 45$), correlations were made between density (urometer) and density (test stick), and the ionic strength from all urine parameters was calculated (computer program

according to Finnlayson). In 22 patients, self-control of the urine density was carried out with urometer and test stick and the results statistically evaluated.

The results show that a combination of the urometer and test sticks is a good patient self-control method.

31 The Significance of Hyperuricemia and Hyperuricosuria in Calcium-Oxalate Stone Formers

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Serum uric acid and the excretion of lithogenic substances (uric acid, calcium, and oxalic acid) were examined in 116 calcium-oxalate stone formers.

The first measurements were made on patients who were still on individual diets. Of the calcium-oxalate stone formers, 70% showed hyperuricemia or latent hyperuricemia, and 67% of the patients showed hyperuricosuria.

After 5 days on a standard diet during a stay in the Rehabilitationskrankenhaus in Bornheim-Merten, the second measurements were made. Now 66% of the stone formers showed hyperuricemia and only 33% of the patients showed hyperuricosuria.

These results confirm the significance of diet in calcium-oxalate stone formation.

The excretion of calcium and oxalic acid was measured in 116 calcium-oxalate stone formers on individual diets and after 5 days of a standard diet. An attempt was made to find a correlation between the excretion of uric acid, calcium and oxalic acid in these 116 calcium-oxalate stone formers.

32 Rate and Composition of Recurrent Urinary Concrements

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A study was made of 415 consecutively collected urinary concrements from patients treated in our clinic or sent in by other hospitals or urologists. Of these, 201 were primary stones, 168 were recurrent concrements, and no information about recurrence was available on the remaining 46 stones. This amounts to an adjusted relative recurrence rate (ARRR) of 44%.

Divided into four main stone classes (i.e., Ca stones, infection-induced stones, uric-acid-containing stones, cystine-containing stones), the ARRR of Ca stones (Ca oxalate/phosphate mixed stones) was 41% and that of cystine-containing stones was 40%, which are not significant deviations from the mean value. The ARRR of uric-acid-containing stones was 75%, however, which could be explained by the fact that these stones have a considerably faster growth rate compared with the very slow rate of Ca stones. The lowest ARRR (35%) was calculated for infection-induced stones. These are known to be fixed within the renal pelvis (a) by a mucous matrix substance and (b) by their very rapid growth rates, which in a short time leads to calculi too big to be washed out and causes symptoms such as colic or macrohematuria. These so-called staghorn calculi as a rule cause only mild discomfort and continue growing for a long time until they normally have to be removed surgically.

In most cases of recurrence, the composition of the primary and recurrent stones stayed constant within one stone class. Some exceptions to this rule are presented and attempts are made to explain these irregularities.

33 Drug-Induced Stones: Detection and Diagnosis

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Drug-induced urinary stones are generally unrecognized because of the inadequacy and inaccuracy of stone analysis. Only in-depth physical methods allow them to be characterized without ambiguity.

We have been routinely using IR spectrophotometry for 7 years and have analyzed 2,200 adults' stones by this method. If the spectrum revealed the presence of a drug, the corresponding layers of the stone were extracted by the appropriate organic solvents and studied by thin-layer chromatography and mass spectrometry.

Out of 2,200 stones, we detected 24 stones (1% of the total) either totally or partially made up of drugs. We identified: 8 cases of metabolites of glafenine (glafenic acid and hydroxy-glafenic acid); 7 cases of triameterene and derivatives (hydroxy-triameterene); 4 cases of phenazopyridine (in one of which the stones was pure, i.e. entirely made up of drug); 3 cases of sulfamides, one N-acetylsulfamethoxazole chlorhydrate, one N-acetylsulfaguanidine, and one N-acetylsulfadiazine; 1 case of flumequine; 1 case of calcite.

In France, it is estimated that 100,000 stones are eliminated or removed every year, of which 1,000 (1%) probably contain drugs and are not detected. It would seem essential, therefore, that stone analysis be improved so as to detect the presence of drugs. This would permit the interruption of treatment by the drug revealed, thus reducing the risk of aggravating the disease and preventing relapses.

In patients with known calculus disease any previous use of these drugs should be established. For all these patients such drugs should not be prescribed.

34 The Diurnal Renal Excretion of Cystine and Its Significance for Treatment with Chelating Agents in Patients with Cystinuria

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Excretion of cystine in classic cystinuria can be influenced by treatment of patients with D-penicillamine (DPA) or α-mercaptopropionylglycine (MPG). As cystine stone formation can be diminished by appropriate therapy, we studied free-cystine excretion in a number of patients under therapy. The relation ship between fluid balance and cystine excretion was also investigated by subjecting the patients to a strict fluid regime (500 ml water intake every 4 h).

The analysis of free cystine in urine was performed by a modification of the procedure according to Haux and Natelson, in which free cystine is separated from the complex bound form by ion-exchange chromatography. The highest levels of cystine excretion in the untreated patients were observed 4–8 a.m. During this period the urinary concentration of cystine amply exceeded 300 mg/l, being the crucial level for precipitation. Adjusting the dosage and time of administration of the drugs to this diurnal variation in excretion, it was possible to keep the concentration of urinary cystine below the critical 300 mg/l.

With this treatment scheme, it was possible to obtain good results with a dosage considerably lower (1,250 mg/day for DPA and 1,500 mg/day for MPG) than advised and used in clinical practice (2,000–4,000 mg/day for DPA). Since this regime has been introduced to new stone formation has occurred in all patients studied.

35 Heredity, Serum Phosphate and Urinary Calcium in Calcium Urolithiasis

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Familial patterns have been found in some studies of renal calcium stone disease. The question as to whether certain risk factors are

inherited has not been extensively studied. Since stone formers often have low serum phosphate, we designed a study to examine if low serum phosphate or hypercalciuria is a familial feature of stone formers.

In 183 stone formers, heredity for stones in first-degree relatives was registered in 77 patients (42%). Low serum phosphate (<0.94 mmol/l, i.e. mean – SD for controls) was found in about half of the stone patients. This was independent of stone heredity. Eighteen randomly selected patients and 60 of their first-degree relatives were studied in more detail regarding serum phosphate and 24-h urinary calcium excretion. In 9/18 stone formers a family history of stones was evident. Twelve out of eighteen stone formers had low serum phosphate. In their relatives, low serum phosphate and hypercalciuria were noted only occasionally. Thus, relatives of normo- as well as hypophosphatemic stone patients generally had normal serum phosphate and urinary calcium levels.

Serum phosphate (mmol/l, mean ± SD)/urinary calcium (mmol/24 h, mean ± SD)

	Stone formers (n = 18)	Parents (n = 14)	Siblings (n = 32)	Children (n = 14)
A.	0.85 ± 0.17/ 6.8 ± 2.8	1.01 ± 0.14/ 4.6 ± 2.9	1.04 ± 0.12/ 5.9 ± 1.8	1.15 ± 0.25/ 4.1 ± 1.1
B.	0.90 ± 0.26/ 6.2 ± 1.6	1.02 ± 0.18/ 4.6 ± 4.3	0.95 ± 0.08/ 5.4 ± 1.6	1.08 ± 0.14/ 4.0 ± 1.0

A = Stone formers with heredity for stones (n = 9)
B = Stone formers without heredity for stones (n = 9)

Conclusion. There was a familial accumulation of renal calcium stone formers. The stone formers had a tendency towards low serum phosphate. However, low serum phosphate or hypercalciuria was not a familial feature.

36 Bacteriological Aspects of Renal Stone Surgery

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It is well recognised that gram-negative organisms are related to the development of struvite calculi. The presence of organisms within oxalate calculi (OC) is not commonly noted,[1] though the presence of OC within infected urine is well recognised.[2] The bacteriological characteristics of 62 patients undergoing surgical removal of OC were studied. Prior to operation, the urine was cultured and at operation, both an aspirate of the pelvic urine and the removed calculus were cultured for bacteriological growth. Postoperatively, samples from the wound drain, wound swab and urine were cultured on the 1st, 4th and 6th day. A late urine culture was performed at 3 weeks.

Results. Thirteen per cent of patients had a bacteriologically positive preoperative urine, and at operation 18% had a positive pelvic urine aspirate and 22.5% of removed calculi demonstrated bacteriological growth. It was found that *E. coli* was the predominant organism isolated from both the pelvic urine aspirate (73% of positive cultures) and the removed stone (64% of bacterial isolates).

Postoperative wound and urine cultures demonstrate a clear early postoperative bacteriological washout phenomenon (Table 1), and the likelihood of a continuing postoperative wound or urinary infection may be identified by appropriate sampling on the 6th postoperative day.

[1]Dajani AM, Shehabi AA (1983) Bacteriology and composition of infected stones. Urology 21:351–353; [2]Scott R (1975) Urinary tract stone disease, classic studies. Urology 6:667–675

Table 1. Percentage positive bacteriological samples from the wound and urine following surgical removal of 62 oxalate calculi

	Early postoperative cultures		Late postoperative cultures	
	Days 1 & 4	Days 6+	Late positive urine	Wound complications
Urine	21%	14%	16.5%	–
Wound	20%	7.6%	–	7.6%

37 Serum Parathormone and Urine and Nephrogenous Cyclic AMP in Idiopathic Hypercalciuria and in Primary Hyperparathyroidism

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Parathyroid activity was evaluated in 40 patients with idiopathic hypercalciuria and stones (31 absorptives and 9 renals) and in 15 surgically proved primary hyperparathyroids (10 hypercalcemic and 5 normocalcemic), as well as in 20 age- and sex-matched normal controls. On a 1,000-mg and 400-mg calcium diet and on fasting, morning total and ionized calcium, phosphate, and creatinine, parathormone (PTH) and cyclic AMP in the serum were determined, as well as calcium, phosphate, creatinine and cyclic AMP in the urine. All hypercalciurics, either absorptives and renals, for any given level of calcium intake did not show differences in terms of serum calcium, phosphate, PTH, urine and nephrogenous cyclic AMP. Hypercalcemic primary hyperparathyroids showed, other than low serum phosphate ($P < 0.001$), an increased fasting urine calcium ($P < 0.001$), as well as urine and nephrogenous cyclic AMP ($P < 0.001$). In normocalcemic primary hyperparathyroidism, mean ionized calcium was borderline and the second normal standard deviation, serum phosphate, reduced ($P < 0.05$), whereas serum parathormone was within normal limits in all. In such patients urinary calcium was higher compared to normal controls either on different calcium intake and during fasting ($P < 0.001$). Urine and nephrogenous cyclic AMP were normal in all but one patient. It is concluded that: (a) parathyroid activity is normal in idiopathic hypercalciuria subgroups; (b) in subtle primary hyperparathyroidism, serum PTH and cyclic AMP have a poor diagnostic validity, whereas serum ionized calcium points out such patients better; (c) high fasting calcium excretion in the urine may represent the first step in suggesting subtle primary hyperparathyroidism among a stone-former population.

38 Urinary Excretion Pattern of Main Glycosaminoglycans in Stone Formers and Controls

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Total daily excretion of urinary glycosaminoglycans (GAG) in stone-forming subjects, measured as hexuronic acids, hexosamines and sulfate, has been investigated by several workers with different results. The possible presence in urine of non-GAG macromolecules which may contain hexosamines or hexuronic acids can contribute to an explanation of these differences. The interpretation of these findings has led to contrasting hypotheses on the effects of GAG on calcium oxalate (CaOx) growth and/or aggregation. In order to clarify whether the excretion of GAG is significantly

different in the two populations, the excretion of the main GAG species in 20 CaOx stone formers was compared with that of controls by means of electrophoresis on cellulose acetate membranes. The identification and quantitation of GAG alcian-blue-positive materials was achieved using specific GAG enzymatic and chemical procedures.

39 Hypercalciuria in Patients with Normocalcemic Hyperparathroidism (HPT) and Stone Disease

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Hypercalciuria in primary (p) HPT is considered to be only a consequence of severe hypercalcemia, because PTH stimulates renal reabsorption of calcium. Therefore, relatively low urinary calcium excretion in pHPT can be observed. In contrast, the evaluation of patients with pHPT and urolithiasis has revealed a considerable number of patients who are hypercalcuric while having a marginal elevation of serum calcium.

In order to investigate the relationship between serum calcium and urinary calcium in pHPT with stone disease, we studied 36 patients with calcium stones and elevated immunoreactive PTH (carboxyl-terminal parathyroidhormone) in the serum. Patients with elevated creatinine were not included. Serum calcium and the urinary 24-h calcium excretion under individual and standard diets, as well as in a fasting urine sample, were determined.

Patients were grouped according to the 24-h urine calcium (UCa) under individual diet (elevated > 6 mmol/24 h) and the serum calcium (SCa, elevated 2.55 mmol/l). We observed 11 patients with normal SCa and elevated UCa (group I), whereas in only 9 were both elevated (group II). In 14 both were normal (group III) and in 2, UCa was normal and SCa elevated. The fasting urinary calcium excretion of group I with normocalcemia and hypercalciuria was normal (<0.11 UCa/UCreat mg/mg) in 8 patients: 0.047 ± 0.03.

According to these data, the combination of elevated immunoreactive PTH and hypercalciuria in the presence of normal serum calcium is not a rare finding. Because of a normal fasting calcium excretion, a "renal leak" with secondary HPT can be excluded in the majority of patients. It is likely that this group of patients represents pHPT in whom it is the hyperabsorption of dietary calcium which results in a hypercalciuria.

40 Renal Tubular Function in Patients Following Intestinal Bypass Operations

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The formation of kidney stones is a very common complication following intestinal bypass operations. In patients forming idiopathic calcium-containing kidney stones, about 20% of the patients have been found to have renal tubular defects, such as defective acidification of urine, tubular proteinuria, and phosphate leak.

The aim of the present study was to investigate a group of 16 patients who had undergone intestinal je-unoileostomy because of obesity with respect to risk factors of calcium stone formation and renal tubular function. Two surgical techniques were used, i.e. end-to-side jejunoileostomy and end-to-end jejunoileostomy. The operation reduced the length of the small intestine to about 10% of the original. The patients were investigated 5–11 years after surgery. *Results.* Nine patients, i.e. 56%, had recurrent kidney stones after the operation. One of the patients had had one stone before surgery. The body weight was stable in all patients (x = 90 ± 12 kg). The average weight loss after surgery was 39 kg. Seven of the patients (44%) had distal renal tubular acidosis (dRTA), of which one was complete, compared to about 20–25% in patients with idiopathic calcium stones. Five patients (31%) had pathological excretion of β_2-microglobulin. Urinary citrate was low and the urinary oxalate high in all patients compared to healthy controls.

Conclusions. A high incidence of renal tubular defects and low urinary citrate and high urinary oxalate was found among patients following intestinal bypass operations. The changed metabolism following intestinal bypass operations seems to induce an increased risk of stone formation which, among other factors, may be due to such factors as low urinary citrate and high urinary oxalate.

41 Is 24-H Calciuria of Predictive Value for Calcium Stone Recurrence?

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Measurements of 24-h calciuria (Uca) are often performed in recurrent calcium stone formers (CaSF). However, the predictive value for stone recurrence of this parameter remains uncertain. The aim of these studies was to look both retrospectively and prospectively to see if 24-h Uca is related to the formation of new calcium stones. In a first study, 103 recurrent CaSF have been divided into two groups according to their retrospective stone disease history: 46 SF had formed de novo at least three stones or one staghorn calculus within the 5 years preceding the study ("severe" lithiasis), whereas the 57 remaining SF did not ("benign" lithiasis). The 24-h Uca was determined during 3-day hospitalization while on a controlled diet and did not significantly differ between the two groups (5.8 ± 2.3 and 6.6 ± 2.9 mmol/day, SD, in "severe" and "benign" lithiasis, respectively). In a second study, new stone-formation rate (SFR, as stones/year) was determined in 38 CaSF before and during treatment with water diuresis either alone (WD, 19 patients) or with hydrochlorothiazide, 50 mg/day (HCT, 19 patients). Patients were randomly assigned to WD or HCT. Before treatment, SFR was 0.72 ± 0.10 and 0.88 ± 0.11 (SD) in WD and HCT patients, respectively. In both groups, SFR was unrelated to 24-h Uca determined immediately before treatment initiation. During treatment, SFR dropped significantly in both groups, independent of 24-h Uca, which remained unchanged in WD patients and decreased only slightly and transiently in HCT patients. In conclusions, on the basis of retrospective and prospective studies, 24-h Uca appears of little, if any, value for predicting the formation of new calcium stones in recurrent calcium stone formers.

42 Urinary Citrate Excretion of Calcium-Oxalate Stone Formers Before and During Uralyt-U Therpay Compared with Otherwise Normal Adults

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From the beginning of the century citric acid excreted in the urine has been known to form a weakly dissociating salt complex with calcium.

Hence, the question is discussed as to whether lack of organic acids is involved in stone formation. Somewhat different results on the citrate excretion of calcium-oxalate-formers in comparison with normal persons have been reported by Schwille, Butz, Hesse, Bach and our clinic. In 200 healthy test subjects it was investigated whether there is a difference in citrate excretion between males and females. Further, the citrate excretion of a stone-forming population was compared with a control group. In addition, the effect of a sodium-potassium-citrate mixture (Uralyt-U) on the urinary citrate-concentration was explored. The question will be examined as to whether increased calcium excretion frequently correlates with decreased citrate excretion.

43 Renal Calculi and Acetazolamide Therapy

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Renal calculi occurred in 28 of a total of approximately 350 patients during treatment of glaucoma with acetazolamide (0.25–1.0 g/day). The interval between the start of acetazolamide and the first stone episode ranged from 3 months of 8 years. The acetazolamide was discontinued when renal calculi occurred. Ten of these 28 patients were studied 3–16 years after acetazolamide withdrawal. The protocol included 24-h urine collections and a standard 1-g oral calcium-load test. The data are compared with those from a local age- and sex-matched normal control group (n = 10) with no history of glaucoma or stone disease. No significant differences were observed in mean fasting serum levels of calcium, phosphate, magnesium, sodium and potassium, or in mean creatinine clearances. Urinary uric acid and oxalate were within the normal range but 3 out of 10 patients had a 24-h urinary calcium in excess of 250 mg. In response to the oral calcium load, both fasting urinary calcium/creatinine ration (mg/mg) and increments in this ratio following the calcium load were greater in patients than in the normal subjects (fasting ratios 0.11 ± 0.01 vs 0.04 + 0.004, $P < 0.001$; Δpost-load 0.14 ± 0.02 vs 0.08 ± 0.01, $P < 0.05$). Fasting hypercalciuria was present in 4 out of 10 patients. Two of these subjects, in addition to one other, also demonstrated calcium hyperabsorption. These observations suggest that many patients who form renal calculi during acetazolamide therapy may have a pre-existing abnormality of urinary calcium excretion. The alternative possibility that chronic acetazolamide therapy causes persistent hypercalciuria after withdrawal of the drug seems unlikely and is currently being investigated. The screening of glaucoma patients for hypercalciuria before starting chronic acetazolamide treatment may permit the exclusion of many of those at risk for stone formation and thus greatly reduce the incidence of this complication of treatment.

44 Restricted Calcium Diet and Calcium Oxalate Urolithiasis

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Does the prescription of a low calcium diet in hypercalciuric renal stone formers promote the formation of calcium oxalate urolithiasis? Thirty-seven patients with calcium urolithiasis and hypercalciuria (24-h urine calcium > 0.1. mmol/kg) were studied during two periods of time, P1 and P2. During P1 the patients were fed a daily 1-g calcium diet. P2 corresponds to a daily intake of 400 mg of calcium for 4 days. Oxalic acid intake was not changed between P1 and P2.

The average 24-h urine calcium and urine oxalic acid (chromatography in gaseous phase) were, respectively: 10.10 ± 2.5 mmol (mean ± SD) and 0.47 ± 0.41 mmol during the P1 period and 5.69 ± 1.91 mmol ($P < 0.001$) and 0.44 ± 0.28 (NS) during the P2 period. The urine oxalate/urine calcium ratio during the two periods was on average 0.048 ± 0.042 at P1 and at P2 0.085 ± 0.079 ($P < 0.001$). The urinary saturation of calcium oxalate (CaOx) expressed by log 10 (relative CaOx supersaturation) (Marshall PW, Robertson WG: Clinica Chemica Acta 72:253; 1976) was at P1 0.950 ± 0.237 and at P2 0.850 ± 0.220 (NS). Fifteen patients had a ratio higher than 1, i.e. above the formation product (FP) at P1 but only 9 patients at P2.

In conclusion, a restricted calcium diet, without modifying the oxalate intake, does not raise the urinary saturation of calcium oxalate but increases the urine oxalate/urine calcium ratio. Thus, expressed in term of urinary saturation, the lithogenic risk is not increased. However, on a low calcium diet some patients may have an oxalate saturation higher than the FP. In such a case the high urine oxalate/urine calcium ratio may be responsible for producing CaOx crystals of a larger size, increasing consequently the risk of urinary stone formation.

45 Bone Mineral Content in Calcium Nephrolithiasis

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The role of altered skeleton mineral metabolism in calcium nephrolithiasis has not been determined. In vivo neutron activation analysis and whole body counting were used to measure vertebral calcium content (calcium bone index, CaBI) in 109 patients with recurrent calcium nephrolithiasis (average 9 stones in 10 years). CaBI in stone formers (SF) was 0.91 ± 0.13 (SD), significantly lower than 115 normal adults of similar age, 0.97 ± 0.13, $P < 0.01$. There was no distinct group of SF with low CaBI values. This decrease in bone mineral in stone formers was largely due to a decrease in male SF (0.91 SF vs 0.98 normals, $P < 0.02$) rather than female SF (0.93 SF vs 0.97 normals, P = NS). CaBI in male SF was lower than normals from age 20–39 ($P < 0.02$) and age 40–59 ($P < 0.02$), with no correlation with age. Repeat CaBI measurements after an average follow-up of 3.5 years showed no change in CaBI during treatment with dietary calcium restriction, thiazides, or phosphate. CaBI did not correlate with indices of aprathyroid function; serum Ca, PTH, % TRP, or urine cyclic AMP. Fasting urine calcium/creatinine (FUCa/Cr) ratios, however, were higher in patients with lower CaBI, $r = -0.39$, $P < 0.01$.

The decrease in bone mineral content in recurrent stone formers suggests negative calcium balance and is not compatible with a primary increase in intestinal calcium absorption. The decrease in CaBI was not progressive with age or follow-up. The negative correlation of CaBI with fasting urine Ca/creat suggests a renal calcium leak or increased bone turnover, but the lack of correlation with indices of parathyroid activity does not support a primary renal leak of calcium with secondary hyperparathyroidism. The results suggest, therefore, that patients with calcium nephrolithiasis may have a significant disorder to bone metabolism which requires further study.

46 Pyelonephritis and Hypercalciuria

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According to some authors, hypercalciuria (↑Cau) occurs in 30–50% of patients having infection-induced stones. Struvite stones grow secondarily on a preexistent stone, formed as a consequence of the underlying ↑CaU. This paper describes a patient who offered the opportunity of being investigated during active stone formation caused by infection of the urinary tract. RA, 30, in January, 1977, because he had passed two calcium-oxalate stones, underwent a metabolic work-up for urolithiasis which did not demonstrate any disorder. In August, 1978, after a bilateral ureterolithotomy because of recurrent calcium-containing stones, he came under our care with a severe acute pyelonephritis with positive urine culture for Proteus R. During the hospitalization he passed 80 small stones composed of struvite and apatite with a small component of calcium oxalate and underwent left ureterolithotomy for recurrence of obstructing stone. ↑CaU (440–640 mg/day) appeared in association with the infection. In a follow-up of four years, it did not recur as long as the urine remained sterile. ↑CaU was due to renal leak, shown by increased fractional calcium excretion per cent (FECa%) and low levels of serum ionized calcium (Ca^{++}) (table). The fractional sodium excretion per cent (FE Na%) was normal as were the serum total calcium, iPTH, uric acid, phosphate, HCO3, creat. clearance, urinary uric acid and TmPO4/GFR.

Immobilisation or an infection-mediated defect in sodium reabsorption were not the cause of ↑CaU. Our observation suggests that

	CaU mg/day	FeCa %	Ca^{++} mmol/l	FeNa %
Acute phase	480	7.8	1.07	0.7
Follow up	214	1.8	1.15	0.6

urinary tract infection, with a possible contribution from partial obstruction, may have temporarily impaired calcium tubular reabsorption in this patient. Whether this applies to other patients remains to be confirmed.

47 Metabolic Fate and Solubility of Triamterene are not Causative Factors of Triamterene Nephrolithiasis

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Since our initial report of a case of triamterene lithiasis (Ettinger et al., Ann Intern Med, 91:745, 1979) several reports have documented the widespread occurrence of triamterene deposits in kidney stones. Our report concerning the percentage of triamterene was based entirely on a microscopic crystallographic method. Recently, Werness et al. (J Lab Clin Invest 99:254, 1982) suggested that the drug and its metabolites diffuse into the stone matrix. Parts of that hypothesis were based on our preliminary stone analysis (Ettinger et al., JAMA 244:2443, 1980). We now report a more extended analysis of the kidney stones available to us, including the metabolites of triamterene. In addition, we show the results of a study which looked into the possible causative factors of triamterene nephrolithiasis.

Aims of the study. Exact analysis of kidney stones passed during therapy with triamterene. Attempt to explain or exclude the triamterene stone diathesis on the basis of urinary excretion patterns of unchanged triamterene and its metabolites.

Method. Triamterene (T), hydroxytriamterene (T-OH) and hydroxytriamterenesulfuric acid ester (T-O-SO_3H) were measured in urine and buffer solutions by high-pressure liquid chromatography, using reversed phase columns and an acetonitrile/0.02 H_3PO_4 solvent. T, T-OH and T-O-SO_3H analysis in kidney stone material was performed using quantitative thin-layer chromatography.

Results. In the sixty-six stones analyzed the average stone composition of triamterene-derived material was as follows: 42.3% T, 34% T-O-SO_3H, 23% T-OH. In 49% of the stones less than 5% was total triamterene-derived material. Approximately one-fourth of the specimens contained more than 20% of the drug and metabolites. In 85.3% of the stones, T-OH had the lowest percentage of the three compounds tested. Eleven stone-forming patients exhibited concentrations of T and T-O-SO_3H similar to that found in hypertensive patients and normals. However, a unique metabolic pattern was seen in one stone-former, which happened to be our index case. T-OH was not found in urine of either patients or healthy volunteers. In urine we could not observe a dependency of solubility on urinary pH (T: 4 to 36.5 μg/ml, T-O-SO_3H: 3.8 to 64 μg/ml). The solubility in urine was on the average twice as high as in buffer. In buffer, pH dependencies for T and T-O-SO_3H were observed.

Conclusion. The information provided in our studies indicates that the metabolic pattern of triamterene is probably not a causative factor for triamterene nephrolithiasis. The saturation of urine with T and especially with T-O-SO_3H may be related to stone formation, but other physical factors play a role in determining the relative amounts of drug found in calculus material. Together with clinical data from us and others, we also suggest that triamterene-derived material is admixed to kidney stones rather than causing or enhancing their formation.

48 Relation of Severity of Renal Impairment to Tissue Calcium Concentration in the Human Kidney

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MacKay, Oliver and many subsequent investigators have firmly established that renal failure is accelerated in experimental renal disease by procedures which increase calcium deposition in the kidney. Such procedures as high phosphate diet, administration of parenteral calcium, parathormone or vitamin D all lead to nephrocalcinosis, with intense interstitial inflammatory reaction, rapid loss of renal function and death. In contrast, survival is prolonged in experimental renal disease by a low-phosphate diet which markedly reduces the rate of deposition of calcium within the kidney and preserves renal function. The report by Ibels et al. (Am J Med 71:33, 1981) that kidneys from patients with end-stage renal disease have calcium contents that are eightfold greater than that in normal kidneys has focused attention on the possible influence of calcium deposition in the kidney on the progression of human renal disease. Whether this is a later occurrence or an early event which may accelerate the loss of renal function in diffuse renal disease remains to be determined.

We have studied this relationship in renal biopsy material obtained in 71 patients with a variety of renal diseases. Serum creatinine obtained at the time of biopsy provided the index of renal function for comparison with tissue calcium content measured by atomic absorption spectrophotometry on acid digests of a portion of each biopsy. Renal tissue from biopsy specimens from patients with serum creatinine of 1.5 mg/dl or less yielded a mean renal calcium content of 11.9 ± 1.5 μg/100 g wet weight of tissue; those with serum creatinine greater than 1.5 mg/dl had a mean calcium content of 14.7 ± 2.0 μg/100 g wet weight, and for serum creatinine greater than 2 calcium content was 16.1 ± 2.8. In contrast, control tissues from human renal tissue obtained at autopsy from patients without renal disease was 8.9 ± 1.1 μg Ca/100 g tissue. Most importantly, a small but significant positive correlation was demonstrated between serum creatinine and calcium concentration in biopsied renal tissue ($r = +0.24$, $P < 0.5$ ($n = 71$). These data demonstrate that calcium deposition in renal tissue begins early in the course of renal disease.

49 Treatment of Idiopathic Hypercalciuria by Prostaglandin Synthetase Inhibitors

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Idiopathic hypercalciuria is significantly associated with calcium oxalate stone of the kidney, and dietary manipulation to reduce the nutrient density of the diet has been shown to reduce the calcium excretion in the urine [2]. However, about 30% of the patients fail to respond to these dietary measures for one reason or another, and in these it is necessary to have recourse to drug treatment. There is evidence that renal prostaglandin E2 influences tubular excretion of calcium and that prostaglandin synthetase inhibitors may play a role in the control of idiopathic hypercalciuria [1]. Twenty-three calcium stone formers with idiopathic hypercalciuria who fialed to respond adequately to dietary manipulation were included in an open study of flurbiprofen. On at least 3 days, 24-hour specimens of urine were analysed for calcium, magnesium, uric acid, oxalate, and creatinine prior to commencement of the treatment. The pateints were given flurbiprofen at a dose of 50 mg three times a day and urine analysis was repeated 2 and 6 weeks

later. In ten patients urine analysis was also repeated 3 -4 months after discontinuation of the treatment.

There was a significant reduction in the urinary calcium excretion after flurbiprofen therapy, and a tendency for the calcium levels to return to pretreatment values after the medication was stopped. It is concluded that flurbiprofen reduces urinary calcium excretion and may prove to be a useful alternative for the treatment of idiopathic hypercalciuria resistant to dietary measures.

1 Buck AC, et al. (1982) Br J Urol 53:485–491
2 Rao PN et al (1982) Br J Urol 54:478–483

50 Relative Merit of Various Strategies of Nonsurgical Treatment of Infection Stones in Dogs

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Infection-induced stone formation in dogs, as in man, is secondary to colonization of the urinary tract by urease-producing organisms. Recently a low-protein, low-magnesium, low-phosphate, high-sodium diet (stone diet) combined with antibiotic treatment has been shown to induce rapid dissolution of spontaneous infection stones in the urinary bladder of dogs. Other studies have shown that the rate of stone dissolution in vitro is enhanced by progressively reduced relative supersaturation of the surrounding fluid. In this study, urinary relative supersaturation of struvite (RS-S) in dogs receiving a control diet was compared with RS-S during administration of three different strategies of infection stone dissolution: stone diet, antibiotic treatment, and flurofamide treatment (a urease inhibitor). Strategies were given alone, then in combinations.

Five dogs with experimental infection stone disease were sequentially given (1) normal diet; (2) stone diet; (3) normal diet and flurofamide; (4) stone diet and flurofamide; (5) antibiotics; (6) stone diet and antibiotics; and (7) stone diet and antibiotics and flurofamide. After a suitable equilibration period with each strategy, RS-S in 48-hour urine samples was calculated from chemical estimation of urinary MG^{2+}, NH_4^+, PO_4^{3-}, SO_4^{2-}, Ca^{2+}, Na^+, K^+, citrate, oxalate, and pH using the FORTRAN IV computer program EQUIL. The urinary RS-S in each case was (1) normal diet: 18.87 ± 16.31; (2) stone diet: 0.77 ± 1.63 ($P < 0.005$)[a]; (3) normal diet and flurofamide: 3.63 ± 2.90 (NS)[b]; (4) stone diet and flurofamide: 0.08 ± 0.09 ($P < 0.005$); (5) antibiotics: 2.79 ± 2.85 ($P < 0.01$); (6) stone diet and antibiotics: 0.27 ± 0.23 ($P < 0.005$); and (7) stone diet and antibiotics and flurofamide: 0.05 ± 0.07 ($P < 0.005$).

Dietary modification was the single most powerful strategy for reduction of urinary RS-S. Additional simultaneous treatment with antibiotics, flurofamide, or both appeared to reduce urinary RS-S further. However, the magnitude of further decrements was not statistically significant in this study. The results emphasize the need to consider dietary factors in the assessment of strategies for infection stone dissolution in human patients.

[a] *P* values for comparison against normal diet
[b] NS = not significant

51 Effects of Long-term Thiazide Treatment of Bone

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Thiazide diuretics are used to treat renal stone formers with hypercalciuria. They may induce hypercalcemia which is not explained by hypocalciuria. We suggested previously that thiazides may act directly on bone and enhance the release of calcium (Ca). This hypothesis is relevant, since patients undergoing chronic thiazide therapy may develop bone changes.

The effect of thiazides on bone was studied in 28 inbred beagle dogs divided into 4 groups of 7 dogs each. Group 1 was a control group, group 2 received hydrochlorothiazide (HTZ) at a dose of 1.25 mg/kg per day, group 3 received the same dose of HTZ with magnesium (Mg) supplementation, and group 4 was parathyroidectomized (PTX) prior to HTZ treatment. The duration of the study was 12 months. Oral Ca carbonate was given to PTX dogs to maintain normocalcemia. Serum Ca increased in the HTZ-treated PTX dogs and in the HTZ-treated dogs with MG supplementation Serum MG increased and serum potassium decreased in HTZ-treated dogs with MG supplementation. There were no significant differences in serum levels of parathyroid hormone, phosphate, and protein in the dogs with intact parathyroid glands (groups 1–3). Urinary Ca and Mg increased in HTZ-treated dogs with and without circulating parathyroid hormone. However, dogs receiving HTZ treatment and Mg supplementation failed to show an increase in urinary Ca and Mg. The effects of HTZ on quantitative parameters of bone are shown in Table 1.

	1 Control	2 HTZ
Osteocytic density (#/mm^3/cm^3)	223 ± 14	325 ± 40*
Bone mass (mm^3/cm^3)	206 ± 9	209 ± 9
Osteoid volume (mm^3/cm^3)	12.8 ± 2.4	12.0 ± 1.6
Osteoblastic index	1,139 ± 210	959 ± 182
Osteoclastic index	99.5 ± 11.6	91.2 ± 10.4
Mineralization rate (μm/day)	0.65 ± 0.04	0.63 ± 0.03
	3 HTZ + Mg	**4 PTX + HTX**
Osteocytic density (#/mm^3/cm^3)	251 ± 9	303 ± 10[a]
Bone mass (mm^3/cm^3)	221 ± 3	210 ± 8
Osteoid volume (mm^3/cm^3)	11.2 ± 2.3	10.4 ± 2.9
Osteoblastic index	832 ± 97	920 ± 140
Osteoclastic index	96.5 ± 17.9	98.4 ± 10.8
Mineralization rate (μm/day)	0.68 ± 0.06	0.68 ± 0.05

Results are given as mean values ± SE; [a] $P < 0.01$

There was a significant increment in osteocytic density in all HTZ-treated dogs without Mg supplementation, irrespective of circulating parathyroid hormone. It is of note that Mg supplementation prevented the HTZ-induced increase in osteocytic index. No changes were observed in static parameters of bone such as bone mass and osteoid volume, or in static or dynamic cellular parameters of bone formation and resorption such as bone-osteoclast interface, osteoid-osteoblast interface, or mineralization rate. The results provide evidence that HTZ exerts an effect on bone which is demonstrable in the presence and absence of parathyroid hormone. However, this effect is blunted when Mg is added to HTZ therapy. These observations are paralleled by an increase in urinary Ca observed with HTZ treatment. This increase is not seen if Mg is added to HTZ treatment. The data of increased total osteocytic activity and increased urinary Ca with long-term HTZ administration raise the question as to whether other mechanisms than hypocalciuria are responsible for the value of HTZ in the management of renal stone formers with hypercalciuria.

52 Comparison of Medical Treatments for the Prevention of Recurrent Calcium Nephrolithiasis

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In patients with recurrent stones, the important clinical questions of which patients to treat with long-term drug therapy and which

drugs to use remain difficult to answer. A randomized prospective open trial of five medical regimens was carried out in patients with idiopathic recurrent calcium nephrolithiasis. Regular treatment (extra fluids, moderate diet calcium, and oxalate restriction) was compared with regular treatment (Rx) and hydrochlorothiazide (Hct) 100 mg, or sodium phosphate (Phos) 1.5 g, or magnesium oxide (Mg) 400 mg, or allopurinol (Allo) 300 mg daily. In this study, 16% of patients were lost to follow-up; 26% of patients had side-effects, with a higher frequency in the phosphate group (57%, $P < 0.02$). In 94 patients, pre-Rx data for 11.3 years (mean) were compared with treatment results > 1 year (mean 2.8 years).

	Regular	Hct	Phos	Mg	Allo
No. of patients (Pts)	21	23	17	16	17
Pre-Rx stones/years	0.84	0.82	0.81	0.69[b]	1.53[b]
No. of Pts to relapse	8	7	6	7	8
Stones/year with Rx	0.32	0.15	0.32	0.63	0.48
Actual/predicted	44%[a]	21%[a,b]	42%[a]	102%[b]	34%[b]

[a] Different from pre-treatment value, $P < 0.01$
[b] Different from other groups, $P < 0.05$

Pre-treatment stone recurrence rate was higher in patients with relapse on treatment (1.24 stones/years vs 0.063, $P < 0.001$) and pre-Rx urine calcium, uric acid, and oxalate were also higher ($P < 0.05$). Thus these parameters may be helpful in selecting patients for drug treatment. Regular treatment reduced the stone recurrence rate by 56% and phosphate or allopurinol had no additional effect. Magnesium was ineffective. Hydrochlorothiazide was significantly more effective than other treatments, reducing the recurrence rate by 79%, and was the only treatment which also reduced the recurrence rate in patients with relapse.

53 Relation of Clinical Outcome to Urine Biochemistry and Physical Chemistry Under Various Forms of Therapy for Idiopathic Calcium Stone Disease

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In a recent paper we reported that the addition of drugs such as hydrochlorothiazide (HCT) and/or allopurinol (ALP) to a controlled low-calcium-oxalate diet did not seem to add any further benefit as far as the clinical outcome after a mean of 18 months follow-up was concerned. The aim of this paper is to relate the clinical behavior to the biochemical and physical chemical changes induced by each of the above therapies, to explain why different kinds of treatment have failed in providing different results.

All the patients considered had been instructed to eat a low-calcium-oxalate diet and to increase fluid intake. For 40 patients no drug was added, while 105 received HCT and/or ALP in association with the diet. Urine samples were analyzed before any treatment and at 3- to 6-month intervals during treatment for the main ionic constituents and for the saturation levels with respect to calcium oxalate monohydrate (β_{ww}) and to brushite (β_{bsh}).

Most of the changes induced by diet alone did not significantly differ from those obtained by the addition of drugs. In particular, urine volume ($P < 0.05$) and oxalate concentration and excretion ($P < 0.05$) decreased by a similar extent. Calcium and uric acid excretion decreased in all the treatment groups, although decreases during HCT (for calcium) and during ALP (for uric acid) medication were mildly higher. The excretion of citrate, magnesium, and zinc, some of the known inhibitors for which data are available, were not affected by any treatment. β_{ww} and β_{bsh} decreased slightly during all the treatment programs, the decrease being a little more pronounced with HCT.

In conclusion, our biochemical and physical chemical investigations do not fully explain why patients receiving different treatments behave in a similar way. The increase in urine volume, the decrease in calcium and oxalate excretion, and the consequent disappearance of peaks of urine oversaturation, which were quite similar in all the treatment groups, might be the major features to which the beneficial effects of therapy can be attributed. It seems, from our results, that a favorable clinical outcome in the individual patient is linked to the ability to maintain for a long time, rather than to achieve, a urine environment less conductive to stone formation. This seems to depend on the patient's compliance with a stated therapy rather than a selective therapeutic approach.

54 Prevention of Calcium Oxalate Stones by Alkaline Treatment

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Introduction. Citrate is a potent inhibitor of calcium crystal formation. In recurrent oxalate stone formers hypocitraturia and hypercalciuria are frequent findings. Enhancement of renal citrate excretion by the intake of alkaline salts is a well known metabolic mechanism. Thus we inaugurated alkalinizing therapy for prevention of oxalate stones. Results of a 4-year follow-up are presented.

Methods and Results. Twenty-one recurrent oxalate stone formers with an incidence of 1–18 stones/year were treated with Na-K citrate (daily dose 8–12.5, urinary pH 6.8–7.4). The mean period of treatment was 32 months (7–48 months). Citrate excretion increased by 98%, while calcium excretion decreased by 32%. Activity products of Ca oxalate, apatite and Na urate were significantly lowered. The stone incidence dropped from 59 to 19 stones and 5 relapses occurred. The overall success rate was 76%.

Conclusions. The prophylactic effect of long-term citrate therapy in oxalate stone formers is demonstrated for the first time. It emphasizes the pathogenetic role of urinary citrate in calcium stone disease. Compared with alternative therapeutic measures (e.g., thiazides), side-effects and restrictions are rare in citrate therapy.

55 Further Reduction of Oxalate Excretion by Allopurinol in Stone Formers on Low-Purine Diet

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Allopurinol may reduce calcium oxalate stone formation. Small elevations in urinary oxalate are implicated in calculus production, and increased dietary purine increases oxalate excretion [2] Preliminary studies elsewhere [1] showed that allopurinol reduced urinary oxalate in normal subjects and the effect was greater with a high purine intake. The present study was undertaken to determine whether oxalate excretion in stone formers could be further modified by allopurinol when a low-purine diet was adhered to.

Ten patients with recurrent calcium oxalate stones completed the study. The patients had normal renal function and no obvious metabolic abnormality. Excessive oxalate ingestion was avoided and calcium intake controlled. Allopurinol 300 mg daily was given during the third and fourth weeks and the seventh and eighth weeks. At the end of each 2-week period 24-h urine collections were made. Urine oxalate and glycollate were measured by enzymatic methods. Urinary creatinine, urate, and calcium were measured by standard laboratory methods.

The 24-h creatinine excretion remained constant during the study period. Allopurinol treatment produced a significant reduc-

tion in urate and oxalate excretion (Table 1). Urine glycollate and calcium remained unchanged. Mean oxalate and urate excretion for each patient showed a significant correlation ($r = 0.51, P < 0.001$).

Table 1. Urine constituents on low purine diet before and after allopurinol (mean ± SEM in μmol/mmol creatinine excretion)

Weeks	Diet alone		
	1–2	5–6	
Calcium	466 ± 74	344 ± 68	
Urate	360 ± 27	320 ± 26	
Oxalate	19.9 ± 1.8	16.9 ± 1.4	
Glycollate	18.7 ± 2.1	17.8 ± 1.7	
Weeks	Diet + allopurinol		P^a
	3–4	7–8	
Calcium	429 ± 74	451 ± 58	NS
Urate	220 ± 28	200 ± 22	<0.00005
Oxalate	15.3 ± 1.6	14.2 ± 1.0	<0.005
Glycollate	22.0 ± 2.7	17.6 ± 1.2	NS

[a] Comparing mean levels with diet alone against mean levels with diet + allopurinol

Previous work has produced conflicting evidence about the effect of allopurinol on oxalate excretion. Even with the reduced urinary oxalate produced by purine restriction in our patients we were able to demonstrate a further reduction with allopurinol treatment. Zarembski and Hodgkinson (1969) suggested a metabolic pathway from purine to oxalate and it may be that allopurinol reduces oxalate excretion by interfering with this pathway rather than having any direct effect upon oxalate absorption or metabolism, as the changes in urate excretion were proportionally higher than changes in oxalate. The effects on oxalate excretion would be more pronounced with a high-purine diet.

[1] Simmonds Ha, et al (1981) In: Clinical and Basic Research. Plenum Press, New York, pp 363–367

[2] Zarembski PM, Hogkinson A (1969) Clin Chim Acta 25:1–10

56 Dissolution of Phosphate Stones by Percutaneous Nephrostomy and Local Irrigation

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Urine pH plays a critical role in the origin and growth of phosphate containing renal stones. Conversely, it is possible to dissolve phosphate stones in solutions with low pH values. The solutions used for local irrigation are citric acid- and magnesium-containing solutions with pH values of 3.8–4.1 (Suby's solution or Sol. G, Renacidin or Sol. R). Usually, this kind of treatment has been used to dissolve residual stones after operation. However, local irrigation of the kidney may also be an alternative treatment, especially when an operation is not possible or is contraindicated.

In this paper we present our experience and results with local irrigation in 17 kidneys (15 patients) with phosphate stones [calyx or pelvic stones (4), or staghorn calculi (13)].

Twelve kidneys had been operated upon previously (once or several times) at least 6 months before this treatment. In three kidneys (two patients) surgical removal of the stones was judged to be impossible. In three other patients operation was contraindicated by the general condition. The irrigation fluid was Sol. G or R, administered through a percutaneous nephrostomy catheter and a normal infusion system. The maximal flow was 200 ml/h. For the first 2 weeks all the patients received antibiotics. Eight patients had positive urine cultures at the start of the treatment. The duration of the treatment varied from 10 to 101 (mean 36) days. During this period all the patients were ambulatory. Nine patients were treated partly on an out-patient basis.

In seven kidneys with staghorn calculi, the stones were dissolved completely. In six kidneys minimal stone fragments remained. In two of the four kidneys with calyx ro pelvic stones no improvement could be obtained. In no single patient did the GFR worsen. The mean serum creatinin values (+/– SD) before and after therapy were 96 (+/– 36) and 88 (+/– 32) μmoles/liter, respectively. The most frequently noted side-effect was dysuric pain, which could be alleviated by diluting the irrigation fluid with saline. Seven patients had a temporary febrile reaction which necessitating discontinuation of the irrigation for some days. In nine cases the catheter was changed because of displacement of the catheter or to improve the contact of the irrigation fluid with the stones. No other significant complications were recorded.

In conclusion, this treatment proved to be a safe and good alternative to surgical removal of staghorn calculi, suitable for use expecially when an operation is judged to be difficult because of previous operations, or is impossible for other reasons. This approach is not contraindicated by the presence of urinary tract infection.

57 Magnesium in Citric Acid and the Dissolution of Struvite and Hydroxyapatite

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When surgical removal of phosphate stones is contraindicated, impossible, or otherwise rejected, a possible approach is local irrigation with citric acid-containing solutions. As long ago as 1943, Suby suggested that the presence of magnesium was useful in promoting stone dissolution. In addition it proved to be useful in alleviating the symptoms of irritation of the ureter and bladder. The solutions in use today are Sol. G, or Suby's solution, and Sol. R, or Renacidin.

The similarities of these solutions are the final pH of 3.8–4.1, the high content of citric acid (about 300 mmol/liter for Sol. R and 150 mmol/liter for Sol. G) and the magnesium content (about 300 mmol/liter for Sol. R, and 20 mmol/liter for Sol. G). Depending on the particular solution, some minor further additives, such as sodium, calcium, EDTA and gluconolacton are included. One of these solutions, Sol. G, is isotonic. To clarify some questions about the effectiveness of some of the constituents in helping to dissolve urinary stones, in vitro studies on tabletted pure struvite and hydroxyapatite were carried out. The main interest was focused on the effectiveness and presence of magnesium ions.

The experimental set-up consisted of a continuous perfusion of the tablets (positioned in the center of a filter-lined funnel) from a reservoir with the solution to be studied. At regular time intervals samples from the reservoir were taken and analysed for calcium (in the case of hydroxyapatite) and ammonium (in the case of struvite). The effectiveness of Sols. R and G and dilutions of them was compared with that of pure citric acid solutions in the same concentrations.

The results allow the following conclusions: at comparable citric acid concentrations of 150 mmol/liter pure citric acid shows a significantly better dissolution capacity for both hydroxyapatite and struvite, Sol. G Being obviously more effective than Sol. R. These differences are explained by the different magnesium concentrations. Undiluted Sol. R (285 mmol citrate/liter) appeared to be superior to Sol. G (150 mmol citrate/liter) for dissolving struvite,

whereas both solutions appeared to be equally effective for hydroxyapatite.

From these studies we conclude that addition of magnesium ions has a negative effect on the in vitro dissolving capacity, pure citric acid solutions having the highest capacity. Clinical experience, however, has clearly shown that magnesium does indeed exert a substantial alleviating effect of the irritation experienced by the patient. Therefore it seems best to find a compromise between dissolution effectiveness and practicability for clinical use.

58 Methenamine Hippurate (Hiprex) and Potential Urinary Risk Factors of Renal Stone Formation

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Methenamine hippurate (Hiprex) has been used widely for the prevention of recurrences of lower urinary tract infections. It has also been shown to postpone encrustation of urinary catheters and seems to have very few side-effects. We know from micropuncture studies in the rat that there seems to be a decrease in the tubular secretion of oxalate when para-aminohippurate is given IV. Previous studies suggested an increased solubility of calcium oxalate by hippurate (Elliott) and of uric acid by formaldehyde (Vermeulen), the metabolite of methenamine. This prompted us to perform the present metabolic study with Hiprex.

Methods. A total of 15 healthy subjects on the staff each received 3 g Hiprex daily for 2 weeks. For each subject 24-hour urine collections were made before and during Hiprex medication and measurements were made of electrolytes, urate, oxalate, citrate, titratable acids, and ammonia. The pH was measured in freshly voided urine. Oxalate measurements were made with an enzymatic method, as hippurate had been shown to interfere with the color reaction in the colorimetric procedure based on cromotrophic acid. The inhibitory activity of calcium oxalate crystal growth was measured in diluted urines.

Results. There was a significant increase in the urinary excretion of ammonium ions and a decrease in titratable acid ($P < 0.001$). Urine pH increased from 5.6 ± 0.5 to 6.2 ± 0.7 ($P < 0.05$). No significant changes in urinary calcium, magnesium, phosphate, urate, oxalate, or citrate were observed, whereas favorable tendencies were found and the risk index (calcium x oxalate) x magnesium x citrate)$^{-1}$ decreased from 0.124 ± 0.07 to 0.095 ± 0.06 ($P < 0.05$ paired *t*-test). No change in the inhibitory activity of the urines could be shown.

Conclusion. The potential of methenamine hippurate for the treatment of calcium oxalate stones is not very convincing. No decrease in oxalate was seen. The effect on urine pH may be of interest for uric acid stone disease. Whether the described metabolic effects would be different in stone formers remains to be seen.

59 Thiazide Prophylaxis of Urolithiasis: A Double Blind Study in General Practice

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In all 50 recurrent stone formers were included in a double blind randomized study (median 3 years) performed in a Norwegian general practice to compare twice-daily administration of 25 mg hydrochlorthiazide versus placebo.

The number of patients with new stones was significantly higher in the placebo group than in the thiazide group ($P = 0.05$, one-sided test). If a new stone was formed, thiazide, but not placebo had the effect of prolonging the stone-free interval ($P \leq 0.01$). The probability of not forming a new stone during the treatment period was 45% for the placebo group and 75% for the thiazide group. The thiazide effect seemed to be independent of urinacy calcium, but was less beneficial in patients with hyperuricosuria. The placebo group also showed a substantial decrease in the expected number of new stones ($P \leq 0.01$), emphasizing the importance of having an adequate control group.

60 The Effect of Long-Term Treatment with Allopurinol on Stone Recurrence in Calcium Urolithiasis

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The beneficient effect of allopurinol in hyperuricosuric calcium urolithiasis has been shown by Coe and Smith, who assumed a lower excretion limit of 4.5–4.8 mmol/day for hyperuricosuria. It was the aim of this study to define the circumstances in which treatment of calcium urolithiasis would be successful on the basis of a long-term trial in different types of hyperuricosuria, with and without simultaneous hypercalciuria and with no pathological findings. In all 126 outpatients, predominantly with recurrent calcium urolithiasis, were treated with 300 mg allopurinol/day for 1–5 years (mean 2.5). Of these, only 10 have formed recurrent stones up to now. The factors probably accounting for the recurrences are interruption of treatment (2), extreme hypercalciuria (3), inadequate reduction of hyperuricosuria (3), and unknown (2). It may be concluded from the results that treatment of calcium urolithiasis with allopurinol is advisable for patients forming uric acid/urate-containing stones and for those with hyperuricosuria (> 4 mmol/day) and calcium excretion < 10 mmol/day.

61 Lithogenic and Alithogenic Urinary Constituents Before and During Therapy with Allopurinol

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The aim of the present study was to investigate (1) how treatment with allopurinol affects the excretion of urinary constituents which might be of importance to stone formation; (2) in which cases such treatment might be indicated; and (3) how these indications and the effect of treatment could be checked by laboratory measurements.

Allopurinol 300 mg/day was administered to 31 outpatients for 2–18 weeks. The following parameters were determined in portions of 24-h urine before and after the trial: creatinine, calcium, magnesium, phosphate, citrate, oxalate, pyrophosphate, and pH; uric acid was determined in both urine and serum.

The following results were obtained: (1) Treatment with 300 mg allopurinol/day exerts the known effect of a highly significant decrease of uric acid in serum and urine; (2) there is no significant correlation between the decline of uric acid in serum and that in urine; (3) the decreasing effect of allopurinol on uric acid increases absolutely and relatively with increasing urinary excretion. From this can be concluded that treatment of urolithiasis with allopurinol is indicated in hyperuricosuria (and not hyperuricosemia) with excretion of more than 4 mmol/day.

62 Use of Wheat Bran in Prophylaxis of Calcium Oxalate Patients and for Reduction of Calcium Excretion

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Twenty patients with idiopathic hypercalciuria with calcium oxalate stone diathesis were treated with 24 g wheat bran daily. The patients took the wheat bran either in one portion in the morning or distributed over the day in two or three portions. Serum Na, K, Cu, P,

creatinine, and uric acid and Na, K, Ca, P, Mg, Zn, Fe, Cu, citrate, oxalic acid and uric acid in the 24-h urines were determined 1 day before the beginning of the therapy and every 6 weeks during the therapy for about 9 months. At the beginning the patients had no stones and were symptom-free.

Four patients stopped the treatment in the early weeks because of diarrhea and flatulence in 2 cases). Therefore only 16 patients were treated and studied throughout the period envisaged. There was a striking and significant decrease of Ca (11.31 ± 5.30 mmol/24 h before and 6.06 ± 2.52 mmol/24 h after therapy) and Fe (1,094,81 ± 682.88 μg/24 h before and 315.53 ± 162.68 μg/24 h after therapy). Only in one patient did the calcium excretion not alter. He had colic with discharge of a calcium oxalate stone. There was no change in the excretion of Na, K, P, Mg, Zn, Cu, citrate and oxalic acid in the 24-h urines. The uric acid excretion decreased from 1,033.66 ± 409.69 to 893.13 ± 363.49 mg/24 h, but this change was not significant.

This study shows that the wheat bran therapy leads to a decrease in the excretion of Ca and Fe. Wheat bran is able to correct hypercalciuria in the calcium oxalate stone diathesis and it is a 'natural' alternative to the thiazide therapy. We can recommend it for aftercare following treatment for calcium stones.

63 Influence of Magnesium Therapy in Calcium Oxalate Stone Patients on Lithogenous Substances

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Seventeen calcium oxalate stone patients who were free of infection were treated with a magnesium preparation for a period of about 2 years as aftercare to prevent relapse. The patients treated in this study had hypomagnesiuria or an elevated Ca/Mg quotient. Serum creatinine concentrations were normal. Serum sodium, potassium, calcium, creatinine, and uric acid were determined over periods of about 3 months. Magnesium, citrate, and oxalic acid were determined in the 24-hour urines. The influence of the magnesium treatment on the course of these parameters and the effectiveness of the therapy (i.e., absence of recurrence) were studied.

64 Water-Soluble Nondialysable Fraction of Tamarind in Preventing Recurrence of Renal Stones

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The incidence of renal calculi in South India is strikingly low compared with to North India. Sur et al. [1] showed by inhibitor assay that daily feeding of 10 g tamarind pulp to healthy medical students markedly reduced the calcifying propensity of their urine. It appeared this effect might be due to the chelating effect of the large amount of tartaric acid present in tamarind.

When the quantity of tamarind pulp fed daily was reduced to 3 g the tartaric acid in the urine of normal adults diminished to negligible amounts but the inhibitory effect of tamarind intake on the calcifying properties of urine was not reduced. This showed that something other than tartaric acid was the principal inhibitory factor in tamarind. This was confirmed by trials in which tamarind pulp was given after exhaustive dialysis with distilled water. The inhibiting effect of tamarind remained unchanged after complete removal of tartaric acid.

Inhibitory assay studies on urine show that the principal active substance of tamarind is present in a water-soluble, nondialysable fraction comprising 1/30th of the whole gives positive test results for carbohydrate and protein, and reduces Cu^{2+} as used for blood sugar estimation. This fraction, when fed in a daily dose fo 150 mg only to adult normal men rendered their urine strongly resistant to calcium oxalate crystallization. Since the nondialysable fraction must be absorbed after hydrolysis, the finally effective fraction may have to be fed in still smaller amounts.

After two operations for renal stone one patient has been taking tamarind for $6^1/_2$ years without further recurrence. Generally it is difficult to pursuade North Indian patients to take large amounts of tamarind. If the active inhibiting factor of tamarind can be isolated it may provide a practical way of preventing the recurrence of renal stones.

1 Sur BK et al (1980) In: Smith PH, Robertson WG, Finlayson B (eds) Urolithiasis, clinical and basic research. Plenum Press, New York London, pp 333–336

65 Effectiveness of Hemodialysis and Continuous Ambulatory Peritoneal Dialysis (CAPD) in Controlling Plasma Oxalate Concentrations

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The plasma oxalate value was determined in patients before and after hemodialysis and during CAPD. The concentration of oxalate in plasma and dialysate was monitored in a patient undergoing CAPD over a period of 18.5 h, being assayed at the end of each of three exchanges. The total quantity of oxalate removed by dialysis over this period was derived from these results. Urinary oxalate excretion by patients undergoing hemodialysis and CAPD was also determined. Urinary and plasma oxalate were assayed as previously described [1, 2], while oxalate in the dialysate was determined by a modification of the plasma procedure [2]. The plasma oxalate concentration before and after hemodialysis is shown in Table 1 and that of CAPD patients in Table 2.

Table 1. Plasma oxalate concentrations before and after hemodialysis

Subjects	Anhydrous oxalic acid μg/100 ml			Frequency of dialysis
	Pre-dialysis	Post-dialysis	% Decrease	
M. C.	852	476	44.1	5 h 2 times/week
J. M.	560	250	55.4	5 h 2 times/week
I. T.	620	360	41.9	5 h 2 times/week
M. K.	828	455	45.0	4 h 3 times/week
C. D.	710	436	38.6	4 h 3 times/week
A. C.	971	522	46.2	4 h 3 times/week
D. H.	495	270	45.5	4 h 3 times/week
P. L.	490	230	53.0	4 h 3 times/week
			46.2 ± 5.5 (mean ± SD)	

Table 2. Plasma oxalate concentrations in CAPD patients

Subjects	Anhydrous oxalic acid (μg/100 ml)
C. B.	490
T. W.	450
J. G.	470
B. G.	410
B. C.	380
D. M.	290
M. G.	490

The total oxalate removed by dialysis in a CAPD patient over 18.5 h was 20.66 mg, equivalent to 26.8 mg/24 h. Urinary oxalate excretion was 23.74 ± 11.5 mg/24 h (mean ± SD) in eight hemodialysis patients and 4.92 ± 2.94 mg/24 h in five CAPD patients.

Plasma oxalate decreased sharply with hemodialysis (46.2% ± 5.5% mean decrease), while with CAPD plasma values stayed more constant. The oxalate removed in one patient with CAPD over 18.5 h amounts to 26.8 mg/24 h, which is close to the endogenous synthesis of oxalate in man. This finding supports the view that CAPD is capable of maintaining a reasonably constant plasma oxalate concentration. Predialysis plasma oxalate values in hemodialysis patients are significantly higher than in those treated with CAPD ($P < 0.005$). Because of the higher plasma oxalate values achieved in hemodialysis, these patients appear to be at greater risk of depositing calcium oxalate in the tissues.

1 Costello J, Hatch M, Bourke E (1976) J Lab Clin Med 87:903
2 Maguire M, Fituri N, Keogh B, Costello J (1981) Urolithiasis: Clinical and basic research. Plenum Press, New York, p 963

66 The Influence of Flurbiprofen on Calcium Excretion and Vitamin D3 in Recurrent Calcium Lithiasis – A Double Blind Study

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Hypercalciuria and hyperoxaluria in idiopathic renal stone formers is mainly attributed to calcium oxalate hyperabsorption. Vitamin D3 is the principle hormone that determines the capacity of the gut for active calcium absorption and serum 1, 25 (OH) 2 D3 levels are indeed raised in some hyperabsorptive hypercalciurics. Recent experiments have shown that the stimulus to the renal 1 α-hydroxylation of vitamin D3 is prostaglandin mediated [1]. The aim of this study was to evaluate the effect of prostaglandin inhibition with flurbiprofen on urinary calcium excretion and vitamin D3 metabolism in stone formers.

Method. Forty recurrent idiopathic stone formers were entered into a double blind placebo-controlled cross-over trial with flurbiprofen (50 mg t.d.s.). The patients were randomly allocated to either placebo or flurbiprofen, with a cross-over at 4 weeks. The following investigations were carried out before treatment at 4 weeks and at 8 weeks: 1. Three checks of 24-h urinary calcium, oxalate, phosphate, urate, Mg and electrolyte excretion. Creatinine clearance (GFR), cAMP and prostaglandin metabolites were assayed. 2. Two-h renal threshold phosphate concentration according to Walton and Bijvoet [2]. 3. Serum calcium, 1, 25 (OH) 2 D3, and PTH.

Results. There was a significant reduction ($P < 0.01$) in urinary calcium excretion and in calcium oxalate/phosphate activity products following flurbiprofen compared with placebo and pretreatment values. A fall in uric acid excretion was observed with both placebo and flurbiprofen. There was no significant change in Mg or Na excretion or in GFR with flurbiprofen.

Serum 1, 25 (OH) 2 D3 levels were significantly reduced in 73% of patients, but there was no change in PTH urinary cAMP or TmP/GFR, indicating that this fall was unrelated to parathormone activity.

These results confirm our previous observation that PG inhibition reduces urinary calcium excretion. The influence of NSAIDs on vitamin D3 levels has important implications for the management of hyperabsorptive hypercalciuria.

1 Wark JD et al (1981) Regulation of 25-hydroxy-vitamin D-hydroxylase in chick isolated renal tubules: Effect of prostaglandin E2, frusemide and acetylsalicylic acid. Clin Sci 61: 53–59
2 Walton RJ, Bijvoet OLM (1975) Nomogram for derivation of renal threshold phosphate concentration. Lancet II:309–310

67 Treatment of Hyperoxaluria in Patients with Jejunoileal Bypass: Effects of Calcium, Aluminium, Magnesium, and Cholestyramine

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Hyperoxaluria and calcium oxalate urolithiasis are common following intestinal resections and jejunoileal bypass operations. High intestinal absorption of dietary oxalate appears to be responsible for the hyperoxaluria. Because oxalate has to be in solution before absorption, one way to decrease its absorption is to reduce the amount of free oxalate in the intestinal lumen. Oral administration of calcium, aluminium, magnesium, and cholestyramine have all been reported to decrease the oxalate excretion in patients with enteric hyperoxaluria. None of these studies was performed in outpatients and therefore such studies are desirable.

Aim. The aim of the present investigation was therefore to study the effects on urinary composition in patients with hyperoxaluria after jejunoileal bypass operations following administration of calcium, aluminium, magnesium, and cholestyramine under outpatient conditions.

Methods. Seven patients with hyperoxaluria following jejunoileal bypass were investigated. The study was performed on an outpatient basis before and during daily administration or 38 or 113 mmol calcium, 28 mmol aluminium, 20 mmol magnesium, or 16 g cholestyramine. Each substance was administered for 7 days, with free intervals of at least 7 days.

Results. The mean urinary oxalate excretion was not reduced with any of these regimens. Administration of 38 mmol calcium/day resulted in increased magnesium excretion. Increased of both calcium and citrate was observed during administration of 113 mmol calcium/day. Calcium and magnesium excretion was increased with aluminium. An increased magnesium excretion was also observed during administration of magnesium, resulting in a decreased calcium/magnesium ratio. Cholestyramine resulted in increased oxalate and decreased citrate excretion.

Conclusion. An increased risk of recurrent stone formation, oxalate deposits, and renal failure attributable to hyperoxaluria exists in patients with jejunoileal bypass. It is therefore important to reduce the oxalate excretion in this group of patients. In our hands calcium, aluminium, magnesium, and cholestyramine all failed to have such an effect.

68 Dietary Treatment of Hyperoxaluria Following Jejunoileal Bypass

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Hyperoxaluria is common following intestinal resection and jejunoileal bypass (JIB). Besides a high incidence of urolithiasis, the hyperoxaluria may result in renal failure due to oxalosis. High intestinal absorption of dietary oxalate seems to be responsible for the hyperoxaluria. Diets low in oxalate and fat decrease oxalate excretion during metabolic ward conditions. However, little is known about the effect of such diets in outpatient conditions.

Aim. The aim of the present investigation was to study the effects of diets low in oxalate and fat on oxalate excretion in patients with hyperoxaluria following JIB.

Method. Ten patients who had undergone JIB > 3 years before the investigation because of obesity were studied on an outpatient basis. A diet aimed to reduce the intestinal load of oxalate and fat was designed individually for each patient. Urine was collected before and after 1 week with the regimen.

Results. All pateints had hyperoxaluria before starting the dietary treatment, with a mean of 1.10 ± 0.11 mmol/24 h (± SEM, normal < 0.45 mmol/24 h). During treatment oxalate excretion decreased in all ten patients to a mean (± SEM) of 0.70 ± 0.07 mmol/24 h.

Conclusion. This study shows that oxalate excretion in patients with hyperoxaluria following JIB might be reduced by diets low in oxa-

late and fat even during outpatient conditions. We think that efficient regular dietary information, preferably, provided by a dietitian, is of great value in the treatment of these patients.

69 Experience with Long-Term Thiazide Treatment in Calcium Oxalate Stone Disease

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During recent years conflicting evidence has been presented concerning the clinical effects of thiazides on renal stone formation. Although urinary calcium reduction is the rationale for this treatment, some studies have shown that the clinical effect is independent of the pretreatment calcium level. An effect on urinary oxalate has been suggested as an additional explanation for the reduced stone formation.

Patients. In an 85 patients with recurrent calcium oxalate stone disease or operated on because of calcium oxalate stones were treated with 2.5–5 mg bendroflumethiazide daily. Of these patients, 26 stopped therapy within 2 years because of side-effects, while 59 continued treatment with a mean observation period of 3.7–1.0 years. Before treatment, 40 patients had hypercalciuria, 6 had a high calcium/magnesium quotient, 4 had hyperoxaluria, and 6 had a normal urine composition; 3 could not be classified.

Results. Of the 59 patients who continued with the treatment, 21 had side-effects. Eight patients had recurrence of stone formation and another two patients had significant growth of stones. Patients with recurrence had higher pretreatment stone rates, but no correlation with biochemical findings could be demonstrated. The mean stone rate decreased from 0.31 to 0.12 stones per year. The biochemical composition of serum and urine changed during the first year of treatment, but was then stable. In serum, potassium and magnesium concentrations decreased, whereas urate increased. In urine, calcium excretion and the calcium/magnesium quotient decreased, while citrate and oxalate were unaffected. The calcium oxalate risk index (an estimate of the risk of forming supersaturated urine) decreased. No effect on urine flow was recorded.

Conclusion. A reduced rate of stone formation was apparently the result of treatment with bendroflumethiazide, but 17% of the patients continued to form stones. Side-effects were surprisingly common. A reduced calcium excretion was recorded, but the oxalate excretion was unchanged. Further studies with biochemically and clinically well defined control groups are highly desirable.

70 Urine Composition in Calcium Oxalate Stone Formers During Treatment with Alkali

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There is experimental evidence that the rate of calcium oxalate (CaOx) crystallization in urine decreases at pH values above 6.0–6.5. The inhibition of CaOx crystal growth also appears to be increased in this pH range. Furthermore, several stone formers have a low citrate excretion.

Aims. This study was undertaken to obtain information in the effects of alkali on urine composition.

Methods. Fifteen patients with recurrent CaOx stone disease were given 2.5 g of a mixture of sodium and potassium citrate (Renapur) three times daily. The 24-h urine composition was analyzed with reference to calcium, oxalate, citrate, magnesium, urate, and inhibition of CaOx crystal growth rate. In a few patients the effect on pH was studied in fractionated 24-h urine collections.

Results. The most pronounced finding was an increase in urinary citrate ($P < 0.002$). This resulted in a decreased calcium/citrate quotient ($P < 0.002$), and a lower CaOx risk index ($P < 0.005$). A slightly higher urine flow ($P < 0.01$) contributed to the reduced AP(CaOx) index (an estimate of the ion activity product of CaOx). Urinary oxalate, magnesium, urate, and inhibiting activity (at pH 6.0) were unaffected by the treatment.

Conclusion. Administration of alkali in this form favorably affected urine composition regarding risk factors of CaOx crystallization. Such treatment might thus be a useful alternative in prevention of CaOx stone formation, particularly if administered during the periods of the day when a high CaOx supersaturation coincides with a low pH. In as much as an increased risk of calcium phosphate stone formation cannot be excluded, carefully supervised long-term trials with alkali are imperative.

71 Efficacy and Safety of Non Steroidal Anti-Inflammatory Drugs in Ureteral Colic: A Double Blind Controlled Trial

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Renal PGE_2 values rise during ureteral colic (UC). PGE_2 enhances ureteral wall tonus and produces an increase of both renal vascular resistances and glomerular filtration rate, probably because of an improvement of natriuresis. Since nonsteroidal anti-inflammatory drugs (NSAIDs) act through an inhibition of PGs biosynthesis, preliminary studies have been performed to assess their activity in UC: diclofenac and indomethacin previously proved to be superior to placebo. The aim of the study was to compare these two potent well known NSAIDs, i.e., diclofenac (D; 75 mg/amp) and indomethacin (I; 50 mg/amp), with a widely used assocation of a NSAID with two spasmolytic agents: noramidopyrine 1 g, pitofenon 0.4 mg, fenpiverine 0.04 mg/amp (C).

Seventy-five consecutive patients (pts) entered a randomized, double blind (DB), interpatient, controlled trial. The diagnosis was confirmed by urine analysis, IV pyelogram, or voiding of a calculus. Pain intensity was measured according to a 100 mm analog chromatic continuous scale (ACCS), i.e., a modified form of the standard VAS, in basal conditions and 30, 60, 120, 180, 240, and 300 min after treatment. Each pt received an IM injection of D, I, or C according to a randomization list. If the pain intensity (ACCS) had not decreased by 50% of the initial value 60 min after the injection, the patient received a second injection of the same drug, always in DB manner. Any patient not experiencing a decrease of pain intensity according to the same criterion after the second injection was dropped from the trial.

Statistical analysis was performed with either parametric or nonparametric tests according to both distribution and quality of the data. The three treatment subsamples were homogeneous for age, sex, weight, height, and severity and duration of initial pain. Complete relief of pain at 30 min (ACCS = 0 mm) was obtained in 74% (20/27) of pts with D, in 79% (19/24) with I, and in 41.7% (10/24) with C (χ^2-test, $P < 0.02$). The split-plot ANOVA of pain intensity at the different times showed a significant difference between treatments ($P < 0.05$) and between times ($P < 0.01$). The partitioning of the comparison between treatments showed that both D and I were superior to C ($P < 0.05$), while no significant difference was detected between I and D.

Eight pts dropped out, three in the D group, one in the I, and four in the C group (χ^2-test, N.S.). No undesirable side-effects were reported during the trial.

These results confirm the good efficacy of IM injection of NSAIDs, such as diclofenac and indomethacin, in UC. The antispasmodic component of the association C seemed not to produce any advantage over the NSAID component, which is probably the reason for the weak activity of this combination. These data seem to be very encouraging in terms of both efficacy and tolerability, suggesting the use of NSAIDs as drugs of choice in UC.

72 Effect of Orthophosphate Treatment on Urine Composition in Idiopathic Calcium Urolithiasis

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Oral neutral orthophosphate (OP) has been shown to be effective in the treatment of patients with idiopathic calcium urolithiasis (ICU). To determine the effect of therapy on urine composition, we studied a group of 19 patients with ICU, while receiving no treatment and while being treated with OP in a dose providing the equivalent of 1.5–2.0 g elemental phosphorus per day. Each study period was conducted in a clinical research unit, while the patients were consuming their normal diet and fluid intake as determined by diet history.

Urine pH and major ions were determined, and the EQUIL program was used to calculate supersaturation. Inhibitors of crystal growth of calcium oxalate and hydroxyapatite were assayed by means of seeded crystal growth systems. Crystalluria was assessed in each freshly voided urine sample.

Urine volume, osmolality, and ionic strength did not change during therapy. Total urine calcium excretion decreased (mean change −0.25 mole/mole creat., $P < 0.001$), and ionized calcium concentration decreased (mean change 1.3 mmoles/liter, $P < 0.001$). The ratio of ionized calcium concentration to total calcium concentration decreased (mean change 0.2, $P < 0.001$), indicating increased complexation of calcium with treatment. Total oxalate and urate excretion did not change, and total phosphate excretion increased (mean change +2.87 mole/mole creat., $P < 0.001$). The urinary pH increased. Supersaturation decreased for calcium oxalate and uric acid, but did not change for hydroxyapatite, brushite, and sodium urate.

There was no change in inhibitor activity of calcium oxalate crystal growth; inhibitor activity of hydroxyapatite crystal growth increased (mean change +15 IU/liter $P < 0.005$). Excretion of the individual inhibitors pyrophosphate and citrate increased (mean change +3.0 mmole/mole creat. and +0.06 mole/mole creat., respectively, $P < 0.001$ for both). Magnesium excretion decreased (mean change −0.11 mole/mole creat., $P < 0.001$). The crystalluria score decreased (mean change 0.55, $P < 0.005$).

The beneficial changes brought about by orthophosphate therapy included a decrease in urine calcium excretion with an increase in calcium complexation secondary to increased urinary phosphate, citrate, and pH. This resulted in a decreased supersaturation of calcium oxalate, without an increase in supersaturation of the phosphate-containing phases, hydroxyapatite and brushite. The excretion of the inhibitors pyrophosphate and citrate increased, as reflected in the increased inhibitor activity of hydroxyapatite crystal growth. Crystalluria decreased to normal.

73 Placebo-Controlled Double Blind Study of Allopurinol in Severe Recurrent Idiopathic Renal Lithiasis. Preliminary Results

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Starting in 1973, a group of 30 patients with severe recurrent idiophatic calcium oxalate stone disease, entered a double blind study of allopurinol 300 mg/day versus matching inert tablets. Criteria for entry included a minimum recurrence rate of two stone episodes a year for 3 years prior to entry. Only 15 patients have completed 3 years' observation, 8 receiving allopurinol and 7 placebo. The rates of stone formation for 3 years before and 3 years after entry were compared in both groups. A striking fall in the rate was observed in both, from 3.29 to 0.96 stones per patient/year with allopurinol and from 1.81 to 0.66 stones per patient/year with placebo.

The reduction rate was marginally better in the allopurinol group but the difference was not of statistical significance. These results confirm the importance of irgorous control in such trials and the benefit of intensive management of such patients. The study continues, with the remaining patients still being treated under double blind conditions.

74 Appraisal of Methodology in Studies of Either Thiazide or Orthophosphate Therapy for Recurrent Calcium Urolithiasis

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Both thiazides and orthophosphates are generally considered effective in the prevention of recurrent calcium urolithiasis, but several recent clinical trials have failed to confirm this effectiveness. Therefore, clinical trials evaluating the treatments were reviewed. The criteria for review were English-language publication, a well defined clinical event as the outcome measure, and a well described treatment. The study design was then classified and evaluated. Fourteen studies (9 thiazide and 5 orthophosphate) satisfied these criteria. Eleven had a quasiexperimental design (i.e., lacking a randomly allocated control group); 3 had a true experimental design. Of the 11 quasiexperimental design studies, 1 (orthophosphate) Trial determined the outcome in a treated group only after the treatment, while 6 (3 thiazide and 3 orthophosphate) compared the rate of the outcome in a treated group before and after treatment. These one-group quasiexperimental designs fail to consider the important effects of statistical regression and co-intervention (e.e., diet and fluid therapy) as determinants of outcome. In four quasiexperimental studies (4 thiazide) a nonequivalent comparison group was added to the design. This design fails to protect against differences due to selection factors between treatment and comparison groups (e.g., compliance). All 11 quasiexperimental studies concluded that treatment was effective. There were 3 randomized controlled trials (2 thiazide, 1 orthophosphate). None found a statistically significant difference in outcome between treated and control groups. These 3 studies lack the statistical power to conclude that treatment is ineffective. These 14 clinical trails fail to either establish or disprove the effectiveness of thiazides or orthophosphates.

75 Effectiveness of Long-Term Treatment for Recurrent Kidney Stones Using the D.I. Method

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The recurrence rate of kidney stones in stone formers in the Jerusalem population is high. Patients without evidence of metabolic disease who were treated with a high fluid intake had a 29.6% recurrence rate during the first 18 months of follow-up. A prospective study of the prevention of recurrent kidney stones is presented. Patients were followed-up with reference to the Discriminating Index (D.I.) method, the D. I. values representing the inhibitory potential of the urine.

Fifty-eight Ca-oxalate stone formers had their urines analysed after removal of the stone, and the D.I. values were recorded. The patients were treated with orthophosphates, thiazides, or allopurinol. The choice of drug and the adjustment of its dosage were in accordance with the response of the D.I. values of the urines. The patients were followed-up for periods ranging from 9 to 36 months, or on average 22 patient-months. Only one patient (1.7%) had a recurrent kidney stone during the follow-up period. These encouraging results may indicate a new approach to the management of the prevention of recurrence of kidney stones.

76 Phosphate Treatment of Calcium Urolithiasis

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Dietary supplementation with phosphate has been proposed as an alternative method of preventing renal stones in patients with or

without hypercalciuria. Sixty-four patients with idiopathic recurrent stone formation were treated with neutral Na-K orthophosphate salts (Phosphate Sandoz, 1.5 g phosphorus/24 h) for 1–5 years (mean 3 years). Thirty-eight patients had hypercalciuria. Before treatment mean stone episode rate (SER) was 0.56 stones/year. On treatment SER decreased to 0.05 stones/year ($P < 0.01$). Of the 64 patients, 50 (78%) remained free of recurrence during follow-up. The corresponding figure for patients with hypercalciuria was 76%. TmP/GFR decreased, but serum phosphate did not change. A marked reduction in urinary calcium excretion was recorded. Pyrophosphate excretion increased significantly. There was no consistent change in serum concentrations of calcium, alkaline phosphatase, or PTH. However, in two patients a rise of PTH to supranormal levels was noticed. Side-effects of phosphate intake with gastrointestinal discomfort were common. In controls SER did not change significantly during follow-up.

Conclusions. Phosphate treatment is an effective alternative for management of calcium urolithiasis regardless of whether hypercalciuria exists or not. There seems to be no major risk of parathyroid stimulation.

77 Biochemical Alterations in Urinary Stone Patients Receiving Chemoprophylaxis

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Aims of Study. Various chemoprophylactic regimens are administered in urinary stone patients to correct the primary biochemical abnormality. This study aims at identifying the biochemical changes produced by certain drugs.

Methods Employed. Patients with biochemical anomalies were studied. Thiazide was given for hypercalciuria, allopurinol for hyperuricemia and/or hyperuricosuria, and magnesium for hypomagnesiuria. A combination of thiazide and allopurinol was given vor hypercalciuria and hyperuricemia/hyperuricosuria, and thiazides and magnesium for hypercalciuria and hypomagnesiuria. The biochemical changes that occurred in urine and blood were studied daily for 4–10 days and at monthly intervals for 2–6 months.

Summary of Results. Thiazides produced a reduction in the urine calcium level immediately and this was maintained later. The urinary magnesium level showed a marked reduction immediately and then tended to increase minimally. The serum uric acid level increased in the case of normo- or hypouricemia, but fell minimally in hyperuricemia. The urine uric acid level showed a similar pattern. Allopurinol produced a significant decrease in the urinary uric acid level, but not in the serum level in short-term therapy. In long-term treatment the levels of uric acid in both serum and urine fell.

The immediate hypercalciuria produced by magnesium alone masked its hypermagnesiuric effect. The combination of thiazide an allopurinol produced a lasting reduction of urine calcium and uric acid. Thiazide and magnesium produced the desired ideal biochemical change.

Conclusions. Combination chemotherapy is ideal for calcium and magnesium abnormality. Magnesium alone is better avoided. Allopurinol is good if uric acid alone is a problem.

78 Five-Year Treatment in Hyperoxaluric Stone Forming Patients

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Gas chromatographic procedures show that hyperoxaluria is common in patients who have recurrent renal stones and that hyperoxaluria may be present alone or associated with hypercalciuria. Hyperoxaluria, alone or associated with hypercalciuria, was detected in 69 of 450 patients with recurrent stones (15.3%). Hyperoxaluria was investigated with ^{14}C-oxalate and three main mechanisms of hyperoxaluria were detected: (1) Oxalate hyperabsorption (51.1%); (2) hyperoxalemia (21.9%); and (3) increased or decreased oxalate clearance (17.0%). A borderline group was also detected (9.7%). Hyperoxaluria was corrected by administering 15 g DEAE-cellulose daily to the hyperabsorptive group. The hyperoxalemic group received 6 g daily PO. Orthophosphate 1 g and magnesium chloride 10 g were given to those with oxalate clearance alterations. The risk of stone formation in each group was calculated from the equation: risk = L x Ns/Np, where L is the duration of disease or treatment, Ns is the number of stones, and Np is the number of patients. Correction of oxalate hyperabsorption (DEAE administration) was successful insofar as oxaluria decreased and the risk of stone formation was reduced. The administration of succinimide and/or magnesium phosphate did not have such good results.

79 Intestinal Phosphate Binding by Phosphate/Sulphate- Exchange as a Therapeutic Principle in Phosphate Renal Stone Disease: Effects on Calcium–Phosphate–Vitamin D Metabolism and Urinary Saturation

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Currently, the principle of dietary restriction of phosphorus by means of intestinal phosphate binders (SHORR regimen) has not definite place in the treatment of phosphatic renal calculi, since it entails the risk of phosphate depletion (PD): PD induces resorptive and 1,25-DHCC-mediated absorptive hypercalciuria, which tends to increase the activity product of brushite or struvite. The aim of the present investigation was to define the effects of moderate dietary phosphate restriction on calcium-phosphate-vitamin D metabolism in normal controls and patients with recurrent phosphate stone formation. A new oral phosphate binder with high PO_4-binding capacity [$Al_7(OH)_{17}(SO_4)_2$] and with a novel pharmacological principle (intestinal sulfate/phosphate exchange) was used.

Patients and Methods. Eleven controls (10 male, 1 female, age 32 ± 5.4 years, body weight 76 ± 8.9 kg) and 19 patients (6 male, 13 female, age 42 ± 11 years, body weight 74 ± 17 kg) were treated with aluminium oxyhydrate sulfate ($AlSO_4$) mean dose 4 x 2 g; sulfate content 18.71 mmol/d). Urinary chemistry: Ca^{2+}, PO_4, PTH, 25-HCC, 1,25-D-HCC.

Results. a) Urine. Both in controls and in patients, there was a significant ($P < 0.01$) decrease in urinary phosphate (40%), an increase in urinary sulfate (50%), and a decreased PO_4/SO_4 ratio in the urine (controls: before 1.74 ± 0.47 M/M, after 0.73 ± 0.33; patients: before 1.32 ± 0.54, after 0.67 ± 0.44 M/M, $P < 0.01$). Urinary calcium and oxalate were both unchanged in patients and controls. The relative supersaturation for brushite fell to below 0 in 20/30 individuals (undersaturation). cAMP was also unchanged (controls: 3.35 before, 268 x 10^{-6} M/M Cr during $AlSO_4$ medication). *b) Plasma.* No changes were recorded in Ca^{2+}, PO_4, PTH, or 25-HCC. 1,25 DHCC levels increased in controls from 62 ± 23 to 72 ± 22 pmol/liter and in patients from 52 ± 25 to 64 ± 32 pmol/liter during $AlSO_4$ medication ($P < 0.05$).

Conclusions. 1) Oral application of $AlSO_4$ leads to a significant (50%) reduction in urinary phosphorus without significant side-effects. 2) The degree of saturation for brushite decreased in two-thirds of the individuals, since in spite of slightly elevated 1,25-DHCC-levels neither urinary calcium nor urinary pH increased. 3) Intestinal complexation of Ca^{2+} by SO_4^{2-} and intestinal, absorption of acid may be possible mechanisms for the absence of hypercalciuria and alkalinization with phosphate withdrawal.

80 Effect of Alkalinizing Agents and Chelating Agents on the Dissolution of Calcium Oxalate Kidney Stones

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An attempt to elucidate the role of alkalinizing and chelating agents in reducing calcium oxalate stone formation has been made. The relative effectiveness of these agents in the dissolution of calcium oxalate dihydrate crystals and calcium oxalate renal stones has been measured and compared in various concentrations over a pH range of 4–8. The alkalinizing agents used were sodium orthophosphate and sodium bicarbonate. The chelating agents were sodium ethylenediaminetetraacetate (EDTA) and sodium citrate.

The equilibrium solubility of calcium oxalate dihydrate crystals was reached after 10 minutes, whereas that of calcium oxalate renal stones was reached after 48 hours. The equilibrium solubility of calcium oxalate dihydrate crystals and of renal stones was increased six-fold in trisodium citrate solution compared with the equilibrium solubility of monosodium citrate solution. Twenty-fold enhancement of the equilibrium solubility was obtained in tetrasodium EDTA compared with disodium EDTA. The dissolution of calcium oxalate dihydrate crystals in both sodium orthophosphate and sodium bicarbonate solutions yielded low values of calcium and high values of oxalate.

Results suggest that the rate of dissolution of calcium in chelating agents depends on the concentration of anions in these agents, which effects the formation of a soluble calcium complex. It also appears that the difference between the mechanisms by which these chelating and alkalinizing agents dissolve calcium lies in the variability of dissolution/reprecipitation processes.

81 Effect of Alkalinizing Agents on Calcium Oxalate Stone Formation in a Rat Model

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In an attempt to determine the relative efficacy of therapy directed at the reduction of calcium oxalate stone formation by enteric hyperoxaluric patients, the effects of alkalinizing agents such as sodium bicarbonate, sodium orthophosphate, and sodium citrate were studied in an experimental animal model capable of rapid enteric hyperoxaluric stone formation.

In a group of 50 rats made hyperoxaluric by feeding a diet supplemented with ammonium oxalate calcium oxalate renal stones were produced after 4 weeks in 100% of the animals and bladder stones in 30%. The urine of stone-forming animals contained 554 μg oxalate/ml and 11.8 μg calcium/ml at a pH of 5.7, and contained many dihydrate and monohydrate calcium oxalate crystals. In addition to free stones, micro- and macro-deposits of crystals were seen at the papillary tip, at the corticomedullary junction, and in the renal fornices.

A group of animals receiving the same lithogenic diet and also oral supplementation with sodium bicarbonate or sodium orthophosphate for the 4-week treatment period showed a 30% incidence of renal stones and no bladder stones. A group of lithogenic animals supplemented with sodium citrate showed a 15% incidence of renal stones and no bladder stones. The urine of these animals contained 1117 μg oxalate/ml 30.8 μg calcium/ml, and a pH of 7.78, and had rare calcium oxalate crystals.

Sodium citrate has been shown to be a more effective drug for the prevention of calcium oxalate stone formation than either sodium bicarbonate or sodium orthophosphate. These experiments demonstrate the relative effectiveness of various therapeutic agents in reducing the incidence of calcium oxalate stone formation and the relative protection offered by urinary alkalinization, which favors the formation of soluble calcium salts in preference to the formation of insoluble calcium oxalate.

82 Allopurinol and Calcium Oxalate Stones, 15 Years Later

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In 1968, we reported that 14 of 21 patients with recurrent calcium oxalate stones stopped making stones when receiving allopurinol. This work was confirmed by Coe. In 1970, a prospective blind study of placebo versus allopurinol was begun. In 1975 the code was broken, and 92 patients who had been followed-up for a minimum of 6 months were reported on. It was clear that there was a placebo effect in the management of recurrent stone disease. The only event that separated these groups completely was cessation of further calculus formation; however, this only occurred in 61%. This group of patients has been monitored continuously. The placebo group was switched to allopurinol 300 mg q.d.

Careful analysis of the results has shown that there is only a very small group of patients in whom allopurinol will be effective. These patients are those who excrete urinary uric acid in concentrations that exceed 40 mg/100 ml urine in 24 hours. However, when this group is analyzed further it is apparent that increased fluid intake and alkalinization of the urine played a significant role in the good results that have been reported by our group. It is apparent that allopurinol has a limited application in the management of calcium oxalate stones.

Years on study	Allopurinol			Placebo		
	No.	Imp	Lost	No.	Imp	Lost
7	31	8	10	1	2	40
8	28	4	12	1	2	33
9	19	4	13	1	2	29
10	18	3	7	1	1	24
11	14	3	9	1	0	16
12	9	1	6	1	0	7

83 Clinical Manipulation of Urinary GAGs – A New Method of Stone Prevention (?)

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The urinary glycosaminoglycans (GAGs) are potent inhibitors of calcium oxalate crystallization. Unfortunately, it has not so far been possible to increase their excretion in the urine for the purpose of preventing the recurrence of calcium oxalate stones.

A new synthetic sulphated polyanion known to be excreted in the urine following oral administration was studied in vitro and in vivo. First the inhibitory activity of the compound on crystal nucleation, growth, and aggregation was measured in a continuous crystallizer system. The polyanion was found to be active at low molar concentrations. Secondly, urine was collected prior to and following a course of this drug in a group of human volunteers. Urinary risk factors were measured in all samples, with particular reference to the concentration of the alcion blue precipitable polyanions and its constituent glycosaminoglycans, ribonecleic acid and Tamm Horsfall mucoprotein. A significant increase in the glycosaminoglycan concentration and inhibitory activity occurred. This sulphated polyanion may offer a new and exciting method of stone prevention.

84 A Multicentre Trial to Evaluate Three Treatments for Recurrent Idiopathic Calcium Stone Disease – A Preliminary Report

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A trial on the treatment of idiopathic calcium stone disease was set up, involving five centres throughout the United Kingdom. So far, 120 male recurrent idiopathic calcium stone formers aged between 20 and 60 have been entered and followed-up for between 3 and 5 years. After a period of observation before treatment, during which the patients were assessed clinically and biochemically, they were allocated at random into one of the four treatment groups. These consisted of patients receiving (1) no treatment at all, *not even dietary advice,* (2) allopurinol (300 mg Zyloric oral dose), (3) thiazides (2.5 mg Centyl K t.d.s.), and (4) orthophosphate (1 tablet Phosphate Sandoz containing 0.5 g phosphorus t.d.s.). The urinary risk of stone formation (P_{SF}) was measured in at least two 24-hour urine specimens before treatment and at regular intervals during therapy. Fasting blood and urine samples were also analyzed to ensure that no patients with disorders of calcium metabolism were included in the study. Stone recurrence rates were calculated before and during treatment.

Analysis of the data so far indicates that the P_{SF} values were higher than normal in all four subgroups prior to treatment. During treatment they were not significantly reduced in the untreated group and only slightly in the allopurinol group, but significantly so in the groups receiving thiazides and orthophosphate. The corresponding stone recurrence rates were not significantly reduced in the untreated group, marginally reduced in the allopurinol group, and significantly reduced in both the thiazide and orthophosphate groups after 2–3 years of treatment. Later data from this study will be reported.

85 Additive Effects of Magnesium and Tartrate Upon Inhibition of Calcium Oxalate Crystal Formation in Whole Urine

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Aims. 1. To evaluate the effect of tartrate on inhibiting calcium oxalate crystal formation in whole urine undergoing rapid concentration by removal of water. 2. To find out whether in this system the effect of adding tartrate and magnesium would be additive or whether they would be negated by the formation of magnesium tartrate complex or ion pairing.

Methods. Random fresh urine samples were collected from normal subjects into vacuum flasks. ph values were adjusted to either 5.3 or 6.0 and ^{14}C-oxalate was added. Duplicate aliquots were withdrawn for both the untreated urine control samples and the 'test' samples. Enough cold oxalate was added to each sample to raise the final concentration after evaporation by 0.22 mMol/liter. To the test sample was added D(−), L(+) or mesotartaric acid (previously adjusted to urine pH with NaOH) or $MgCl_2$ sufficient to raise the postevaporation concentrations by 5 mMol/liter and 4 mMol/liter, respectively. Samples were rapidly evaporated on a rotary evaporator to 1250 mosmol/kg and ^{14}C-oxalate in the precipitate was measured by scintillation counting.

Results. 1. Tartaric acid. The addition of L(+) tartaric acid to urine at pH 5.3 produced a fall in calcium oxalate precipitation in 11/11 cases. The mean recovery of precipitated calcium oxalate from tartrate-containing urine was 69.9% of the control values. In 20 further tests at pH 5.3, 10 with the D(−) isomer, and 10 using the meso-form, the mean recovery with D(−) form was 77.6% and that with the meso-form was 84.8%. Only in one instance for each isomer was a rise in the oxalate recovered found, but the size of the rise is very small. At urine pH 6.0 falls in recovered ^{14}C-oxalate followed addition of the L(+) acid in all 10 cases tested (mean recovery 51.2%), addition of the D(−) tartrate in 9/10 cases (mean recovery 82.7%), and addition of the meso-form in 6/10 (mean recovery 90.9%). *2. Magnesium with Tartrate.* For this investigation, counts from aliquots at pH 5.3 containing both magnesium and tartrate were compared with samples containing one of these substances alone. At the time of writing, the 9 tests carried out so far clearly indicate that the effects of tartrate and magnesium are additive. Urine with both substances yielded 37.9% of the recovery of ^{14}C-oxalate yielded by urine with tartrate alone and 58.3% of that yielded by urine with magnesium alone.

Conclusions. If the L(+) tartrate levels of urine could be increased this would reduce the risk of calcium oxalate crystalluria and presumably of stone formation. It is already known that raising urinary magnesium alone is also effective, and it is now proposed that raising both urinary magnesium and tartrate would be even more effective than raising either alone. Magnesium tartrate might be a safe and effective therapy for calcium oxalate stone prophylaxis.

86 Controlled Studies in Stone Prophylaxis: Comparison of Placebo Versus Allopurinol/Chlorthalidone/Magnesium Hydroxide

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We have enrolled 126 subjects in 5 double-blind, placebo-controlled clinical trials of kidney stone prophylaxis. We randomly assigned subjects with recurrent and active calcium oxalate stone disease to either 1) magnesium hydroxide 625 mg or 1,300 mg, 2) chlorthalidone 25 mg or 50 mg or 3) placebo. In a parallel study, 72 subjects with hyperuricosuria and active, recurrent calcium oxalate stone disease were randomly assigned to either 1) allopurinol 100 mg t.i.d. or 2) placebo.

In an "open study", we are also observing 97 stone formers who either 1) refused any treatment or 2) insisted on known therapy. After 2 years of study, we can compare the success rates of various regimens. Although these subjects had previously passed an average of 5 stones and had a pre-treatment rate of 0.6 stones per patient per year (pppy). The rate observed (prospectively) with no treatment or placebo was significantly less (0.20 stones pppy). This "clinic effect" may be benefitted further by chlorthalidone therapy (0.07 stones pppy) allopurinol therapy (0.06 stones pppy) but not by the administration of low doses (325 mg b.i.d.) or high doses (650 mg b.i.d.) of magnesium hydroxide (0.21 stones pppy). The 25 mg chlorthalidone dose was slightly more effective than the 50 mg dose – yet only the larger dose produced a significant reduction in urinary calcium (75 mg/24 h). The lower dose reduced urinary oxalate significantly (13 mg/24 h).

87 Procedure and Results of Conservative Treatment of Ureteric Calculi

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Pathophysiologic knowledge of the upper urinary tract is a prerequisite for adequate therapy of an obstructing ureteric stone. Urodynamic disorders of the upper urinary tract caused by stone obstruction will be demonstrated as well as modern therapeutic measurements.

In the stage of acute colic therapy with a potent spasmoanalgesic is essential. The aim of this initial therapy is to obtain freedom of pain and recovery of physiological tonus of the urinary tract. To releave the stone passage, periodical administration of spasmoanalgesics is recommended, completed by an adjuvant therapy (diuretic and antiedematous measurements etc). The administration of the different adequate medicaments depends on patients constitution and individual tolerance.

Providing equal efficacy the drug with the least side effects should be administered. From that point of view we performed a multicentric randomised and prospective study to compare classic spasmoanalgesic with a phytotherapeutic medication (Urol). In the group with classic spasmoanalgesic 85.5% of the stones passed spontaneously in the group with Urol administration 89.3% of the stones. There was no significant difference concerning passage time of stones between the two groups, but side effects and costs were less in the Urol group.

88 Operative Ureterorenoscope for Endoscopic Therapy of Urethral Calculi

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Aims of the study. Progress in the miniaturization of optical systems made ureterorenoscopy possible. This method was first of benefit in diagnosis, and especially in the differential diagnosis of carcinoma of the ureter or nonopaque stones. Following these diagnostic endeavors we soon felt the need of instruments to manipulate ureteral calculi (loop extraction and ultrasonic fragmentation). Working with the Wolf companys we developed a suitable instrument that allowed direct-vision ultrasonography and use of a stone basket. Because of the narrow spatial relation between stone and ureter we had to take into account that side-effects would follow the application of ultrasonic energy. Appropriate animal experiments were necessary.

Methods. We tested the effect of ultrasound in 10 beagles exposed for 30, 60, 120 and 180 s. We were concerned to record both the immediate and the delayed effect. The reaction of the urothelium, tunica muscularis, and tunica adventitia was examined histologically. The instrument is 11.5 F in diameter with a large oval wash and tube canal. It also involves two further instruments, an ultrasonic tube (ultrasonodrode 1.5 mm) and a Dormia stone basket (size 3.5 F).

Summary of results obtained. The animal experiments demonstrated that the application of ultrasound for a maximum of 30 s through the tube does not cause permanent irreversible damage to the urothelium, the tunica muscularis, or the tunica adventiitia. Fragmentation of concrements in the ureter requires applications of ultrasound for 5–10 s, which is below the safe limit we found in the experiments. Up to now we have successfully extracted concrements in 6 cases. The instrument is an important step forward in the endoscopic surgery of ureteral stones under direct vision. We apply this method with concrements that cannot be passed spontaneously and when urethral obstruction or stone size would otherwise make ureterolithotomy necessary.

89 Renal Autotransplantation with Direct Pyelocystostomy in Patients with Recurrent Renal Calculi

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Despite recent advances in the prophylactic treatment of patients with recurrent calculi of the upper urinary tract, and despite modern facilities for clearing a kidney of stones, frequently recurring stones may still be a problem in some patients. This report describes our current experience of an operative procedure which not only makes radical stone removal possible but also provides for spontaneous passage of stones occuring subsequently.

Renal autotransplantation with direct pyelocystostomy was performed in eight patients, all male, with recurrent renal calculi. All patients had a history of recurrent renal calculi with two to nine prior operations. Two of them had only one kidney. At the time of the autotransplantation three had a staghorn calculus and the other five had multiple calculi. Three patients had a stricture of the ureteropelvic juntion. Four had a prior history of urinary infections. Four patients had struvite calculi, two had calcium phosphate calculi, one had cysteine calculi, and one had a calcium carbon apatite and oxalate calculus with cholesteatoma formation.

Pre- and postoperatively the patients were studied with IVP, cystoscopy, cystography, determination of concentration ability and glomerular filtration rate, renography, frequent urine cultures, and determinations of residual urine and micturition flow-rate. Renal angiography was carried out preoperatively and about 1 week postoperatively.

The kidney was carefully excised and lithotomy was performed extracorporeally. The kidney was autotransplanted and a wide direct anastomosis was made between the pelvis and the bladder.

During a follow-up period of 4–53 months (mean 30 months) one patient has spontaneously passed stones, one has a stone which not yet has been passed, and the others have remained free of stones, as confirmed by x-ray. None has complained of any discomfort from the kidney in the unusual site. One kidney was lost in a patient with a normal contralateral kidney, due to a vascular complication; this kidney was severely damaged by multiple previous resections. The renal function, both glomerular and tubular, has remained unchanged during the follow-up period. Three patients have occasionally had bacteriuria postoperatively; all had the same kind of bacteria preoperatively.

The procedure may be indicated in patients with disabling frequent stone recurrences to promote future spontaneous passage of stones.

90 Intraoperative Polaroid Roentgenogram

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Complete eradication of calculi from the kidney can be difficult and frustrating. The use of intraoperative radiography for the localization of small residual stones has been a useful adjunct. However, the time factor and need for repeated exposure have turned this technique into a frustrating experience, so that many have abandoned it. The recent introduction of Polaroid radiographic film type TPX in both the 18 x 24 cm and 9 x 11.5 cm sizes has allowed the production of superb-quality films with excellent definition. In the laboratory, we have shown that 1-mm calcifications can be identified. We have compared Kodak X-omat KS film, Mammography film, and Polaroid types 52, 57, and TPX. These films have also been compared in the operating room. The convenience and rapidity of film production has returned the control of intraoperative radiography to the surgeon. It is the personal opinion of the author that Polaroid type TPX gives the best results.

91 Ultrasound in Renal Stone Surgery with Specially Constructed Mini-Probes

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Ultrasound was used in renal stone surgery as long ago as 1961 by Lytton. But it was another 20 years before this technique became a routine procedure. Another step forward was the detection of larger vessels by Doppler stethoscope.

Our technique of removing staghorn calculi by surgical means without ischemia and cooling is presented. All caliceal parts of the staghorn stone not to be extracted via a pyelotomy are located by renal time ultrasound. Their location is then marked with a thin needle and the area is examined with the Doppler stethoscope to define a poorly vascularized area for nephrotomy. Our new special-

ly constructed mini-transducers for intraoperative use are presented. With these multielement mini-probes even small stones can be detected, and ultrasound-guided intrarenal manipulations are possible. This technique has given good results with respect to total stone extraction and renal function; we have abandoned ischemia and cooling in staghorn stone surgery, and have had more success in the location and extraction of small stones in caliceal systems with difficult access.

92 Three-Dimensional Intraoperative Renal Radiography by Means of a Combination of Polaroid Films and Intensifier Screens

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Aims of the study. Pyeloscopy, ultrasound and radiography are applied for the exact intraoperative localization of stones in kidney stone surgery. In this paper we report on an improved method of intraoperative renal radiography with Polaroid films.

Methods employed. Intensifying screens and a single-film plate which can easily be constructed from the plastic frame of a commercially available Polaroid film pack were used. A newly developed plate holder allowing the film boxes to be changed intraoperatively was used. The plate holder shows a reticulum Ito be positioned in front of the exposed kidney) and a film box holder behind the kidney. Double imaging of the kidney with different radiation angles shows the stone and the reticulum. The shifting of the calculus and the reticulum in the picture after double exposure leads to exact determination of the stones position. The site of the stone can be easily estimated or calculated exactly and then marked with pins. For the experiments we used different intensifier screens (universal and rare earth screens of Kodak, Agfa and Dupont), various types of Polaroid films (type 667, 611 and 665), and different stones (uric acid, cysteine and calcium oxalate stones).

Summary of results obtained. Rare earth screens in combination with Polaroid film type 667 proved to be best. The quality of the pictures was at least as good as with conventional radiographs on mammography films and could even visualize tiny stone fragments or poorly opaque calculi with an extremely reduced x-ray dose.

Conclusion. A new technique is introduced for visualization of kidney stones in the exposed kidney during surgery. With this method a quick (about 40 s) and distinctive Polaroid paper picture can be obtained. This procedure is a cheap and easily performed method of localizating of calculi in the kidney. It is an alternative for conventional radiography.

93 Alterations in Kidney Location with Changes in Patient Position: Implications for Percutaneous Renal Procedures

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Ultrasound and fluoroscopy in conjunction with IV contrast administration are proven methods for guiding initial puncture of the collecting system preparatory to performing percutaneous renal procedures. Nonetheless, many practitioners continue to rely on supine plain abdominal or excretory urogram films to localize the kidneys for a 'blind stick' using surface landmarks or fluoroscopy without contrast administration. To test the reliability of this approach, we perform CT scans of normal kidneys in ten patients. Each was scanned in the supine, prone, LAO, and RAO positions. We then measured patient position-related alterations in the location of the pertinent pyelocalyceal system(s). Kidney location was altered greatly by positional changes in most patients. Alterations varied considerably among patients and between kidneys in the same patient. Relative to the supine position, movement in the cephalocaudad direction ranged from 12.7 and 7 cm more cephalad to 5.8, and 5 cm more caudad in the prone, LAO, and RAO positions, respectively. In only three patients was cephalocaudad motion insignificant. Both anterior horizontal motion occurred, thus changing the depth the needle must traverse before puncturing a collecting structure. Alterations ranged from 3.7 cm more to 1.6 cm less than the distance measured with the patients supine. There was no relationship between renal mobility and body habitus.

This study demonstrates that the position of the kidneys varies significantly and unpredictably with alterations in patient position. Percutaneous puncture of the collecting system should therefore be performed using a method of continuous guidance to optimize patient safety and the likelihood of success. Review of our CT scans indicates that puncture of the right collecting system is best with the patient in a modestly LAO position and a modestly RAO position is best for the left collecting system. These positions have added virtues: the needle passes through the less vascular renal anteroposterior division; there is also added stability for nephrostomy tubes and reduced chance of leakage; and finally there is a lower likelihood of puncturing the distal wall, and greater postprocedural comfort for the patient.

94 Computed Tomographic Analysis of the Composition of Renal Calculi

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The purpose of this study is to determine, ex vivo, the feasibility of employing computed tomography to evaluate the composition of in vivo renal calculi. We also sought to determine the optimum measurements. Sixty-three coded calculi were scanned while suspended in a water bath. Region of interest measurements provided the mean, standard deviation, minimum, and maximum pixel values for each stone. These parameters were then correlated with infrared spectrophotometric analysis of the stones' composition. By performing a multivariate analysis, we found that the mean and standard deviation of the stones' pixel values were the best CT parameters for differentiating among three different types of renal calculi. With computerized zone mapping techniques, uric acid calcui could be perfectly differentiated from struvite or calcium oxalate calculi. The latter two types also were differentiable with 87% certainty. Our results suggest an important role for CT as an adjunct in determining externally the composition of renal calculi. This information may then be used in decision regarding optimal treatment.

95 Renacidin and Some New Oxalate and/or Phosphate-Dissolving Irrigation Systems: A Direct Comparison of Litholytic Capacity

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The improved technique of the percutaneous nephrostomy opens new ways for the local chemolysis of urinary concrements, as can be deduced from the successful litholysis of residual struvite calculi reported by Nemoy, Smith, Royle, Stamey, Jacobs, Gittes, Fam, and Rossier. In our own experiments we compared the dissolution kinetics of renacidin (hemiacidrin), used by the cited investigators, with some new irrigation systems developed in our laboratory. In addition, we were interested in oxalate-dissolving irrigation systems with comparable oxalate-dissolving capacity to that of renacidin for struvite concrements. In fact we found several irrigation systems with an essentially higher litholytic capacity for struvite concrements than that of renacidin. Alternation of the oxalate- and the calcium-complexing irrigation solutions made it possible to attain a calcium/oxalate-dissolution rate that was the same order as the dissolution rate of struvite concrements treated with renacidin. These

in vitro experiments are of special interest in connection with the new computer-monitored irrigator recently described by Japanese investigators.

Our study comprised the gravimetric analysis of over 1,000 single calculi, subdivided into classes according to composition as determined x-ray diffractometry (wh, wh/we, ap, str, ap/str, and ox/ph concrements) and size (2, 4, 6, and 10 mm diameter). In all, 44 single and combined irrigation systems have been tested. As reference systems we used renacidin and citrate. The concentrations were 0.001, 0.005, 0.010, 0.100, and 0.250 moles/liter; the flow rate was 1.0 ml/min (1,440 ml/24 h). Of special interest were some compounds that markedly increased the Ca^{2+}-binding capacity of urine and/or the citrate excretion when given PO to Wistar rats.

96 Progress in the Technique of Renal Parenchymal Resection

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Suture techniques in renal surgery depend on the surgical procedures. Optimal surgical techniques aim at stopping hemorrhage without decreasing the renal function.

A safe procedure to avoid bleeding and loss of functional parenchyma has been renal pole resection, as practiced in our department since 1979. This differs from conventional techniques in that only three adapting superficial parenchymal sutures are used. For blood coagulation we use infrared irradiation. The resection areas are adapted after spreading a fibrin adhesive over the cut areas. To prevent ischemia 3 ampules of dipyridamol are given by IV injection. The renal function of nine patients is shown before and after the pole resections performed according to the operative method just discribed. It is then compared with the results obtained with the conventional method. Statistical evaluation showed a highly significant reduction of the loss of function in the kidneys that were subjected to the new technique compared with those in which the conventional technique was used.

97 Postoperative Recurrence of Idiopathic Calcium Urolithiasis

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High recurrence rates have been reported in idiopathic calcium stone disease. Repeated surgery is often very difficult in patients who have already received surgical treatment, especially those who have undergone nephrolithotomy. We continue to make every effort to detect the causes of stone disease, in the hope that it will ultimately be possible to prevent recurrence in surgically treated patients.

In the past 7 years we have treated 307 patients with idiopathic calcium stones. Of these, 165 underwent stone surgery. The rate of residual calculus was 6.25%. During the same period we observed a much higher recurrence rate of calcium stones in the surgically treated group (24.0%) than in the nonsurgically treated group (5.3%). No relationship between recurrence and type of operation was demonstrated. We examined several biochemical indicators in the surgically treated subjects. In the group of 46 patients with postoperative recurrence of calcium stone the serum calcium level averaged 4.92 ± 0.21 meq/liter and there were no cases of hypercalcemia. Hyperuricemia was found in 5 of 46 patients (10.9%) and hypercalciuria in 11 of 29 (37.9%). On the other hand, in the group of 129 patients who underwent stone surgery without postoperative recurrence the average serum calcium was 4.93 ± 0.25 meq/liter; this included two patients with hypercalcemia. Hyperuricemia was noted in 9 of 119 patients (7.5%) and hypercalciuria in 12 of 84 (14.2%). Hypercalciuria was more frequently observed in the patients with postoperative recurrence ($X^2 = 7.4349, P < 0.01$).

We concluded that calcium stone formers who require surgery for their first episode have a higher recurrence rate than conservatively treated patients, and that those who have developed postoperative recurrence are more likely to have hypercalciuria or hyperuricosuria. Treatment of postoperative patients with thiazide or allopurinol is justified in the presence of any indications.

98 In Vitro Determination of Optimal Conditions for Coagulum Pyelolithotomy by Means of a Thrombelastograph

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Several methods are described for coagulum pyelolithotomy, using combinations of cryoprecipitate (cryo), platelet-rich plasma (prp), calcium, and thrombin. The purpose of this study was to determine the optimal conditions for clot formation in vitro by means of thrombelastography. Experiments were carried out with several combinations of cryo, prp, saline, calcium ion, and thrombin at varying concentrations and temperatures. The thrombelastographic results were expressed as reaction time (r) and maximal amplitude (ma). The r value is comparable to a clotting time and the ma value is related to the elasticity of the clot. The highest ma values were obtained with cryo (fibirnogen concentrations ± 10 g/liter citrate concentrations 32 mM) and calcium ions. Good results were obtained with calcium concentrations between 10 and 40 mM (final concentration). At the optimal calcium concentration of 20 mM the r value was 3 min and the ma value 8 cm. A standard 10% calcium levulate/$2H_2O$ solution (0.325 *M*) produces this effect at a dilution of 1:16 (15 ml cryo and 1 ml calcium levulate solution). There is no clear difference between the results at room temperature and 37 °C. Addition of prp or saline to the cryo gives acceptable values up to a dilution, respectively, of 2.5:1 and 1:1. If only prp is used the calcium concentration is very critical, in that acceptable values are obtained only at concentrations close to 20 m*M*. If thrombin is used to induce clot formation extremely short r values are found and high thrombin concentrations (25 U/ml) are repuired to obtain an ma value of 4 cm. Addition of calcium ions to the thrombin does improve the results but ma values remain lower than with the use of calcium ions only. It is concluded that according to thrombelastographic criteria the combination of cryo and 20 m*M* calcium ions gives the best results.

99 Vesical Calculi in Neurogenic Vesical Dysfunction – An Avoidable Problem Even in Developing Nations

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Calculogenesis in neurogenic vesical dysfunction is believed to occur in the patient fraction in whom bladder management is achieved initially by prolonged indwelling catheterization and/or resumption of voiding with an 'imbalanced' bladder. The aim of our retrospective study was to ascertain the influence of these two factors leading to urinary infection and bladder calculus formation in a small group of chronic-care patients with spinal injury or neurogenic bladder at this institute. Cystolithotomy or endoscopic stone removal was accomplished, the urinary pathogen identified and treated, and each patient's vesical emptying improved with the aid of intermittent catheterization or pharmacological therapy directed at this purpose. The stones were chemically analysed and proved to be of a mixed variety. None of the patients seen initially at this hospital immediately after injury and for whom sterile intermittend catheterization was advised as the first line of management for urinary retention during the spinal shock phase developed calculi. The urinary infection rate was low in this group and the infections responded to therapy. In contrast, the patients who had with stones had been managed elsewhere, initially be indwelling

catheterization. Some reported prolonged use of this mode of management. Others were voiding inefficiently after catheter-free trial, in that they had large bladder residual volumes or high intravesical pressures and undetected detrusor-sphincter dyssynergia. After assessment and reorganization of their management as above following stone removal and therapy for urinary infection, none has returned with recurrent stones or reinfection during their varying follow-up periods.

100 Disintegration of Urinary Calculi by Means of Laser Beam

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Aims of the study. Disintegration of urinary calculi by means of a laser beam was studied in vitro.

Methods employed. Neodym-YAG lasers were used for laser application in three different ways: 1. Continuous-wave laser; 2) Pulsed laser; direct irradiation; high-power laser, Q-switched laser; 3) Pulsed laser optomechanical coupling.

CaOx and uric acid stones were used. The wave guide of the laser was fixed vertically with a stand so that the stone below could be irradiated. The diameter of the laser beam was variable (distance stone to wave guide 2–8 cm, focus 1–7 mm. Irradiation was performed under water, oil, carbon dioxide, nitrogen, or vacuum in an Erlenmayer flask. Optomechanical coupling was carried out according to the method of Fair: a pulsed laser irradiated a thin aluminium film confined between a glas disk and a metal capsule. Laser energy was thus changed into mechanical energy.

Summary of results obtained. (1) Continuous-wave laser. Direct irradiation of various types of calculi resulted in heating of the stone and subsequent burning or melting. Urate stones often burst after a few seconds due to thermal tension, but this result depended on the crystalline structure and was not exactly reproducible. Heating of the medium or bubble formation and consequent spread of laser energy was the effect of irradiation under oil or water. We used laser power from 5 to 88 W and an irradiation time between 1 s and 10 min. 2) Pulsed laser. Irradiation of a stone with single pulses from 24 mJ and 200 μs to 20 J and 10 ms blew off stone fragments. This effect was also due to a thermal process. The application of laser power from a Q-switched laser (12 ns, 13.6 mJ) induced bursting of stone parts. 3) Optomechanical coupling. A mechanical shock wave was generated with the aid of a pulsed laser (Q-switched power 13.5 mJ, 12 ns). Fragmentation of the whole stone was the result. This proved to be unfavorable because not only the stone, but also the wave guide and coupler were destroyed.

Conclusion. Disintegration of urinary calculi by means of a continuous wave and pulsed laser is due to thermal effects. Only uric acid stones sometimes showed fragmentation before burning. Application under water was not possible. According to our experiments, in vivo application of laser beams for the destruction of urinary, calculi does not seem to be suitable. Stones cannot be destroyed without thermal or mechanic damage to the surrounding tissue. Only optomechanical coupling seems to be favorable if the release of energy can be directed. Possibly high-energy lasers with even more power (>100 million W) can generate a shock wave that would disintegrate stones.

101 Calculus Renal Failure

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Pakistan has a high incidence of calculus disease. For a number of reasons the incidence of renal failure due to stones (calculus renal failure) is exceptionally high.

The present study, based on 238 patients over a period of 10 years, has given us an understanding of management problems. We were able to classify 230 patients with calculus renal failure into four types. This classification is based on the degree of calculus obstruction and renal functional mass at a given moment. However, an individual patient can move into other types over a period of time. Renography and ultrosonography have proved most valuable in recent years. Our classification is as follows:

Type I (109 patients). These patients experience sudden almost total obstruction in the upper urinary tract, leading to anuria or oliguria. Early relief of obstruction produces excellent results. This group of patients is classed under calculus anuria in the literature.

Type II (32 patients). In these patients one kidney suffers from chronic renal damage due to stones. Obstruction of the 'good' kidney by a stone does not produce anuria or oliguria. However, the patient gradually slips into chronic renal failure. Early removal of the stone from the good kidney is indicated.

Type III (87 patients). These patients have large non-obstructing calculi in both kidneys (or the only functioning one), leading to renal damage over a period of time. In our opinion stones must be removed in these case.

Type IV (2 patients). In these patients with bilateral nonobstructing calculi no cause for anuria could be ascertained. We feel anuria was probably due to severe secondary infection.

102 Doppler Ultrasonography in Surgery for Renal Stones

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Total clearance of the stone from the kidney is important for the prevention of recurrence with staghorn calculi. Multiple nephrotomies are usually required to do this using either anatrophic or hypothermic techniques with occlusion of the main vessel to minimise blood loss and renal damage. However, there is still the risk of damage to blood vessels and resultant segmental renal ischaemia with these techniques. Furthermore, there is a time limit for the completion of stone removal, even with hypothermia.

The use of Doppler ultrasonography is well established in determining flow characteristics in blood vessels in the investigation of peripheral vascular disease. It can also differentiate arterial and venous flow. This technique has been applied to localise intrarenal arteries and veins peroperatively during surgery for staghorn calculi. This has effectively defined suitably avascular areas for placing the nephrotomy incision to gain access to the stone. Blood loss has been minimal and confined only to capillary oozing in all nephrotomies without occlusion of the renal artery.

The film will depict the use of Doppler ultrasonography in nephrotomy for the removal of staghorn calculi, demonstrating its speed, simplicity, and reliability in use. A further attraction is that the equipment is inexpensive.

II. Genetics, Epidemiology, Dietary, Environmental Factors, Animal Models

103 The Inhibition of Experimental Nephrocalcinosis with a Prostaglandin Synthetase Inhibitor

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There are essentially two theories of calculogenesis: the anatomical and physicochemical. However, the relationship between intranephronic focal calcification and the "risk factors" of urinary crystal aggregation due to mineral supersaturation remain obscure. Our previous studies showed that prostaglandins are important hormones in the intrarenal regulation of calcium excretion [1]. The aim of

these experiments was to study the effect of prostaglandin inhibition on the process of renal parenchymal calcification.

Method. Nephrocalcinosis was induced in a group of experimental rats (group 1, $n = 5$) by means of an intraperitoneal (IP) injection of 10% calcium gluconate for 10 days according to the technique described by Fourman [2]. Two further groups of rats were treated with oral indometacin (10 mg/kg; group 2, $n = 5$) and flurbiprofen (2 mg/kg; group 3, $n = 5$) daily for 4 days prior to receiving IP calcium gluconate, and indomethacin and flurbiprofen continued during the 10-day course of IP. calcium gluconate administration. The animals were killed and the kidneys studied for differential localisation of nephrocalcinosis by means of contact microradiography and histology and for quantitative calcium concentration by energy-dispersive analysis of X-rays (EDAX).

Results. There was marked inhibition of cortical nephrocalcinosis and significantly reduced calcium concentration ($P < 0.005$) in the animals treated with a prostaglandin inhibitor (groups 2 and 3) compared with the group (group 1) given IP calcium gluconate alone. The prevention of experimental nephrocalcinosis with non-steroidal anti-inflammatory drugs (NSAID) suggests that prostaglandins are implicated in the process of renal parenchymal calcification and may be aetiologically significant in the pathogenesis of stone formation.

1 Buck AC et al (1981) The influence of renal prostaglandins on glomerular filtration rate (GFR) and calcium excretion in urolithiasis. Br J Urol 53:485–491

2 Fourman J (1959) Two distinct forms of nephrocalcinosis in the rat. Br J Exp Pathol 40:464–473

104 Reduction of Urinary Calcium Excretion by Prostaglandin Synthetase Inhibition

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The kidney is a crucial organ for the regulation of calcium metabolism; however, the exact mechanism for the renal tubular handling of calcium was proved ellusive. It is now well recognised that prostaglandins influence intrarenal haemodynamics and electrolyte excretion. Therefore, a study was carried out to investigate the role of prostaglandins in the mechanism of renal tubular calcium excretion. Experiments were performed in conscious Sprague-Dawley rats with previously implanted carotid cannulae. An infusion of 0.9% NaCl containing inulin and PAH was given via a tail-vein cannula. Urine and blood samples were collected at hourly intervals over a 6-h period. An experimental group of animals ($n = 10$) received indometacin (10 mg/kg body weight) at 4 h. Urine calcium output was significantly reduced ($P < 0.02$) after indometacin, as compared with a control group ($n = 10$) which did not receive indometacin. The clearance of inulin and PAH was constant in both groups. In experimental animals an intra-arterial infusion of exogenous PGE2 (0.2 μg/kg/min) for a 30-min period resulted in a marked calciuretic response ($P < 0.001$). These results indicate that prostaglandins influence calcium excretion by a direct action on renal tubular function which appears to be independent of haemodynamic changes.

105 Microstructure of Calcium Oxalate Foreign-Body Stones Produced in Rat Bladder

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Implantation of a foreign body in the rat urinary bladder is a standard method of producing urinary calculi for experimental studies. Usually a zinc disc is used as a foreign body, and the diet is modified or ethylene glycol added to the drinking water to develop a stone of desired composition. In order to study the microscopic architecture of such stones, zinc is inappropriate since it cannot be sectioned. Therefore, we used discs, 4 mm or 6 mm in diameter, made of a plastic normally used for embedding tissue in transmission electron microscopy. The discs were implanted, one each, in bladders of male Sprague-Dawley rats. The rats were given drinking water containing 0.75% ethylene glycol. After varying lengths of time the discs were extracted and studied by scanning (SEM), transmission electron microscopy (TEM), and light microscopy.

Within 3 days of implantation, encrustation of the disc surfaces started and within a week two-thirds of the disc surface was covered with crystals. Within 2 weeks both surfaces of the discs as well as the margins were encrusted. Both calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) crystals were present. No other types of crystals were identified. The urines were free of bacteria and the kidneys showed no sign of histological damage. Light microscopy and TEM revealed the presence of a thin proteinaceous layer between the plastic disc and the encrustation. Examination of the fractured stones by SEM showed areas that appeared to be the nucleation sites of crystals. Nucleation of crystals on the proteinaceous layer at the disc surface was followed by crystal growth which resulted in the coverage of disc surface. This process of nucleation and growth was followed by subsequent nucleation on the encrusted surface and growth of the crystals. Also, there were indications that some crystal aggregation had also occurred. Thus it appears that these foreign-body stones grew by confluent crystal growth, with crystal aggregation playing a minor role.

106 Urinary Calculi Formation in Rats Fed High-Silicate Diet

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Involvement of high silicate intake has been suspected in the genesis of urinary calculi disease. Therefore, studies were undertaken in rats to delineate the role of high-silicate diets in urinary calculi formation.

Weanling male rats were fed diets based on starch (80%) and casein (10%), which provided adequate vitamins and minerals. The ratio of calcium to phosphorus in the diet was 4.6:1. The control group received the basal diet alone (Si: 50 ppm) and the experimental group was fed the basal diet enriched with sodium silicate (Si: 1,000 ppm). The diets were fed ad libitum for 20 weeks. By 3 weeks crystalluria was observed in both the groups. The incidence of crystalluria was far greater in the high-silicate group. Calcium oxalate was the main crystalline species in the urine of controls. In the high-silicate group both calcium phosphate and calcium oxalate were present. Urinary pH of silicate group was significantly higher (8.5) than the controls (6.8). Urine of high silicate group contained higher amounts of total non-dialysable solids and mucoproteins.

The incidence and weight of urinary calculi were far higher in rats fed a high-silicate diet. Calculi formed in controls contained mainly calcium-oxalate, whereas both calcium-oxalate and calcium phosphate were found in the stones of high-silicate groups. The kidneys and bladders of high-silicate fed rats contained a higher concentration of calcium. These studies clearly demonstrate the deleterious effects of high-silicate ingestion on the incidence of urinary calculi.

107 Effect of Wheat and Rice Diets on the Solubility of Uric Acid in Urine Studies in Man

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In India, the incidence of urolithiasis is high in some areas where wheat is the main dietary staple, as compared to those areas where the main dietary cereal is rice. The reason for these differences is not known. Investigations were therefore undertaken in normal adult male volunteers to investigate the effect of rice and wheat diets on some properties of urine. Metabolic studies were conducted in two series. In the first study, the diets employed were identical, except for the cereal, which was either wheat (400 g) or rice (400 g). The diets were isocaloric. Since both diets contained rice or wheat in equal amounts, the protein content of the rice diet was lower (55 g) than the wheat diet (65 g). The mode of formulation and preparation of the individual diets confirmed to the methods used in the practical situation.

The same subjects were kept on a rice diet for 15 days, followed by a wheat diet for the next 15 days. In some other subjects the order of feeding was reversed. After each dietary regime, urine was collected and some of its properties studied.

On the wheat diet the subjects excreted less urine than when on the rice diet. Consumption of a wheat diet led to a fall in urinary pH (5.6). There was more excretion of uric acid on the wheat diet than on the rice diet. Most importantly, the urine of subjects on a wheat died had a lower capacity to hold uric acid in solution than the urine from a rice diet. Differences in the solubility properties of uric acid observed were not entirely due to variations in urinary volume and pH.

The protein content of the rice and wheat diets used in the above study is different. Unequal intake of proteins is known to modify the properties of urine. Therefore, a second study was undertaken, in which the protein content of wheat and rice diets was equalized. This was achieved by including more skimmed milk in the rice diets. In this study, no difference in uric acid excretion was observed in wheat or rice diets. However, consumption of wheat diets resulted in a fall in urinary pH, as well as in the solubility of uric acid in urine in this study.

All the above properties of urine observed in wheat diets are conducive to the formation of uric acid crystals. Therefore, consumption of diets based mainly on whole wheat by those prone to urolithiasis might aggravate the disease.

108 Do Stone Formers Accept Dietary Advice?

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Increased urinary excretion of calcium, oxalate and uric acid is significantly associated with idiopathic urolithiasis, and dietary advice to reduce the nutrient density of the diet has previously been shown to produce a reduction in all these risk factors for stone[1]. It is, however, essential that this change in dietary style be maintained over a long period of time if it is to reduce the recurrence of stone.

We assessed the dietary habit of 62 idiopathic stone formers (males, 46; females, 16) by means of a questionnaire before giving dietary advice[1]. This assessment was repeated after a period ranging from 6 to 43 months (mean 16.6 months).

We found that the median cereal fibre intake increased from 9 g (range 1.3 to 27 g) to 15 g (range 5 to 34 g) per day and that of sugar decreased from 86 g (range 12 to 408 g) to 49.5 g (range 4 to 158 g) per day. There was no significant reduction in the consumption of animal protein (mean before advice 56 g/day; after advice 57 g/day), but the number of patients assessed for animal protein was small for a valid statistical comparison.

We conclude that idiopathic calcium stone formers are a well-motivated group of patients and have shown a significant change in their dietary habit following advice. We therefore suggest that dietary manipulation should form the first line of management in idiopathic urolithiasis.

1 Rao PN et al (1982) Br J Urol 54:578–587

109 Tea Drinking – A Risk Factor for Urolithiasis?

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Urinary oxalate is an important risk factor for calcium oxalate stones of the upper urinary tract. Although the highest concentration of oxalate occurs in foods such as spinach and rhubarb, a major source of oxalate in the diet is tea. It has been suggested that the high prevalence of urolithiasis in areas of soft drinking water might be due, in part, to increased absorption of oxalate from the intestine. The effect of tea ingestion on the risk of being a stone former was evaluated in Newfoundland, an area with soft drinking water and a high prevalence of calcium oxalate-phosphate kidney stones. The study design was case-control. The cases were 117 stone formers identified among 1,112 adult respondents interviewed during a random sampling of households in 6 of 39 health administration districts in Newfoundland. An individual was defined as a stone former if a stone had been passed, removed at operation or seen radiologically. The 117 control subjects were chosen from among the same 1,112 respondents and matched to a case by district, sex and age ± 4 years. Sibling matches were excluded. Tea consumption (i.e. the exposure) at the time of the survey was recorded by the interviewer who had no understanding of the relevance of the question. The extent of tea drinking was graded as mild (0–2 cups), moderate (2–5 cups) and large (> 5 cups/day). The mean number of cups of tea ingested per day was 4.00 for stone formers and 4.18 for control subjects ($P > 0.05$, paired t-test). When single stone formers and multiple stone formers (> 1 stone) were compared with their matched controls, there was no difference in either case ($P > 0.05$). The null hypothesis of no difference in the proportion of tea drinkers between stone formers and their matched controls was not rejected ($\chi^2 = 0.45$, $P > 0.10$). Similarly, the null hypothesis of no difference between pairs with increased exposure was not rejected ($\chi^2 = 0.00$; $P > 0.01$). The amount of oxalate provided by a 3-g tea bag was 21 mg per cup when the infusion time was either 5 or 10 min. We have found no evidence to support the hypothesis that oxalate contained in tea will increase the risk of stone formation even in areas with soft drinking water.

110 Drinking Water Quality and Urolithiasis

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The relationship between drinking water hardness and urolithiasis is the aim of many studies done in different countries in the world. A negative correlation between water hardness and lithiasis has been demonstrated in Czechoslovakia, the southeastern part of the United States, some regions of the United Kingdom and Yugoslavia. From investigation in these regions we have concluded that regions with soft water have a higher incidence of nephrolithiasis than regions with hard drinking water. Hardness of drinking water is determined largely by the concentration of Ca and Mg cations.

Today water hardness is measured by American, English and German grades. In our area where the study was done, we measure water hardness by German grades (one grade is 10 mg/l CaO or 7.19 mg/l MgO). The hardness of water drunk in our area is 0.45 grades (2.2 mg/l Cao and 1.6 mg/l MgO). In spite of very soft drinking water we have a high incidence and prevalence of stone disease. That is one reason why we are studying water quality to see whether or not it has an influence on stone disease.

We have made a statistical study on 1,240 operations done over 35 years in our area where about 138,000 people live. Operations are the best registered data for this problem over a long period of time, but in our opinion these operations represent only a small percentage of urolithiasis in this area, as 75% of the patients admitted to the division of urology are admitted for stone disease. Of 1240 operations in our small division of urology, 496 (40%) were

done on kidneys, 228 (18.40%) on ureters, 462 (37.25%) on bladders and 54 (4.35%) on urethras. The highest percentage of operations were on kidneys but ureteral stones with few exceptions also originate in the kidneys, which means that renal stones numbered 724 or 58.40%. Bladder and urethral stones may be stones from kidneys passed to the bladder and urethra or they may originate in the bladder primarily.

Our investigations show that the right side is more affected than the left and that the male-to-female ratio is 1:1.07, which is practicaly equal. This means that the causative factors in our area are common for both sexes. In the last 5 years in 75% of the stones in our area of extremely soft drinking water, calcium oxalate was the main constituent.

We consider that the mineral content of the drinking water has an influence on the etiology of calcium oxalate urolithiasis and that water hardness is a indicator for the presence of calcium, particularly magnesium in an environment where both stone formers and other people live. There is a correlation between the percentage of magnesium in the drinking water and the incidence and prevalence of urolithiasis. In our area the drinking water has a very, very low level of magnesium cation and high incidence and prevalence of urolithiasis, which conforms the hypothesis about soft drinking water poor in Ca and Mg and the high incidence and prevalence of urolithiasis.

111 The Effect of an Increased Intake of Various Constituents of a High Animal Protein Diet on the Risk of Calcium Oxalate Stone Formation

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It has been shown that a diet containing a high proportion of animal protein and purine aggravates the risk of calcium oxalate stone formation by increasing the urinary excretions of calcium, oxalate and uric acid. The object of this study is to determine which constituents of such a diet are responsible for the various biochemical changes observed in urine. The paper contains the results of studies on the effect on the urinary risk factors of (1) a high purine intake and (2) an increased intake of certain amino acids which are present in higher proportions in animal protein than in vegetable protein.

Groups of six normal men were studied during 1 week on their basal home diet and then during the following week on the same diet supplemented with one of the following amino acids: methionine (2.5 g/day), glycine (4 g/day), tryptophan (1.5 g/day) and hydroxyproline (0.45 g/day). The quantities were selected with a view to doubling the normal daily intake of these amino acids. They were divided and taken with meals. Twenty-four-hour urines were collected and analysed for risk factors during the last 3 days of each diet period. A similar study was carried out in three normal men to determine the effect of a high-purine diet (2 g RNA/day).

The results show that methionine significantly increased the urinary excretion of calcium and that the other amino acids tested each increased the excretion of oxalate. RNA increased uric acid excretion (as expected) but had no effect on oxalate excretion. It is concluded that a high animal protein diet increases the risk of calcium oxalate stone formation through the combined actions of purine and several amino acids on the urinary excretions of calcium, oxalate and uric acid.

112 Calculogenic Potential of the Diet – A new Concept

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Aims of study. Prescription of a diet for the stone patient is riddled with inconsistencies. Many dietetic substances contain a combination of calculogenic and anticalculogenic agents. The present study aims at identifying the total potential of the dietetic substances to promote or inhibit calculogenesis.

Methods employed. The foodstuffs were analysed based on their ingredients and each ingredient was alloted positive or negative points based on its property of promoting or inhibiting calculogenesis, depending upon previously recognised and accepted information. The points ranged from 0 to the maximum value according to ingredients in the foodstuff.

The calculogenic potential of a foodstuff is the total value of calcium (0 to +12 points) + Ca/P ratio (0 to +20) + uric acid (0 to +32) + Vit D (0 to +12) + oxalates (0 to +20) + Ox /Ca ratio (0 to +10) + proteins (0 to −8) + Vit A (0 to −16) + pyridoxine (0 to −8) + thiamin (0 to −4) + citrate (0 to −12) + magnesium (0 to −4) + sodium (0 to −32) + chloride (0 to −4).

Summary of observations. It was possible to calculate the calculogenic potential of all common foodstuffs. From the dietetic history a calculogenic index was determined for each patient. This was found to be very useful for imparting dietetic advice by identifying the ultimate effect of the dietetic material to promote or inhibit stone formation.

Conclusions. Diet intake with low calculogenic potential and avoidance of diets with high calculogenic potential have been found to have a definite impact on reducing the progress and recurrence of calculogenesis.

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113 The Effect of "High Fibre Biscuits" on Urinary Risk Factors for Stone Formation

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Idiopathic hypercalciuria is commonly associated with urolithiasis. Recent reports suggest that supplements of dietary fibre reduce urinary excretion of calcium. There is, however, the theoretical risk that this may induce hyperoxaluria as a secondary phenomenon. Commonly used fibre supplements such as soya or wheat bran contain oxalate in varying proportions, but it is not known if this oxalate is available for absorption. There are no reports to date on the effect of dietary fibre on urinary risk factors other than calcium.

Fifteen healthy male volunteers with no history of stone disease participated in the study. After collecting 24-h specimens of urine on 3 separate days, they were given "high fibre biscuits", two biscuits three times a day, for 6 days. The biscuits were specially formulated for palatability and each biscuit consisted of: dietary fibre (soya bran), 4–4.5 g; carbohydrate, 7 g; fat, 2.6 g; protein, 1.3 g – approximately. Twentyfour-h specimens of urine were collected again for 3 days beginning on the 4th day. In seven subjects, after a washout period of 6 days, the study was repeated using biscuits made of wheat bran.

There was no increase in the urinary excretion of oxalate and, contrary to expectation, urinary calcium excretion was unaltered. Similarly, there was no change in other risk factors. The reasons for this will be discussed with particular reference to the constituents of fibre and other nutrients in the diet.

114 Is Salt Restriction Necessary to Reduce the Risk of Stone Formation?

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Idiopathic hypercalciuria is the most common urinary abnormality in calcium stone formers. It is well recognised that renal tubular transport of sodium and calcium are closely related and some recent

reports imply that dietary salt restriction may reduce urinary calcium excretion.

To examine the relationship between sodium and calcium excretion, urinary electrolyte results of 363 stone formers were analysed retrospectively. In all these patients 24-h specimens of urine were collected on 3 days when they were at home on their usual diet. They were not on any form of dietary restriction at the time of urine collections.

In a separate prospective study the effect of dietary salt restriction on urinary calcium excretion was investigated in 10 stone formers with idiopathic hypercalciuria; 24-h specimens of urine on 3 days were collected prior to an interview when daily intake of salt was assessed by means of a questionnaire. They were advised a low-salt diet and 7 days later 24-h specimens of urine were collected. Urine specimens were analysed for creatinine, sodium and calcium.

The results suggest that there is a weak but statistically significant correlation between urinary excretion of sodium and calcium (Spearman $r = 0.361; P < 0.001$). There was a tendency for patients with hypernatriuria also to have hypercalciuria, but hypercalciuria was not necessarily associated with increased excretion of sodium. The effect of a low-salt diet on urinary calcium excretion will be presented.

115 Epidemiological Aspects of Urolithiasis in the Federal Republic of Germany

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Differences between the experimental findings of the same urinary parameters, found by different laboratories gave rise to the question of whether they could be caused by epidemiological factors. Therefore, a large series of data on stone patients has been evaluated under the aspects of epidemiology. The following factors were taken into consideration: kind and number of stones formed, socioeconomic groups of stone formers, age and sex. Data on 1,290 stone formers were classified in correspondence with these factors and we evaluated by a special program described elsewhere. Documentation of the data and a computerized statistical evaluation were carried out together with the Institute of Medical and Biological Information and Statistics in Marburg.

Regarding the kind and occurence of stones, sex and age distributions, and urinary parameters like uric acid and calcium differences were found between the following socioeconomic groups: (1) farmers, (2) workers, (3) employees, (4) self-employed, (5) civil servants and (6) graduates. According to this sequence (1–6), the prevalence of calcium oxalate and phosphate stones increases from 66 to 89% while that of uric-acid/urate-containing stones decreases from 34 to 9%. The differences between the first groups and group 6 were significant. While the maximum stone incidence occurs for groups 1–5 at 50–64 years of age, group 6 reaches its maximum between 25 and 34. Ca-excretion increases in groups 1–6. Consequently, in order to compare statistical data on stone-forming subjects, socioeconomic aspects always should be taken into account.

116 Transmembrane Oxalate Flux of Red Blood Cells in Idiopathic Calcium-Oxalate Nephrolithiasis

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The frequency at which there is a family history of this disease, together with the observation of higher than normal intestinal absorption and urinary excretion of oxalate, combine to make it feasible to consider idiopathic calcium oxalate nephrolithiasis as a metabolic disease characterized by a disorder in oxalate transport. To test this hypothesis the flux of ^{14}C-oxalate through the membrane of red blood cells (RBC) was investigated in 18 controls and 18 idiopathic calcium oxalate stone formers. To this end, 10 ml of heparinized blood was washed three times in a solution containing NaCl 150 mM, KCl 10 mM, Tris HCl 20 mM, pH 7.4, which was resuspended to an hematocrit of 50% in the above solution with the addition of 10 mM sodium oxalate and then incubated at room temperature for 2 h. After centrifugation, RBC were resuspended at an hematocrit of 20% in the same solution and subdivided into many fractions, to which a tracer amount of ^{14}C-oxalate was added. At 10, 20, 30, 60, 90, 120 min and 24 h the corresponding fraction was centrifuged and the ^{14}C-activity of the supernatant was counted in a β-counter. The flux rate was calculated according to the following formula:

$$\ln (A_t - A_\infty) = \ln (A_0 - A_\infty) - Kt$$

in which t is the time, K the flux constant and A the quantity of labelled oxalate at time 0, t and ∞.

The K value was 0.25 ± 0.15 SD min^{-1} in controls and 1.30 ± 1.85 SD min^{-1} in stone formers, with a statistically significant difference between the two groups ($t = 2.40; P < 0.025$). Furthermore, 10 of the 18 stone-forming patients showed K values greater than the normal range ($\bar{x}$ + 2 SD in controls) ($X^2 = 13.85; P < 0.005$).

Our data seem to support the hypothesis that idiopathic calcium oxalate nephrolithiasis is a metabolic disease characterized by a cellular defect in oxalate transport.

117 Urine and Serum Levels Related to Lithogenesis in Rats on an Atherogenous Diet

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Aims of the study. Animal models of lithogenesis induced by different diets have repeatedly been reported. For this purpose, atherogenous diets have often been used[1]. This rapidly leads to renal calcification and formation of concrements in the urogenital system. The pathophysiological steps resulting in these alterations were not yet clear. Besides renal histology, we therefore examined a number of urine and serum levels related to urolithiasis.

Methods employed. Male rats were fed an atherogenous diet for 8 weeks. Serum and urine levels of calcium, magnesium, sodium and potassium were measured by flame photometry and phosphorus, creatinine and citrate by test kits. We determined uromucoid by rocket immunoelectrophoresis, GAG by the method of Blumenkrantz. Concrements were analyzed by polarization microscopy.

Summary of results obtained. Uromucoid excretion had already decreased significantly by the second week of the experiment. Furthermore, an atherogenous diet resulted in hypercalciuria. Excretion of phosphate was reduced. Serum creatinine was increased significantly. The other serum levels showed no significant differences. The concrements found in the renal pelvis consisted of calcium phosphate and struvite.

Conclusions. Our examination demonstrates that an atherogenous diet results in renal failure caused by nephrocalcinosis. The early decrease of uromucoid excretion, hypercalciuria without hypercalcemia and hypophosphaturia indicates a primary tubular disorder. Hyperparathyroidism seems to be improbable in this phase of study. These results together with the histological findings (calcifications in the outer zone of medulla) point to the importance of the distal tubuli in the pathogenesis of urolithiasis caused by an atherogenous diet. The possible pathophysiological alterations of this region are discussed.

1 Schwille PO, et al (1975) Urologe A 14:306

118 Histopathological Changes of the Rat Kidney Under an Atherogenous Diet

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Aims of the study. The genesis of urinary calculi is for the most part still unclear. On the basis of the investigations of Schwille and others, we worked out a model with laboratory rats that rendered it possible to measure nephrocalcinosis, dependent on parameters that are related to stone pathogenesis. In order to induce nephrocalcinosis we administered an atherogenous diet.

Methods employed. For 8 weeks male rats were fed an atherogenous diet. Besides the determination of serum and urine parameters, the kidneys were examined histologically for calcification. This was done histotopochemically according to the method of Voigt on tissue section. Polarization microscopy was applied on the concrements.

Summary of results obtained. Under an atherogenous diet, the animals developed distinct nephrocalcinosis. Besides stray concrements in the renal pelvis, we found extracellular concrements in the lumina of the efferent urinary tract. The calcifications were located mainly in the outer part of the medulla, but others could be found to a different extent in the area of the medullary papilla. Polarization microscopic analysis demonstrated calcium-phosphate concrements.

Conclusions. Our experiment demonstrated that an athergenous diet can provoke nephrocalcinosis within a few weeks. Artificial stone forming appeared mainly in the area of the kidney in which the tubulary absorption processes are located. Accompanying investigations of certain urinary parameters (calcium, phosphate, uromucoid, etc.) showed that an atherogenous diet results in certain pathophysiological changes. Our investigations suggest that the area occupies an important position in the formation of calculi.

119 Excretion of Calcium and Vitamin D Metabolism in Vitamin A Deficiency in Rats

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Aims of the study. Former studies have demonstrated that 1-α-hydroxylation of 25-OH-D in the kidney is dependent on vitamin A. In Vitamin-A-depleted rats we found an increased excretion of calcium and decreased levels of phosphate and uromucoid in the urine. In the present study we tested the influence of vitamin A on vitamin D metabolism and the possible effect on calcium excretion in the urine.

Methods employed. Female rats were subdivided into two collectives: vitamin A depleted ($n = 20$) and control animals ($n = 20$). Animals were kept under these conditions for 9 months. There were two urine collection periods. The following parameters were determined: (serum) 25-OH, $1,25(OH)_2$-D, creatinine, calcium, phosphate, sodium, potassium; (urine) calcium, phosphate, sodium, potassium, magnesium, creatinine, uromucoid, citrate and GAG. The vitamin A content of the liver was also determined.

Summary of results obtained. The serum levels of $1,25(OH)_2$-D showed decreased vitamin A deficiency; 24-OH-D was enhanced. Serum concentrations of calcium and phosphate were depressed. The creatinine concentration did not indicate renal failure. The depleted animals excreted more calcium and less phosphate than the controls. Uromucoid was decreased in both collectives, as compared with male rats of our former experiments.

Conclusions. Our results demonstrate that vitamin A deficiency influences the metabolism of $1,25(OH)_2$-D. The increased levels of 25-OH-D connected to the decreased $1,25\text{-}(OH)_2$-D values indicate that the 1-α-hydroxylation is affected. The depressed concentration of calcium and phosphate can be interpreted as a result of $1,25\text{-}(OH)_2$-D deficiency. The possible causes of altered urine levels of calcium and phosphate were presented and discussed.

120 Dietary History and Dietary Records in Renal Stone Patients and Controls

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Dietary factors have been claimed to be of importance for the increase of renal stone disease in the Western world. A high dietary intake of animal protein causes an increase in the urinary excretion of certain substances at risk of stone formation. The aim of the present study was to find out if stone formers consume more protein than controls.

Methods. In this study 20 stone patients and 20 controls were both asked to make a 1-week retrospective dietary recall and a prospective 4-day dietary record. The control persons were selected by the stone patients on the basis of equality in age, sex, labour and social conditions but without a history of renal stone disease. Calculations were made of the nutrient contents in the diets from computerized food tables by the National Food Board. Two 24-hour urine collections were also made for the measurement of potential risk factors, and to make a quality control of the methods of dietary intake estimation.

Results. The 4-day redords of dietary intake of protein, sodium, potassium and phosphate were positively correlated to the urinary excretion of nitrogen, sodium, potassium and phosphate, respectively, whereas the dietary recalls were not correlated to the urinary output hereof.

There was no difference between stone formers and controls in the dietary intake of protein, purines, carbohydrates, fat, calcium, phosphate, potassium or iron according to the records. The controls had a 50% higher consumption of vitamin C and a 15% higher fibre intake than stone formers. Stone formers seemed to consume 45% more alcohol than the controls. No difference in the intake of other vitamins was found.

Despite a tendency to lower calcium intake, stone formers had a higher amount of urinary calcium ($P < 0.005$), as well as sodium, phosphate and urate, than the controls. There was no difference in urinary citrate or oxalate.

Conclusions. Diet registration seems to be more accurate than diet recall. No major differences in the diets of stone formers and controls could be found except for tendencies regarding alcohol and fibre intake.

121 Epidemiological Determinations with 377 Recurrent Stoneformers

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The frequency of recurrencies in 217 males and 160 females is presented, from which it is noticeable that more than 10 recurrencies were registered in 11.25% of the males and in only 2.5% of the females. The age limit was between 20 and 30 for the males and between 30 and 40 for the females.

Anamnestically, there was proof of urolithiasis in parents of 15.91% of the patients and in grandparents of 3.18%. For 23.64% of the patients, infection of the urinary system was determined and for 19.62% hypertension.

Gastrointestinal disturbances were anamnestically found in 18.28% of the patients (chronic diarrhea 3.71%, gastric ulcers 5.83%, gallstones 8.74%). Adipositas was revealed in 11.93%, gout in 3.18%, and diabetes also in 3.18%.

Salaried employees (24.41%), housewives, and manual workers (23.08%) formed the largest collection, as opposed to public servants (10.87%), schoolchildren, apprentices and students (8.75%), pensioners (5.85%), and the self-employed (2.91%). All data is graphically depicted in detail and explained.

122 Concerning the Significance of the Sexual Dependency of Lithogenic and Inhibitory Substances in Urine

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During epidemiological studies of recent years, an increase of renal calculi has been registered in females. For specific urine parameters, differences between males and females are given in the relevant literature. In the study submitted, 14 urine parameters were examined for 26 healthy and 71 calcium-oxalate stone formers under individual dietary and standard fare conditions.

For measured values Na, K, Ca, Mg, Cl, P, uric acid and oxalic acid, clear differences were found between male and female stone patients. All results were statistically balanced.

Calculation of relative supersaturation followed the program of B. Finlayson, Gainesville, and shows a strongly increased risk of urinary calculus formation in male patients, whereas in females this risk is no greater than in healthy persons.

123 Calcification Sites in Human Kidney – A REM Study

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Aim of the study was to investigate possible differences in intrarenal calcium oxalate crystallization between stone formers and non-stone-formers. In cases with crystallization the relationship to intrarenal structures was estimated (e.g., inter- or intranephronic crystallization), tissue and urinary saturation with respect to calcium oxalate.

Method. Immediately after nephrectomy or heminephrectomy for different reasons, specimens from renal papilla, medulla, and cortex were stored at 4° C in buffered cacodylate buffer. Specimens were divided 12 h later and prepared for SEM and REM studies. Untreated specimens (papilla, medulla, and cortex) from the same kidneys were analyzed for the content of calcium, oxalate, phosphate, and various heavy metals. Before surgery a 12-h urine sample was collected and analyzed for the concentrations of calcium, oxalate, phosphate, magnesium, urate, citrate, sodium, and potassium; the relative saturation of calcium-oxalate was calculated by a FORTRAN IV program.

Results. Stone-forming kidneys showed intratubular deposition of calcium-oxalate located near the paillary tip. No relationship between crystal formation and tissue concentration was found, but there was evidence of a marked increase of tissue saturation from the cortex to the papilla. There is no relationship between urinary saturation and crystal formation, but in general stone patients show a much higher urinary saturation. In most kidneys of non-stone-formers no crystal formation could be detected; however, two cases exhibited Randall plaques and one internephronic crystal formation.

124 Urolithiasis – Epidemiological Data from the South of Portugal

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Primary prevention, or mass prevention, can only begin after epidemiological and pathophysiological data show this to be necessary and once population treatment areas are defined. When dealing with this data we must take into account intrinsic and extrinsic factors which, combined with abnormal excretion of one or more urinary constituents, will determine the "lithiasis risk" factor.

A total of 1,452 patients from the Department of Urology, Curry Cabral Hospital, Lisbon, were studied. There were 756 males and 696 females; 67% had been living in Lisbon for more than 5 years and the rest in the south of Portugal. Calculi were collected from these patients (1,320 specimens) and were analyzed crystallographically. Age, sex and positive family history were analyzed as intrinsic factors. Climate, passage through equatorial regions, diet, profession, ingestion of liquids and daily urinary volumes diseases and drugs conducive to lithiasis were the extrinsic factors considered.

In 19.8% of the patients there was a family history of lithiasis, 15.2% of the patients had been in hot climates, and 70.5% of these related one episode, at least, to their stay (soldiers in Angola, Moçambique, etc.). Only 2.6% had a profession which might favor chronic dehydration. Of the professions 9.9% were considered active, 83% sedentary, and 7.1% unclassified. In 29.3% of the cases there was a history of drug ingestion and/or associated disease leading to lithiasis. History of diseases and factors known to cause lithiasis was found in 58.7% of the cases. The most common were associated urinary pathology (9.3%), urinary infection (15.5%), and urinary tuberculosis (4.7%). These were followed by prolonged bed rest in 7.9%, gout in 5.5%, and peptic ulcer with ingestion of alkalines in 4.5%.

Of the calculi analyzed, 64.5% were calcium oxalate, 14% magnesium ammonium phosphate, 19% uric acid, 0.9% cystine, and 1.5% the rest. It was possible to identify the nucleus in 63.8%, of which whewellite accounted for 29%, uric acid 3.5%, brushite 2.9%, cystine 0.4%, and ammonium acid urate 0.4%.

This constitutes a valid basis on which to draw up guidelines for the prevention of lithiasis in the south of Portugal. The high incidence of uric-acid stones and magnesium-ammonium-phosphate stones must be emphasized, both of which are easy to prevent.

125 Histochemical Findings in the Kidneys of CFY Rats Treated with Sodium Oxalate

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Using polarization microscopic techniques, we demonstrated a submicroscopically oriented structure of periodate-reactive carbohydrates both in the concentric laminations and in the radial striations of the matrix of human calcium-oxalate renal calculi (Szabó-Földváry and Módis 1981). The origin of this matrix material has not been clearly identified. The present experiment was undertaken to study the possible sources of periodate-reactive carbohydrates of the matrix.

CFY rats were used for the investigations. The rats were injected intraperitoneally with 7 mg of sodium oxalate per 100 g body weight per day for 1, 2, and 3 days. Control animals were injected with 0.9% sodium chloride. Ten treated and five controls rats were used for each time interval. The kidneys were fixed in a 4:1 mixture of ethanol-formaldehyde, embedded in wax, and cut at 8 μm. The following histochemical reactions were carried out: PAS, aldehyde-bisulfite-toluidine blue ("ABT," Romhányi et al. 1975) with and without antecedent diastase digestion. The sections were investigated by common and polarized light microscopy.

The following observations were made. Birefringent crystals appeared in the kidneys of treated animals. The number of crystals increased with time. The crystals were predominantly present in the lumina of the tubules of the cortex, but they were also found in the collecting tubules of the medulla. The brush border of proximal tubules containing crystals was irregular and fragmented. Crystals adjacent to the brush border were frequently surrounded by a periodate-reactive organic material. After 2 and 3 days of sodium oxalate challenge, a number of cells of the distal tubules and loops of Henle were filled with a material stongly stained both with PAS and ABT reactions. These reactions could not be abolished with antecedent diastase digestion. These result suggest that some epithelial cells of the kidney of treated animals produce a high amount of glycoproteids. We found crystals in the lumina of collecting tubules after 3

days of sodium oxalate treatment. These crystals were frequently embedded in a diastase-resistent, PAS- and ABT-positive material.

We suppose that the glycoproteid matrix material, or at least a part of it, may derive from the damaged brush border and from the strongly PAS- and ABT-positive cell population of the nephrons in our experimental model. The exact chemical nature of this material could not be elucidated by the techniques used.

126 Urolithiasis in Manipur (North-Eastern Region of India): Urinary Excretion of Stone-Promotor and Inhibitor Substances

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We have already pointed out earlier that this region in India is maximally affected with urinary calculus disease (incidence varying from 8 to 17% of all general surgery cases in the Medical College Hospital of Imphal from 1968 to 1975) and that their many primitive dietary habits could possibly be the etiological factors. The present paper reports 24-h urinary excretion of calcium, oxalic acid, uric acid, magnesium, sodium and citrate in 26 normal subjects and 38 stone formers to explore further the aetiology of this disease in Manipur.

Calcium excretion was significantly higher in stone formers as compared to normal subjects ($P < 0.05$). Of the former 36.8% and 26.9% of the latter had calcium excretion > 200 mg/24 h. Oxalic acid excretion was also higher in stone formers ($P < 0.05$); 39.4% of the stone formers excreted > 50 mg/24 h. Uric acid excretion did not show any significant difference between stone formers (1,151.9 ± 101.1 mg/24 h) and normal subjects (1,061.6 ± 113.3 mg/24 h), but hyperuricosuria appears to be the most prominent feature in the local population. Of the stone formers 63.1% and 34.6% of the normal subjects excreted > 800 mg/24 h.

The stone formers showed no significant difference in magnesium and citrate excretion. Interestingly, magnesium excretion was < 50 mg/24 h in 53.9% in both stone formers and normal subjects. Citrate excretion was within normal range in all persons. Sodium excretion was very low in stone formers as compared to normal persons ($P < 0.001$).

Our results suggest that hyperuricosuria and hyperoxaluria with concomitant lower magnesium and sodium excretions could be important determinants in the causation of urinary calculus disease. Hypercalciuria appears to play a comparatively minor role.

127 Urolithiasis in Southern Rajasthan (Western Region of India) II. Contribution of Dietary Oxalate to Urinary Oxalate

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Since 1978 we have been facing an alarming problem: the progressively increasing incidence of urolithiasis in the Udaipur region. The aetiology is under investigation. The present investigation was taken up in light of the report by one of the authors (P. P. S.) that consumption of oxalate-rich vegetables is unusually high here in the seasonal diet. This could be one of the contributing factors. Spinach, chenopodium, amaranth, and portulaca leaves are widely consumed here. We are reporting herewith the effect of ingestion of 200 g of green spinach leaves on 24-h urinary oxalic acid and calcium excretion in 14 healthy volunteers and 14 stoneformers. None of the patients had ileal resection and regional enteritis. All the results are expressed as mean ± SE.

The oxalate content of the hospital diet varied from 85 to 150 mg/day and that of 200 g spinach from 1,508 to 2,155 mg. In normal subjects and stone formers, the initial 24-h urinary excretion were 21.0 ± 4.6 and 35.7 ± 6.8 mg, respectively. After ingestion of spinach, the respective excretions were 45.1 ± 5.9 and 77.0 ± 26.1 mg.

The mean rise in oxalic acid excretion on spinach intake was almost double in stone formers (45.3 ± 20.9 mg) as compared to normal subjects (23.1 ± 5.3 mg). In the latter group maximum absorption was 3.2% of the total spinach oxalate. In stone formers, it was almost similar to the normal group except for two cases. In one patient it was 10.3% and in the other 11.4%. The absorption was much higher when only soluble oxalate of spinach was taken into consideration. These two patients appeared to be hyberabsobers of oxalate. Spinach ingestion also invariably increased calcium excretion in both groups, although individual variation was very wide.

Our results indicate: (a) in normal persons the absorption of dietary oxalate is < 5%, (b) hyperoxaluria is a prominent feature in the local population, (c) some stone formers could be hyperabsorbers of oxalate, and (d) increase in oxalate excretion was associated with increase in calcium excretion on spinach ingestion.

128 Urolithiasis in Southern Rajasthan (Western Region of India): III. Urinary Excretion of Calcium and Oxalate in Normal Population and Stone Formers

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We have undertaken a systemetic study to explore the etiology of urinary calculus disease in this region. The present work includes the 24-h urinary excretion of calcium and oxalate in 57 healthy volunteers and 158 stone formers. In the latter group, 63 patients required surgery immediately after the relevant investigations, including that of 24-h urine samples. The other 95 patients were placed on conservative therapy after the collection of urine samples. All patients were given low-oxalate hospital diet before 48 h and during the collection of the samples.

The oxalic acid excretion was < 50 mg/24 h in all 57 normal subjects. On the other hand, 41 out of 158 stone formers suffered from hyperoxaluria (excretion > 50 mg/24 h). Among these hyperoxalurics, 36 patients had stones in the kidney or ureter. The mean oxalic acid excretion in stone formers was almost double that of normal subjects.

Calcium excretion was < 200 mg/24 h in 93% normal subjects, 200–300 mg/24 h in 3.5%, and > 300 mg/24 h in another 3.5%. Among stone formers, the 24-h urinary excretion was 200–300 mg in 10.1% of the cases and > 300 mg in 17.1%.

Our results indicate: (a) hyperoxaluria is the most important etiological factor; (b) hypercalciuria is also another reckoning factor; (c) incidence of hyperoxaluria and hypercalciuria is more common in upper urinary tract stone formers.

In our 3-year study we have come across only one case of primary hyperoxaluria. The patient is a 10-year-old boy with symptoms of this metabolic disease, including multiple renal stone and severe hyperoxaluria.

129 Epidemiology of Urolithiasis and Calcium Metabolism in Human Diabetes Mellitus

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It has been claimed by several authors that human diabetic bone disease is characterized by osteopenia in the presence of normal histomorphological parameters. Cross-sectional studies (McNair 1980) demonstrated glucose-dependent hypercalciuria; others, however, were unable to detect any abnormality of vitamin D status in treated diabetics (Heath III 1980). In the present controlled epidemiological study, the prelevance of urolithiasis and hypercalciuria was examined in diabetics (D) and controls (CO). In addition, in a more detailed metabolic study the regulation of CA-excretion in insulin-treated diabetics was investigated.

Methods. a. Epidemiological study: 339 diabetics (160 M, 179 F) and 327 controls (171 M, 156 F); standardized questionnaire; measurement of volumes of Na, Ca, glucose, and creatinine in 24-h urines. *b. Metabolic study:* 19 male insulin-treated diabetics, 33 male controls; defined exclusion criteria; measurement of Ca, PO_4, Cr, urea, Na, K, protein, AP, glucose, 25-OH-D, PTH, cAMP in plasma or urine (24-h and fasting).

Results. a Epidemiological study: The prelevance of urolithiasis was identical in diabetics and controls (D: 34/339 = 10%, CO: 32/327 = 9.8%). In 3.8% of diabetics and 2.8% of controls urological operations had been performed because of stone disease (NS). Ca-excretion was slightly higher in younger male diabetics than in controls, but not in elder or in female patients. Urinary Ca was not correlated (multiple regression analysis) with either glucose excretion, forms of therapy, or duration of treatment or disease. *b. Metabolic study:* No difference between diabetics and controls existed in urinary Ca/Cr ratio (24 h or fasting), Na, K, or crea., plasma PTH or 25-OH-D. cAMP/L GFR, however, was increased in diabetics (D: 3,5 ± 1.2 nmol/100 ml GF, Co: 2.8 ± 1.2 nmol/100 ml GF; $P < 0.01$). Plasma phosphate was negative correlated with plasma glucose ; high (below 300 mg%) plasma glucose levels were associated with low fasting urinary PO_4.

Conclusions. 1) The risk of non-infected renal stone disease in human diabetic mellitus in the absence of hypercalciuria is comparable to that of the non-diabetic population. 2) The above studies do not support a significant influence of glucose-induced osmotic diuresis on the degree of calciuria. This is possibly due to the hypocalciuric effect of the lack of insulin (De Fronzo 1976). 3) Renal losses of Ca cannot explain the osteopenia of diabetes. 4) Periods of hyperglycemia are associated with very low plasma and urinary phosphate levels, especially in the early morning hours and thus expose diabetics to all risks of low P-levels.

130 Copper Coils and Prevention of Stones

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Aim. The evaluation of copper coils in the prevention of the feline urethral syndrome (FUS) and bladder calculi in human neurogenic bladders.

Methods. a) Male cats were studied under experimental and clinical situations. Copper-balance studies were carried out. The influence of copper coils on implanted struvite stones were studied and compared to controls. Similar studies were carried out in diseased animals and controls. b) Copper coils have been placed in the bladders of four paraplegics who acted as their own controls.

Results. Animals. Every cat that had an implanted copper coil had elevated urinary copper when compared to controls and the copper coil lost weight. In those cats in which struvite stones were implanted, the stone gained weight over the 12-week period. While in those animals with the coil, three-fourths showed complete disappearance of the implanted stones. *Humans.* In all patients encrustation of suprapubic tubes ceased, but bacilluria persisted.

Conclusion. This preliminary experimental study suggests that copper may be useful in the management of calculi associated with infection in humans. There is little doubt that copper coils have a role in the management of FUS when other conventional therapies have failed.

III. Gastrointestinal and Renal Physiology and Pathophysiology

131 The Effects of Oral Glycin and Methionine on Urinary Lithogenic Substances

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A marked increase in urinary calcium and a small increment in urinary oxalate has been reported upon feeding high protein diets to human subjects. Though it has been claimed by several authors that the high protein intake of Western societies is thus responsible for the increasing incidence of nephrolithiasis, the mechanisms whereby hypercalciuria or hyperoxaluria are induced are less clear. In addition, different types of proteins may differ in their calciuretic or oxaluretic potential. Fixed loads of dietary protein might also exhibit different responses, whether applied during a state of previous low or normal protein intake. In the present investigation, the effects of oral loads of glycin (45 g/d = 600 mmol) or methionine (6 g = 40 mmol) on plasma and urinary chemistry were investigated.

Probands and methods. Nine healthy male individuals (aged 31 ± 7 years, weight 74 ± 13 kg) were examined. The study was devided into three metabolic periods (5 days each), during which the individuals were on their usual self-selected and recorded (dietitian) diet. Periods: control (CO), oral glycin (GLY), methionine (METH). Continuous collection of urines under thymol were performed during each period. Measurement in urine of: Cr, urea, uric acid, magnesium, citrate, K^+, Na^+, oxalate, PO_4, cAMP; in plasma: Na^+, K^+, Ca^{++}, albumin, phosphate, Cr, urea, uric acid, phosphate, PTH, insulin, growth hormone (GH).

Results. Oral application of GLY resulted in increased excretion of oxalate (CO: 644 ± 155 µmol; GLY 797 ± 194 µmol/24 h, $P < 0.01$), but not in hypercalciuria (CO: 4.74 ± 2.36 mmol/24 h; GLY: 4.84 ± 1.19 mmol/24 h; *NS*). Neither urinary Mg, Ci, pH, PO_4, Mg or creatinine were altered by GLY. In contrast, METH resulted in significant hypercalciuria (6.9 ± 3.2 mmol/24 h) without increasing urinary cAMP (CO: 4.0 ± 1.18 µmol/24 h, METH 4.3 ± 1.04 µmol/24 h; *NS*). Urinary uric acid excretion was significantly lower under METH (3.45 ± 0.54 mmol/24 h) than under GLY (4.18 ± 0.85 mmol/124; $P > 0.01$). Plasma calcium was unchanged under either CO, GLY or METH. However, plasma phosphorus (CO: 1.46 ± 0.29 mmol/l) decreased under GLY (1.34 ± 0.19) and METH, (1.32 ± 0.27 mmol/l; $P < 0.05$). Citrate excretion was lower under METH, compared with CO. In neither experimental condition was GFR (C_{cr}) changed.

Conclusions. 1. Oral application of high doses of GLY results in mild hyperoxaluria without increasing urinary calcium. This is in contrast to IV administration of GLY, which is without effect on oxalate. 2. Oral METH induces hypercalciuria, low urinary pH, diminished urinary citrate and lowers plasma phosphate, probably by mild metabolic acidosis, since neither GH, insulin, nor PTH were altered. 3. The study explains some of the observed abnormalities of stone formers, though only two of several amino acids with oxaluretic or calciuretic potential were examined.

132 Effects of Metabolic Acidosis and Alkalosis on the Renal Brushborder Membrane Transport of Citrate

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Aims. Urinary citrate excretion increases with metabolic alkalosis and decreases with metabolic acidosis. Alteration of intracellular citrate metabolism may be responsible for these changes. Na^+-gradient-dependent luminal uptake of citrate across renal brush border membrane (BBM) vesicles is reportedly stimulated by in vitro lowering of *extravesicular* pH. However, this may not be responsible for the low urinary citrate excretion found in patients with distal renal tubular acidosis. The *intracellular* pH or bicarbonate concentration may modulate the transport of citrate across renal BBM. Therefore, we studied the effect of in vivo acid-base manipulation on BBM vesicle uptake of citrate.

Methods. Rats were maintained on ad lib. standard chow and drinking water containing 150 mM NH_4CL (metabolic acidosis – Group A), 150 mM $NaHCO_3$ (metabolic alkalosis – Group B), or 150 mM NaCl (control – Group C). After 6 days of treatment, BBM vesicles were prepared from each group, and Na^+-gradient-dependent uptake of citrate was measured.

Results. The urinary citrate excretions (mg/24 h, mean ± SE) on the initial and final days of the experiment are shown in the table.

Group	*N*	Initial (I)	Final[a] (F)	Paired *t* (I vs F)	Final FE(%)
A	5	41 ± 5	1.4 ± 0.4	< 0.005	2.3
B	5	41 ± 5	128 ± 7	< 0.001	114
C	5	41 ± 5	40 ± 2	NS	44

[a] $P < 0.001$ for A vs B, A vs C. B vs C

BBM uptake of citrate (pmole/mg protein/2.5 min, mean ± SE) was greater in Group A (411 ± 43) than that in Group B (164 ± 19, $P < 0.005$, paired *t*) or Group C (204 ± 40, $P < 0.001$, paired *t*). No significant difference was found between Group B and Group C.

Conclusion. The results indicate that increased Na^+-gradient-dependent transport of citrate by renal BBM may, in part, be responsible for decreased urinary citrate excretion during metabolic acidosis.

133 Oxalate and Urate: A Possible Common Secretory Tubular Site?

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Hyperuricuria seems to be very common in calcium-oxalate kidney-stone disease. This observation has led to the suggestion that uric acid plays a hypothetical role in promoting calcium-oxalate crystal growth. However, the prevalence of hyper-oxaluria in stone-forming patients is not less frequent than hyperuricuria. The purpose of this study was to look for a way in which both uric and oxalic acid might be handled in the same way by the kidneys. To this end, the effect of morphazinamide (MFZ) and sulfinpyrazone (SP) on oxalate excretion was evaluated in 15 normal volunteers, as these two drugs have a well-known effect on the renal handling of uric acid. Oxalate secretion was partially blocked by MFZ (0.36 ± 0.19 μmol/min before and 0.26 ± 0.15 μmol/min after drug administration; $t = 4.55$; $P < 0.001$), while SF was completely uneffective on this ion (0.32 ± 0.11 μmol/min before and 0.35 ± 0.12 μmol/min after drug administration; $t = 1.08$; *ns*). These data might indicate a common tubular secretory site for both uric and oxalic acids and suggest that a congenital defect at this tubular site could be present in idiopathic calcium-oxalate stone formers.

134 Are Morning Fasting Urines Adequate for the Discrimination of Absorptive and Renal Hypercalcurias? A Simple Test Using Cellulose Phosphate

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Several investigators have proposed the determination of Ca and cAMP in fasting morning urine samples for the differential diagnosis of absorptive, resorptive and renal hypercalcuria. The use of fasting morning urine is based on the assumption that the calcium in fasting urine samples is derived completely from skeletal resorption. The present study, using short-term application of oral sodium-cellulose-phosphate, does not support this assumption.

Methods: A total of 22 healthy male individuals (aged 42 ± 12 years) were examined; urinary Ca, Na, Cr and cAMP were measured during the following (sequential) periods: (1) 24-h urines (unrestricted diet); (2) fasting urines (2 h-samples); (3) 24-h urines [15 g cellulose-phosphate (CP)]; (4) fasting urines (2-h sample). The same measurements were performed in 20 male idiopathic stone formers without HPT, RTA and normal creatinine clearance.

Results. Both in controls and patients fasting urinary calcium (Ca/Cr) was significant lower after CP than without inhibition of intestinal Ca absorption; controls: 0.31 – 0.23 – 0.21 – 0.15 (M Ca/M Cr); patients: 0.37 – 0.28 – 0.24 – 0.17 (M Ca/M Cr). In stone formers, but not in controls, an increase in urinary cAMP (collection period 4) was observed: 0.33 – 0.31 – 0.40 – 0.38 – 10^{-3} McAMP/M Cr.

Conclusions. The present study documents that oral application of sodium-cellulose-phosphate results in a significant further reduction of the Ca:Cr ratios in fasting morning urines without stimulation of parathyroid activity (controls). It is concluded that even after a 12-h fasting period not all of the dietary calcium absorbed has been eliminated by the didneys. Thus, short-term tests do not fulfil the requirements for the detection of renal or resorptive hypercalcurias. The increase in urinary cAMP in stone patients points to activation of the parathyroids after blocking of the high calcium-turnover state and does not in itself prove a renal calcium leak. At least for the detection of resorptive hypercalcurias (mobilisation of bone mineral) prior inhibition of intestinal calcium absorption seems to be more adequate than currently performed tests in the evaluation of fasting morning urines.

135 The Effect of Ingestion of Megadoses of Ascorbic Acid on Urinary Oxalate Excretion in Normal Subjects and Stone-Formers

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The observations, that megadoses of ascorbic acid are of therapeutic and prophylactic value in the common cold and some other diseases, that in such doses this vitamin may substantially contribute to urinary oxalate, thereby increasing the risk of stone formation, and that some individuals may be oversensitive to oxalate formation from ascorbic acid and high doses will place them in danger of renal calcification and lithiasis, prompted us to evaluate the effect of daily ingestion of 6 g ascorbic acid on urinary oxalate in 10 healthy volunteers and 29 stone-formers under controlled conditions. None of the patients had oligouria. Calcium and ascorbic acid excretions were also measured.

The first 24-h urine sample was collected before ascorbic acid administration. Then 6 g ascorbic acid (in three split doses of 2 g) was given daily for 3 days. On the 3rd day the second 24-h urine sample was collected. The third 24-h urine sample was collected after 48 h of discontinuation of vitamin ingestion.

The serum ascorbic acid level and its urinary excretion were normal in all 39 persons. Ascorbic acid loading increased its excretion to 1,098.2 ± 145.7 mg/24 h in normal subjects and 1,057.9 ± 149.2 mg/24 h in stone-formers. Concomitantly, oxalic acid excretion rose from 20.5 ± 2.9 mg/24 h in the former and 29.3 ± 3.5 to 54.7 ± 5.3 mg/24 h in the latter. In the post-loaded sample the excretion of ascorbic acid and oxalic acid returned to almost pre-loaded levels. The individual variation was extreme. In normal persons ascorbic acid loading increased oxalic acid excretion to between 6.6 and 55.5 mg over initial excretion. In stone-formers it ranged between 4.3 and 119.8 mg.

Calcium excretion also showed a mild rise in both groups on ascorbic acid administration. Interestingly this rise was significant in stone-formers ($P < 0.01$). In the post-loaded sample its excretion decreased. Notably, in stone-formers this decrease in calcium excretion was significantly lower than the pre-loading excretion ($P < 0.05$).

136 The Influence of Glucose and Insulin on Calcium Excretion in the Urine

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In man the administration of sugar [1] or insulin [2] results in an increased calcium excretion in the urine. This phenomenon is exaggerated in a significant number of stone-forming patients [3]. It has been suggested that insulin may alter the tubular handling of calcium [2], but direct evidence is lacking. To obtain such information we developed the following rat preparation.

Male Sprague-Dawley rats, weighing 200–250 g, were anaestetised with Inactin. Catheters were placed in the left jugular vein, for intravenous infusion, and the right carotid artery, to monitor arterial blood pressure. A tracheostomy was performed. Through a flank incision the left ureter was catheterised. Initially all animals received 0.9% saline for 3h; thereafter they were divided into three groups which received: (a) 5.0% glucose in 0.9% saline (n = 10), (b) 2.5% glucose in 0.9% saline (n = 10), (c) 0.9% saline only (n = 10), at a rate of 150 µl/min for a further 4 h; all solutions contained [^{3}H] inulin. Blood was sampled at hourly intervals and urine collected over half-hour periods; measurements of plasma glucose, plasma insulin, sodium excretion, chloride excretion, glomerular filtration rate (as clearance of [^{3}H] inulin) and urinary calcium concentration and excretion rates were made. The significance of glucose-induced changes was assessed by analysis of variance.

Plasma glucose levels were stable for the whole of the infusion period, being 13.2 ± 0.3; 9.6 ± 0.2 and 4.4 ± 0.1 mmol/l for groups a, b and c respectively. The corresponding insulin levels were also stable at 100 ± 5.6, 58 ± 4.7 and 22 ± 1.9 µunits/ml. Glucose infusion had no significant effect on plasma calcium concentration, glomerular filtration rate, sodium or chloride excretion. In glucose-infused animals there was a marked increase in urinary calcium concentration ($P < 0.005$) and in the urinary calcium excretion rate ($P < 0.0001$). Maximal differences were seen in the samples collected between 60 and 90 min after the start of the glucose infusion (Table 1).

Table 1. Urinary excretion of calcium between 60 and 90 min; values are mean ± SEM

Group	Plasma glucose (mmol/l)	Plasma insulin (µunits/ml)	Urinary calcium excretion (µmol/min)	Urinary calcium concentration (mmol/l)
a	13.9 ± 0.7	114 ± 18.7	0.18 ± 0.02	2.6 ± 0.2
b	10.4 ± 0.3	80 ± 9.8	0.16 ± 0.02	2.2 ± 0.3
c	4.5 ± 0.3	19 ± 2.8	0.11 ± 0.01	1.8 ± 0.1

Our results confirm that the rat, like man, has a calciuric response to the infusion of glucose. Experiments are in hand to determine whether an increase in plasma insulin or plasma glucose produces these changes and to investigate by micropuncture the mechanisms responsible for these changes.

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137 The Effect of Acid-Base Changes on Renal Magnesium Transport

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Metabolic acidosis and alkalosis have a profound effect on renal handling of divalent ions. Acute metabolic alkalosis resulting from acute infusions of sodium bicarbonate produce a fall in the fractional excretion of calcium and magnesium, which is mainly due to enhanced reabsorption of these ions at distal nephron sites. On the other hand, experiments performed in our laboratory have shown that chronic administration of ammonium chloride to dogs resulted in an increase in calcium and magnesium excretion. The increment in calcium in the urine is due to a reduction in calcium reabsorption in the distal tubule. Recent experiments performed in rats indicate that this enhancement of magnesium reabsorption may be the pars recta. In the same study, acute administration of acid failed to increase magnesium excretion. Clearance and micropuncture studies were performed in the present study to determine the effects of alkalosis and acidosis on the renal handling of magnesium. To evaluate the effect of plasma and luminal bicarbonate on magnesium transport, tubular fluid bicarbonate concentration was measured in these experiments. Four groups of dogs were examined: group I, normal dogs (n = 29) with P HCO_3 21 mM; group II, chronic acidotic dogs (n = 21) produced by feeding NH_4Cl (10 g/day) for 3 days, P HCO_3 13 mM; group III, acute acidotic dogs (n = 19) induced by intravenous infusion of HN_4Cl, P HCO_3 13 mM; group IV, acute alkalosis (n = 25) by intravenous infusion of $NaHCO_3$, P HCO_3 31 mM. Glomerular filtration rates were similar in groups I, II and IV but significantly lowered in the acute acidotic group (group III). Plasma UF Mg was the same in all groups. The mean FE Na was 9% in group IV compared to 4.3%, 4.8% and 4.3% in groups I, II and III respectively. FE Mg as a function of FE Na demonstrated a reduction of Mg reabsorption in chronic acidosis and an enhancement in alkalosis. The fractional reabsorption of magnesium in the late proximal tubules was 22%, 22%, 24% and 20% in groups I, II, III and IV respectively. In the distal tubule the fractional magnesium reabsorption was 63% in normal animals and 52% in chronic acidotic dogs. Our results suggest that chronic metabolic acidosis increases magnesium excretion, whereas acute metabolic acidosis has minimal effects in dogs. Acute metabolic alkalosis results in enhanced magnesium absorption, whereas chronic metabolic acidosis results in a decrease in magnesium absorption. These effects appear to be due to changes in magnesium absorption in segments prior to the distal tubule: early distal tubule and loop of Henle. Magnesium reabsorption in the distal tubule appears to correlate with the amount of bicarbonate delivered to this segment. In summary, chronic acidosis results in hypermagnesuria and chronic alkalosis in hypomagnesuria. These changes appear to be associated with the bicarbonate delivery to the segment of the distal tubules, principally the loop of Henle. This may reflect direct pH changes within the renal cell or alteration of the ionized magnesium at these segments.

138 Is Magnesium Metabolism Related to Calcium Urolithiasis?

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Some investigators have demonstrated that the urinary ratio of magnesium (Mg) to calcium (Ca) is significantly less in stone-forming persons than in normal persons. Based on this observation, physicians have administered magnesium as a means of preventing new calcium stone formation, but success has been inconclusive. Other reports indicate that Mg:Ca ratios are decreased in stone-former urine only because urinary Ca is greatly elevated; Mg has little importance per se. To test these hypotheses, oral administration of known amounts of calcium and magnesium to 11 normal persons and 94 stone-formers revealed differences in absorption and excretion of both substances by the two groups. The postprandial rate of excretion and urinary concentration of calcium are greater and of magnesium lower for stone-formers than normals, confirming the previously reported differences in urinary Mg:Ca ratio. The postprandial versus

fasting serum to urinary ratios of calcium and magnesium were greater for stone-formers, indicating deficient excretion in a parallel fashion. Discriminant analysis implied that higher serum values of calcium and lower serum values of magnesium in stone-formers reflected the same discrepancy seen in urine, and that in stone-formers intestinal absorption of magnesium may be deficient only in the presence of increased calcium absorption. Of the two methods of magnesium handling known to exist, the absorption mechanism shared with calcium is most probably affected in stone-formers. Continued studies of magnesium metabolism in calcium urolithiasis are warranted.

139 Evidence for Magnesium Depletion in Idiopathic Hypercalciuria

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Because of the suspected role of magnesium deficiency in calcium stone formation, plasma magnesium and urinary calcium and magnesium excretions were measured in 60 controls and 82 calcium stone formers classified according to their calcium excretion in three groups [normocalciuric (NC), dietary hypercalciuria (DH), and in idiopathic hypercalciuria (IH)], the final group being defined by a calcium excretion > 3 mg/kg on calcium restricted diet (CaRD) of 400 mg/day. The measurements performed after 4 days of CaRD are summarized in the table:

Mean ± SEM	$U_{\mu g}V$ (mmol/day)	$U_{Ca}V$ (mmol/day)	$U_{\mu g}/U_{Ca}$	$P_{\mu g}$ (mmol/l)
Controls $n = 60$	3.4 ± 0.16	2.02 ± 0.3	1.68 ± 0.15	0.84 ± 0.01
NC $n = 36$	3.87 ± 0.17	2.84 ± 0.3	1.36 ± 0.08	0.82 ± 0.09
DH $n = 17$	3.94 ± 0.3	3.78 ± 0.4	1.04 ± 0.13	0.85 ± 0.01
IH $n = 29$	4.26 ± 0.28[b]	7.1 ± 0.4[c]	0.6 ± 0.04[c]	0.79 ± 0.01[a]

Comparison patients versus controls: [a]$P < 0.05$, [b]$P < 0.01$; [c]$P < 0.001$

The table shows significant differences only for the IH group, which has a significantly higher U Mg V, a lower P Mg, but a lower U Mg to U Ca ratio.

Conclusions. (1) The coexistence of a higher U Mg V and of a lower P Mg in IH suggests that there is a renal leak of Mg, which might be explained, in the patients with increased intestinal calcium absorption, by the well-known competition between Mg and Ca for tubular reabsorption. (2) The lower U Mg to U Ca ratio may favour a higher propensity for calcium crystallization, suggesting that magnesium supplement may be of therapeutical interest in the IH group.

140 Magnesium Excretion in Recurrent Calcium Urolithiasis

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Magnesium seems to be an important inhibitor of CaOx and $CaPO_4$ crystal formation. Some studies suggest a reduced stone recurrence during oral magnesium treatment. Other investigators, however, remain convinced of the "placebo effect" of magnesium.

Therefore, we measured the magnesium excretion in 122 recurrent non-infectious calcium stone formers (83 males and 39 females) and 30 healthy subjects. All individuals collected a 24-h urine sample on a random diet. On test day a fasting urine sample was collected. Urinary Mg was determined by atomic absorption, urinary Ca by a Technicon SMA 12 autoanalyser. Statistics: one-tailed Student *t*-test.

Preliminary results. Magnesium and sodium excretion was found to be significantly higher ($P < 0.005$) in 60 hypercalciuric subjects (Mg: 140 ± 58.5 mg/day; Na: 237 ± 91.8 mmol/day) as compared to 53 normocalciuric stone formers (Mg: 103 ± 35.1 mg/day; Na: 179 ± 77.9 mmol/day) No such difference was measured between the control group (C) and normocalciuric stone formers (NC). Further analysis of the normocalciuric stone formers showed that in patients without any obvious metabolic abnormality (e. g. hyperuricosuria, hypocitraturia, hyperoxaluria) marked hypomagnesuria was the only consistend disorder (subgroup *X*, $n = 27$): 24-h *urine Mg/Ca (mg/mg):* 1. C: ♂ = 0.49, ♀ = 0.54; 2. NC: ♂ = 0.52, ♀ = 0.67; 3. X: ♂ = 0.47, ♀ = 0.52 ($P < 0.005$ X vs NC). *Mg/Ca in fasting urine (mg/mg):* 1. C: 0.72; 2. NC: 0.78; 3. X: 0.67 ($P < 0.005$ X vs NC, $P < 0.01$ X vs C). *Mg/Cr in fasting urine (mg/mg):* 1. C: 0.033; 2. NC: 0.044; 3. X: 0.037 ($P < 0.01$ X vs NC). *Oxalate (mg/day):* 1. NC: 28 ± 14; 2. HC: 38 ± 19; 3. X: 23 ± 13 (no significant difference between the groups NC and C; $P < 0.0025$ HC vs X).

Conclusion: Our data indicate that urinary magnesium deficiency may be a pathogenetic factor in a metabolic subgroup of sterile calcium urolithiasis. In these patients a trial of oral magnesium supplements seems justified.

141 A Study of Factors Affecting Urinary Citrate Levels

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Aims. Since citrate forms a soluble complex with calcium and reduces crystal formation in urine (in vitro) hypocitraturia may be a factor in the pathogenesis of calcium urolithiasis. While gross hypocitraturia (eg. in complete distal renal tubular acidosis) is easily documented, lesser abnormalities may be more difficult to demonstrate because of a number of physiological and pathological factors which may affect the urine citrate level. Notable among these are the patients' sex, urine pH (about which there is a debate in the literature), renal dysfunction and urinary tract infection. An attempt has been made here to clarify the role of these factors. In addition, the effect of oral citrate loading on urine citrate excretion has been studied, to see if it is possible to manipulate urine citrate levels in this way.

Methods. 1. Effect of urine pH. This was studied using multiple 20-min urines from each of 15 normal subjects (9 males, 6 females), by measuring the changes in urine citrate to creatinine (c:c) ratio induced by: (a) NH_4Cl and $NaHCO_3$ loading; (b) Spontaneous pH changes across the day. *2. Effect of renal dysfunction.* In 20-min timed urines from 34 patients (23 males, 11 females) with established renal failure (plasma creatinine > 0.20 mol/l) but without urinary tract infection, c:c ratios were measured. *3. Citrate loading.* Citric acid (10 mmol) was fiven orally to 7 normal subjects and the c:c ratio studied across the following 4 h in half-hour periods.

Summary of results. 1. Effect of urine pH. In all normal subjects the c:c ratio was positively correlated with urine pH, both following acid/alkali loading and during periods of spontaneous pH change. The values in females were significantly higher than those in males at all pH values. *2. Effect of renal dysfunction.* The c:c ratio was markedly reduced in these patients at all urine pH levels and no sex difference was observed. There was no correlation between the c:c ratio and plasma creatinine or plasma bicarbonate. *3. Citrate loading.* No significant changes in c:c ratio were seen following oral citrate loading when allowance was made for urine pH changes.

The above studies are being expanded and results will be presented from these larger studies as will the data from (a) patients with lesser degrees of renal impairment and (b) patients with documented urinary tract infections.

Conclusions. 1. Urine pH, renal dysfunction and the patients' sex all affect renal citrate handling. Allowance for these factors must therefore be made when studying citrate excretion and this has not been the case in many reports. 2. Attempts to raise urinary citrate by oral supplementation are unlikely to be successful.

142 The Effect of Piretanide Upon the Urinary Excretion of Calcium and Magnesium in Normal Subjects and Hypoparathyroid Patients

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Piretanide, a diuretic with a sulfonamide structure, increased sodium and calcium excretion during the diuretic period. After diuresis ended, calcium was retained, but not sodium. It was postulated that increased secretion of PTH might be the cause of the calcium-saving effect during the post-diuretic phase (Henry Ford Hosp Med J 28:127, 1980).

In order to test this hypothesis a 6-mg oral dose of piretanide was given to five normal subjects and five patients with hypoparathyroidism (Hypo). All subjects collected urine every 4 h during the control and test day. Calcium and magnesium were measured by atomic absorption spectrophotometry and ionized calcium with an ion-specific electrode.

During diuresis the following increments in urinary excretion were observed. Sodium: Normals (Av. ± 1 SEM): 20.2 ± 5.70 mEq/4 h to 128.7 ± 20.0 ($P < 0.001$); Hypo: 16.5 ± 4.1 to 146.7 ± 19.2 ($P < 0.01$). Calcium: Normals: 17.5 ± 3.9 mg/4 h to 65.8 ± 6.7 ($P < 0.001$); Hypo: 7.7 ± 2.6 to 62.2 ± 9.9 ($P < 0.005$). Magnesium: Normals: 8.9 ± 0.7 mg/4 h to 31.5 ± 1.4 ($P < 0.001$); Hypo: 7.0 ± 0.8 to 44.9 ± 7.9 ($P < 0.01$).

During the first 6 h after piretanide administration no changes were observed in the serum levels of ionized or total calcium. Meanwhile, significant diminutions were observed in serum sodium and magnesium in both groups of subjects. Serum creatinine increased in normal subjects.

After diuresis, statistically significant diminutions were observed in the urinary excretion of sodium, calcium and magnesium, in normal subjects as well as hypoparathyroid patients. Thus, the effect of piretanide was essentially similar in both groups and the only difference of significance was the percentage change (increment above basal) in calcium excretion during diuresis: Normals: 354 ± 80% vs Hypo: 1,481 ± 545% ($P < 0.05$).

In summary, PTH secretion seems to moderate calcium excretion during piretanide-induced diuresis, while sodium, magnesium and calcium retention after diuresis is not influenced by PTH.

143 Effects of Benzbromarone on the Pharmacokinetics and Pharmacodynamics of Oxipurinol

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Fixed combinations of therapeutic agents are useful if the single substances act by different mechanisms, if combinations are more effective, or if doses can be reduced. Little is known about the interactions of single substances in combinations, especially on resorption, transformation and excretion.

In an open study healthy volunteers (males, aged 22–29 years) received 100 mg allopurinol per day for 14 days (phase I), then no medical treatment for the same length of time (phase II). In the following 14 days they received 100 mg allopurinol + 20 mg benzbromarone (phase III). At the end of each phase blood specimens and 24-h urine samples were taken and analysed for oxipurinol (HPLC, Brown and Bye), urate (Uricaquant), creatinine (kinetic Jaffé reaction), calcium, sodium, potassium and chloride (Astra-8-Analyser, Beckman), magnesium (Willis), oxalate (Hodgkinson and Williams) and beta$_2$-microglobulin (Phadebas).

In phase III, the oxipurinol serum levels were lower in the morning and before lunch; urate levels were lower and urate and creatinine clearance elevated in phase III. Calcium levels were lower, potassium, sodium, chloride and magnesium levels and the clearances of urea and chloride elevated in phase III; oxalate concentrations in urine were decreased, serum levels of beta$_2$-microglobulin were also lower. The effect of benzbromarone on oxipurinol levels is accompanied by an elevated creatinine clearance. There is possibly no constant action on urate excretion over 24 h. The findings are discussed with regard to the different sites of action of benzbromarone.

144 Effect of Indomethacin and Flurbiprofen on Glomerular Filtration Rate and Calcium Excretion in the Anaesthetized Rat

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The prostaglandin synthetase inhibitors indomethacin and flurbiprofen lower calcium excretion in hypercalciuric stone-forming patients and in conscious laboratory animals, without associated changes in renal haemodynamics [1–3]. These findings imply a role for renal prostaglandins in the control of calcium transport by the tubule. As a preliminary to micropuncture experiments to establish a possible site of action for prostaglandins in the nephron, we have investigated the effects of indomethacin and flurbiprofen on glomerular filtration rate (*GFR*), clearance of *p*-aminohippurate (C_{PAH}) and calcium excretion in the anaesthetized rat.

Male Sprague-Dawley rats were anaesthetized with Inactin [sodium 5-ethyl-5-(1'-methyl-propyl)-2-thiobarbiturate; 110 mg/kg body weight] and the left jugular vein, right carotid artery and left ureter catheterized. Rats were infused with saline (0.14 M NaCl) containing [^{3}H] inulin (1.0 μCi/ml) and sodium PAH (5.8 mg/ml), at 100 μl/min for 8 h. Control animals (n = 12) received saline infusion throughout. After 5 h, experimental animals received indomethacin in buffered saline (10 mg/kg; n = 9) or flurbiprofen (10 mg/kg; n = 8) infused over a 15-min period. Urine and plasma samples were collected hourly beginning 2 h and 2 1/2 h respectively after the start of saline infusion and each was analysed for [^{3}H] inulin and PAH. Urine flow rate was measured and calcium concentration determined in urine and in a terminal plasma sample.

In the 2 h prior to infusion of the drugs there were no significant differences between control and experimental animals in any of the variables measured. Results are presented in Table 1 for the 2nd

Table 1. Haemodynamics and calcium excretion 2–3 h after indomethacin or flurbiprofen

	GFR (μl/min)	C_{PAH} (μl/min)
Control	881.0 ± 31.6	3,580 ± 300
Indomethacin	611.5[a] ± 46.4	3,140 ± 350
Flurbiprofen	640.0[a] ± 48.2	1,686[a] ± 351
	Urine flow (μl/min)	**Calcium excretion (nmol/min)**
Control	36.40 ± 2.80	32.0 ± 4.6
Indomethacin	16.07[a] ± 2.50	3.8[a] ± 1.2
Flurbiprofen	14.66[a] ± 3.80	11.58[a] ± 4.1

[a] Indicates significant difference from control ($P < 0.05$)

hour after infusion of the drugs. There were no significant differences between the 2nd and 3rd hour after indomethacin or flurbiprofen infusion.

We conclude that indomethacin and flurbiprofen reduce calcium excretion in the anaesthetized rat. However, because they also alter renal haemodynamics it is not possible to discern whether these drugs directly affect tubular handling of calcium in this preparation.

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145 Evaluation of Some Stone Promoting and Inhibiting Factors in Fasting and Postprandial Urine

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We were able to demonstrate that the majority of stone patients excrete more oxalate in urine in response to an oxalate-free test meal than healthy control subjects. Conversely, this higher level of oxalate could not be verified in 24-h urine specimens. Our conclusion with respect to increased oxalate in urine during a 3-h postprandial period was that this period might more readily reflect changes in stone-promoting and stone-inhibiting activities. The following investigation was aimed at further characterizing promoters (calcium, phosphate, pH, relative saturation of urine with calcium phosphate) and inhibitors (magnesium, citrate, pyrophosphate) in a 2-h fasting and in a 3-h postprandial urine.

Methods. Patients (Idiopathic hypercalciuria = I–HC, n = 30; normocalciuria = NC, n = 24) with recurrent calcium urolithiasis, and no other diseases, were referred to this laboratory while on a free home diet and subsequently studied under ambulatory conditions. After an overnight fasting period of 12–15 h blood was drawn from a forearm vein without stasis. A 2-h clearance period followed, initiated by drinking of 2 x 300 ml distilled water. Thereafter a liquid test meal supplemented with 1,000 mg Ca^{++} (Vivasorb, Pfrimmer; Erlangen, FRG) was taken. Spontaneous bladder voiding into pre-warmed (37 °C) devices was carried out for quantitation of crystalluria and volume. Healthy control subjects (n = 22) matched with respect to weight, age and sex were studied identically.

Analyses. In serum – creatinine; in urine – creatinine, calcium, phosphate (Pi), pH, magnesium (Mg), citrate (Cit), pyrophosphate (PPi); cyclic AMP (as indicator of parathyroid gland activity). Calculations: creatinine clearance; relative saturation product of calcium phosphate (RSP-CaPi) according to the nomogram of Marshall and Robertson. Crystalluria was evaluated by the filter technique of Werness and Smith. Patients (all with normal creatinine clearance) were divided into two groups according to their calcium to creatinine ratio in fasting and postprandial urine (NC; I–HC) and to their calcium excretion in 24-h urine (I–HC).

Results. 2-h fasting period – Pi (per unit creatinine) was decreased in NC as well as in I–HC ($P < 0.01$); pH was unchanged in both groups. The resulting RSP–CaPi was increased in NC (median/range: −0.23/−1.2 – 0.80) and unchanged in I–HC (−20/−1.3 – 0.10) versus controls (−0.60/−1.3 – 0.20). Mg and Cit (excretion per unit creatinine) were in the same range in NC and I–HC as in controls, whereas PPi (concentration and excretion) in NC was significantly decreased. Also cyclic AMP was unchanged. With few exceptions calcium-oxalate crystals only were detectable, with a slightly higher mean score in NC, I–HC than in control individuals. 3-h postprandial urine – Pi and pH did not differ from control values in NC, I–HC. The RSP–CaPi increased in response to the meal to almost the same level (in the metastable range of supersaturation) in stone patients (NC: 0.20/−0.40 – 0.95; I–HC 0.25/−1.0 – 1.2) and controls (0.15/−1.25 – 0.80). Mg per unit creatinine was decreased in NC only, but increased in I–HC. No changes could be detected in Cit and PPi. Also cyclic AMP was unchanged in stone patients, but was significantly decreased when compared with the 2-h fasting period. Again calcium-oxalate crystals predominated, but the crystal score in stone patients was elevated ($P < 0.05$).

Conclusions. (1) when comparing 2-h fasting with 3-h postprandial urine, instead of examining 24-h urine, there may be differences in factors governing crystal and stone forming and inhibiting processes; (2) only NC patients appear to show a deficiency in stone inhibitors (PPi in 2-h, Mg in 3-h urine), whereas in I–HC important inhibitors (PPi, Mg, Cit) appear normal; (3) crystalluria in this type study is unable to discriminate stone formers and controls in 3-h postprandial urine, but the failure may be related to the concomitant suppression of parathyroid gland activity.

146 Urinary and Serum Sulfate in Idiopathic Recurrent Calcium Urolithiasis (RCU)

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Sulfate (total, inorganic, esterified) is present in considerable amounts in human urine and serum [1]. Little is known about sulfate in RCU patients, although two reasons suggest a relationship between stone-forming processes and sulfate: (1) free sulfate ions in tubular fluid or urine compete with oxalate for free calcium ions and calcium sulfate complex formation; (2) urinary inorganic sulfate under certain dietary conditions correlates directly with urinary calcium, suggesting that so-called idiopathic hypercalciuria (IHC) may be linked with either a high protein level in the diet, enhanced intestinal absorption of dietary sulfate, increased hepatic sulfur oxydation or altered renal sulfate handling.

Subjects and methods. We studied sulfate in 71 subjects (19 controls; 26 IHC patients; 26 normocalciuric patients, NC), grossly matched for age (mean: controls, 37 years; NC, 40; IHC, 41) and sex (37 ♂; 34 ♀), according to the examination program developed for our outpatient stone clinic [2]. It mainly consists of collecting (in subsequent order) 24-h urine, 2-h urine after overnight fast, 3-h postprandial urine (following a test meal in the laboratory), with fasting blood taken prior to the start of the 2-h period. Sulfate in urine and serum was measured by different colorimetric methods, other substances by routine procedures.

Results. 24-h urine – In NC (♂ + ♀) the excretion of total, inorganic, ester sulfate was significantly ($P < 0.05$ – $P < 0.01$) decreased (medians: 22.1, 20.3, 1.9 mM; same order), when compared with controls (medians: 30.6, 28.6, 2.9 mM). In IHC the respective figures for medians were 32.1, 29.9, 2.3 (not significantly different from controls). In general, in females sulfate (total, inorganic) was lower than in males (controls and patients). Normalization for urinary creatinine (as an indirect measure of creatinine clearance and glomerular filteration rate) only eliminated the difference with ester sulfate in NC (♂ + ♀). Fasting serum – Again in NC sulfate was decreased (median 0.346 mM/l) as compared with controls (0.393 mM/l; $P < 0.05$), but was unchanged in IHC (0.384 mM/l). 2-h fasting and 3-h postprandial urine – There were no differences in sulfate level between the groups (excretion rate; sulfate factorized for creatinine). There were direct significant correlations between factorized urinary calcium and inorganic sulfate in 2-h fasting urine (NC; IHC), and these two variables in 24-h urine (NC).

Conclusions. (1) NC subjects appear to suffer from a deficit in 24-h urine of stone inhibitors (and complexors) like citrate [3], magnesium [4], sulfate (this study); (2) because in NC sulfate in serum is lowered but normal in fasting urine, net tubular sulfate reabsorption should be reduced; (3) the role of tubular sulfate in the development of

hypercalciuria is thus unclear in RCU; (4) the correlations between sulfate and calcium in stone disease support the view that their interdependency is not yet understood.

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147 Urine and Serum Potassium in Patients with Recurrent Calcium Urolithiasis – Results of a Pilot Study

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Potassium (K) metabolism in renal stone disease has received little attention [1], although K is a key substance in renal acid-base and citrate regulation, and forms soluble complexes with oxalate [2]. In subgroups of stone formers we found a tendency toward hypokalemia [3], and disturbances of citrate and acid-base metabolism are well documented. These facts necessitate inclusion of K into stone research.

Material. All participants (n = 127) were on an unrestricted free home diet and were examined as ambulatory subjects. The patients (n = 86) with multiple recurring calcium stones (oxalates; phosphates), but no associated disorders, were classified as having normocalciuria (NC; n = 48) or idiopathic hypercalciuria (IHC; n = 38) on the basis of the calcium to creatinine ratio in fasting morning (NC; < 0.12) and postprandial (IHC: > 0.27) urine. The male to female ratio was 44 (NC 25; IHC 19): 42 (NC 23; IHC 19). Age (years): in NC 22–68 (median 40), IHC 26–64 (median 43.5). The controls (n = 41; male:female = 25:16) were metabolically healthy subjects (age 18–70, median 32). Methods: all collected (same order) 24-h and 2-h morning (after a 15-h overnight fast), 3-h postprandial (after a calcium- and carbohydrate-rich synthetic test meal) urine, the latter two in the laboratory.

Analyses. Fasting serum – creatinine, K, citrate, bicarbonate (as crude indicator of metabolic acidosis); urine – creatinine, K, citrate and pH (all by routine procedures). The percentage filtered-excreted K (FE–K) was recalculated after allowing for extrarenal K losses (FE–K–corr; 4). Data are median/range.

Results (some given in Table 1). 24 h urine – K excretion was unchanged (NC; IHC) in the presence of citrate deficiency (not shown), but in patients [K] appeared to be lowered despite the higher concomitant urinary volume (Table 1; A). Fasting serum – [K] was statistically not different in the groups, but in stone disease median [K] was lower (Table 1; B). 2-h fasting urine – only in NC, not IHC, K excretion per unit nephron was reduced (predominantly in female patients; not shown) (Table 1; C); also FE–K and FE–K corr were decreased (Table 1; C), while pH tended toward more alkaline values (NC, IHC; $P < 0.10 > 0.05$) and citrate was unchanged. FE–K and pH were positively correlated in controls (r = 0.53; $P > 0.001$), IHC (r = 0.42; $P < 0.01$), not in NC (r = 0.27; *NS*), indicating a normal K ion for hydrogen ion exchange in the distal tubule of the first two groups only. 3-h postprandial urine – none of the parameters of K and citrate, or the pH, were different among the groups studied.

Conclusions. (1) K abnormalities are most likely present in calcium stone formers and are preferentially detected when studying urines other than from the 24-h cycle or a postprandial period; (2) from the FE–K corr, which is not significantly lower than FE–K, it is evident that renal losses of K do not occur in stone disease; (3) enhanced net tubular K reabsorption as shown by NC patients suggests that the kidney helps keep normal extracellular K, which may be threatened to some degree; (4) in the absence of correlations between urinary K and citrate (all groups) and K and pH in NC, their functional interdependency is unclear at present.

Table 1

	Controls ♂+♀	NC ♂+♀	IHC ♂+♀
A. Volume; ml/24 h	1,465/591 – 2,580	1,705/480 – 3,640	1,855/1,020 – 4,000[c]
[K]; mM/l	40/17–88	31/10–75[b]	22/13–128[c]
B. [K]; mM/l	4.3/3.7–5.5	4.3/3.5–5.1	4.2/3.4–4.9
C. Volume; ml/2 h	200/58–700	198/41–720	325/60–680[b]
[K]; mM/l	42/11–170	36/5–173	25/11–85[b]
$U_K V \cdot C_{cr}^{-1} \cdot 100$; μM	8.9/2.8–21.0	6.8/2.7–17.3[a]	8.7/3.9–15.2
FE–K; %	15.4/5.0–33.8	12.1/5.2–28.4[a]	13.2/7.5–26.9
FE-K-corr; %	16.1/5.0–31.5	11.5/5.0–27.8[b]	11.7/6.8–23.3

[a]$P < 0.05$; [b]$P < 0.01$, [c]$P < 0.001$ versus controls (U-Test)

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148 Metabolic Evaluation of Urolithiasis: Evidence for a Disorder of Phosphate Metabolism in Idiopathic Hypercalciuria

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Controversy persists about the pathogenesis of idiopathic hypercalciuria (IH). This study was therefore corried out in order to gain further information about metabolic defects responsible for this cause of calcium nephrolithiasis; 263 normal subjects (N), 233 normocalciuric (NC) and 180 hypercalciuric (HC) patients with urinary stone disease participated in the study. Metabolic evaluation included a 24-h urine collection on a free diet to determine calcium, phosphorus and creatinine; a blood sample was also obatined immediately following urine collection for measurement of calcium, phosphorus, creatinine and immunoreactive parathyroid hormone (iPTH). Hypercalciuria was defined by normocalcemia and a 24-h urine calcium excretion rate above 300 mg in men or 250 mg in women. Serum and urine calcium was measured by atomic absorption spectrophotometry, phosphorus by a colorimetric technique; serum iPTH concentration was determined by radioimmunoassay using and antiserum having affinity principally for the carboxyterminal region of the peptide. Creatinine clearance values were above 70 ml/min in all the subjects studied; mean ± SEM walues of plasma parameters in the groups are shown in Table 1 (number of ovservations in brackets):

Table 1

	N	NC	HC
Calcium (mg/dl)	9.63 ± 0.030 (263)	9.63 ± 0.032 (233)	9.73 ± 0.037[a] (180)
Phosphorus mg/dl)	3.47 ± 0.035 (248)	3.37 ± 0.039 (233)	3.34 ± 0.044[b] (180)
iPTH (ng/ml)	0.44 ± 0.029 (66)	0.38 ± 0.042 (33)	0.34 ± 0.021[c] (87)

differs from normal $P < 0.05$[a]; 0.02[b]; 0.006[c]

The results obtained in patients classified as having hypercalciuria, raised plasma calcium and decreased iPTH values, seem to

suggest that an intestinal hyperabsorption of calcium, either primary or secondary to phosphate depletion, is a common feature of this disorder. The finding that mean plasma phosphorus values in patients with IH are significantly lower than in normal subjects seems to emphasize the role of a disorder in phosphate metabolism (namely a renal leak for phosphate independent of parathyroid hormone) as a factor promoting lithogenesis in these patients. Finally, when considering the results obtained by using radioimmunoassay for PTH, hypercalciuria from renal calcium leak seems to be quite rare.

149 Mechanism of Sodium Glycolate Absorption in Rat Intestine

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Glycolic acid is a precursor of ocalate in animals and man. In addition to the exogenous sources, mainly from leafy vegetables, glycolate can also be synthesized in vivo from various compounds. Dietary glycolate plays an important role in the formation of urinary oxalate. Under physiological conditions, about 5% of dietary glycolate is converted to urinary oxalate. This conversion rate increases in conditions like pyridoxine deficiency. However, no data are available on the mechanism of glycolate absorption, and its alteration in vitamin B_6 deficiency. The present work was undertaken to elucidate the mechanism of intestinal transport of glycolate in the rat and to study the effect of pyridoxine deficiency therein.

Vitamin B_6 deficiency was produced in weanling rats and the pyridoxine status biochemically ascertained by estimating erythrocyte glutamate pyruvate transaminase (EGPT) by the method of Kishi and Folkares (1976). 1-^{14}C-sodium glycolate transport was studied in normal and vitamin-B_6-deficient rats by the tissue accumulation technique of Alvarado and Mahmood (1974), using intestinal rings. Glycolate transport was expressed as mircromoles of glycolate transported/30 min/g wet wt. tissue. The absorption of glycolate showed a saturation kinetics in the jejunoileal region, with a Km of 6.25 mM for glycolate and V_{max} 5.5 μmol/30 min/g wet wt. The absorption was linear up to 25 min at 37 °C. Jejunum and ileum showed significantly higher absorption of glycolate as compared to colon. Sulfhydryl binding agents viz. *P*-chloromercuribenzoate and iodoacetate, and respiration inhibitors eg. KCN and 2,4-dinitrophenol had no significant effect on glycolate uptake. However, glyoxylate and lactate showed a significant inhibition at 6mM concentration of the inhibitor. Pyridoxine deficiency had no effect on glycolate uptake by rat intestine. Therefore, the enhancement rate of oxalate synthesis in vitamin-B_6-deficient rats is not dependent on the increased intestinal absorption of glycolate, but probably due to induction of glycolate oxidase, the key enzyme involved in the conversion of glycolate to oxalate.

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2 Alvarado F, Mahmood A (1974) Biochemistry 13:2882

150 Intestinal Absorption of Oxalate in Gonadectomized Rats

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Hyperabsorption of oxalate from the gut is knwon to be a primary cause of oxalate urolithiasis, a disease predominant in males. The role of sex hormones on the intestinal absorption of oxalate was investigated in the following groups of rats: males (M), castrated males (CM), castrated males implanted with estradiol (CM + E), females (E), castrated females (CF), castrated females implanted with testosterone (CF + T).

After 1 month of hormone implantation the gonadectomised rats were killed, and intestine from the ligament of Trietz up to the ileoceacal junction was removed. Everted intestinal rings were incubated in 5 ml oxygenated Kreb's-Ringer buffer (containing potassium oxalate and trace amounts of ^{14}C oxalic acid) for 45 min at 37 °C with continuous shaking, after which the incubated rings were blotted, weighed and ^{14}C uptake measured in a liquid scintillation counter. The uptake was expressed as μmol/h/g tissue wt. At 0.5 mM oxalate concentration, rats of the CM group absorbed about 2.2 times more oxalate, while those of the CM + E group absorbed only 1.4 times more than those of the M group suggesting that castration increases the oxalate uptake rate in male rats and that estradiol implantation in castrated male rats is only partly able to suppress the increase in oxalate uptake. On the other hand, in female rats (F, CF and CF + T), ovariectomy and testosterone did not affect the oxalate uptake rates.

A linear rise in the oxalate uptake rate with increasing concentrations of oxalate (0.1 mM – 6.0 mM) in the three groups of male rats suggested that intestinal absorption of oxalate was by passive diffusion. The slope values (as calculated from oxalate uptake vs oxalate concentration plot) of 0.51 for M, 1.07 for CM an 0.77 for CM + E, indicate that though castration enhances oxalate uptake, it can be partly suppressed by estradiol. The study therefore reveals that the sex hormones regulate the intestinal uptake of oxalate only in males, though the mechanism involved therein still remains to be elucidated.

151 Effect of Maleic Acid on the Intestinal Uptake of Calcium and Oxalate in Pyridoxine-Deficient Rats

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Earlier work from this laboratory showed that oxalate uptake in vitamin-B_6-deficient animals is facilitated by a biphasic transport mechanism. Glycolate and glyoxylate competitively inhibit the oxalate uptake by a carrier-mediated component and the induction of the oxalate transport carrier is inhibited by the administration of inhibitors of protein synthesis [1]. The aim of the present study was to envisage the effect of mono- and dicarboxylic acids on the uptake of calcium and oxalate, the two major constituents of urinary calculi. Twelve male albino rats were put on a vitamin-B_6-deficient diet for 45 days. The pyridoxine status of the animals was biochemically ascertained [2]. Calcium and oxalate transport was measured by the tissue accumulation technique described by Alvarado and Mahmood [3]. Among the many mono- and dicarboxylic acids tested viz. pyruvate, lactate, succinate and maleate, only maleic acid had a marked effect on the calcium and oxalate transport rates. Maleic acid (1 mu) in the incubating medium significantly ($P < 0.001$) enhanced the initial (30 s) rate of oxalate uptake and transient accumulation of substrate in the tissue, thereby suggesting that the effects of maleic acid on the oxalate uptake is at the membrane interface. Kinetic analysis of the transport system indicates that maleic acid enhances the capacity of the transport system either by activating the transport carriers or by exposing them to the mucosal oxalate. The affinity of the transport system remains unaltered.

Calcium absorption was significantly increased in vitamin-B_6-deficient rats and the kinetics of calcium uptake in the presence of maleic acid suggest that the dicarboxylic acid competitively inhibits calcium uptake both in controls and in vitamin-B_6-deficient rats.

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152 Interactions of Steroid Hormones and Pyridoxine in the Regulation of Oxalate Metabolism in Rats

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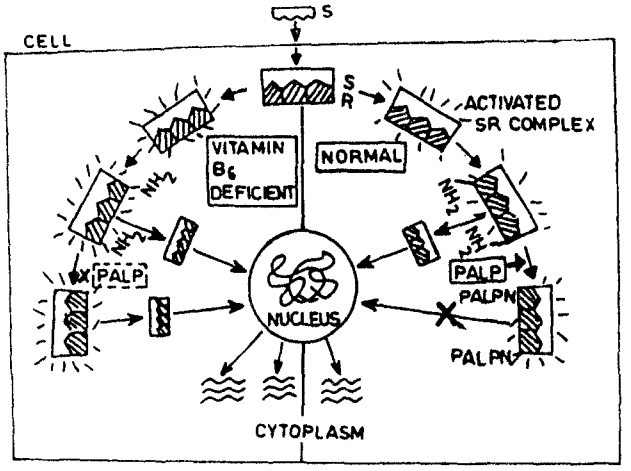
Pyridoxine status is inversely related to the production of oxalate from its precursors, thus regulating the enzymes involved in its biosynthesis. The effect of this vitamin deficiency is eliminated by hepatectomy, suggesting that the mode of action is mediated by the liver enzymes. Calcium oxalate stone formation in pyridoxine deficiency is also known to be sex-related. Interaktions of pyridoxine deficiency and hormones on the oxalate synthesizing enzymes have been elucidated in this study. Young male rats treated for 1 month were grouped accordingly: SB: Fed pyridoxine-deficient diet (ad libitum) only; SPF: Pair-fed of SB made pyridoxine sufficient; EB: Fed pyridoxine deficient diet (ad libitum) + estradiol implantation; EPF: Pair-fed of EB made pyridoxine sufficient + estradiol implantation.

The pyridoxine status of the animals was biochemically assessed from the erythrocyte alanine transaminase (EALT) levels. Glycolate oxidase (GAO), glycolate dehydrogenase (GAD) and lactate dehydrogenase (LDH) were assayed from liver homogenates and the activities expressed as units/mg protein (mean ± SEM of 6–8 rats). The following pattern of the specific activities of GAO SB (8.19 ± 0.52) > SPF (5.35 ± 0.49) = EB (5.05 ± 0.58) > EPF (2.69 ± 0.62) indicated that the elevated levels of GAO in vitamin B_6 deficiency were lowered by estradiol administration, or rather, the effects of this vitamin deficiency and estradiol were counteracted (GAO activities in SPF = EB). A negative corralation was observed between the levels of (i) GAO and EALT ($r = -0.557$, $P < 0.01$), (ii) GAD and EALT ($r = -0.554$, $P < 0.05$), while the LDH activities in all groups were similar. These findings demonstrate that pyridoxine status regulates the two major enzymes of oxalate biosynthesis, i.e. GAO and GAD, and that estradiol has an inhibitory effect on GAO activity in both normal and pyridoxine-deficient animals. Pyridoxal-5'-phosphate (PALP) is known normally to be a cytoplasmic modulator inhibiting the nuclear translocation of steroid receptor complex (after forming Schiff's base with e-lysine residues of the receptor), which is lost in pyridoxine deficiency, leading to an increased steroid translocation to the cells (Fig. 1). It may be hypothesized that steroid hormones may serve as a modulator of pyridoxine-related control of oxalate metabolism in the mammalian system.



S: Steroid R: Receptor
PALP: Pyridoxal-5'-Phosphate

Fig.1

153 Hereditary Familial Renal Phosphate Leak with Bone Lesion, Hypercalciuria and Nephrolithiasis

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A new familial syndrome consisting of kidney stones and bone lesions is presented. The parents are first-degree cousins. The father, two sons and a daughter are affected. Metabolic studies revealed that all the affected siblings were normocalcemic (9.68 ± 0.16 mg/dl, mean ± SE), hypophosphatemic (2.54 ± 0.23 mg/dl), hypercalciuric (376 ± 30 mg/24 h), and had elevated serum alkaline phosphatase levels of skeletal origin (148 ± 17 IU/l). $TmPO_4/GFR$ was low (2.15 ± 0.17 mg/100 ml GF, normal range 2.5–4.2); Uc-AMP and iPTH were in the low normal range. Oral calcium (Ca) and phosphate (Pi) loading tests were performed to assess gastrointestinal absorption. A calcium loading test (1,400 mg Ca) showed the change in urinary calcium excretion, expressed as mg Ca/100 ml GF, between the post- and preloading periods to be 0.28 ± 0.02 compared to 0.12 ± 0.02 in 69 normal controls. A phosphate loading test (1400 mg elemental phosphorus) showed the peak difference in serum phosphate to be 2.56 ± 0.26 mg/dl in patients versus 1.18 ± 0.22 mg/dl in 6 normal controls, including the healthy siblings. A positive linear correlation ($r = 0.85$) between urinary phosphate excretion and SPi was found in both groups; the slope of the line and the intercept on the X-axis (theoretical Pi threshold) were significantly different in the patients as compared to the controls (0.79, 1.4 mg/dl and 0.41, 2.2 mg/dl respectively). A bone biopsy specimen from one of the affected brothers showed changes compatible with osteomalacia. Based on these results, it has been concluded that the primary defect is a primary renal phosphate leak, leading to the following sequence of events: hypophosphatemia → increased 1.25-dihydroxycholecalciferol production → enhanced Ca and Pi gastrointestinal absorption → enhanced Ca filtered load and suppressed PTH secretion → hypercalciuria → tendency to kidney stone formation. Osteomalacia might have been induced by the hypophosphatemia per se. Clinical and biochemical improvement were achieved with continuous oral Pi treatment.

154 Relationship Between Hypercalciuria and Vitamin-D_3 Status in Renal Stone Formers

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Aims of the study. Increased urinary excretion of calcium is common among patients with recurrent urolithiasis. No single causative factor has been identified. A common abnormality besides the high excretion of calcium is low normal or subnormal serum phosphate levels. Low serum phosphate is known to be a stimulus for the renal conversion of 25-hydroxy vitamin D_3 (25-OH-D_3) into the most active metabolite 1,25-dihydroxy vitamin D_3. Other investigators have demonstrated increased serum levels of 1,25-dihydroxy vitamin D_3 in serum from renal stone formers and in serum from patients with hypercalciuria. The major circulating form of vitamin D_3 is, however, 25-OH-D_3. Whether or not the pool of 25-OD-D_3 is increased in hypercalciuria is not knwon. In order to study this possibility we have determined 25-OH-D_3 in stone-forming patients.

Methods employed. Seventy patients (50 males and 20 females) with urolithiasis, mainly recurrent stone formers, participated in the study. The mean age of the males was 43 years (range 15–71), of the females 39 years (range 17–67). They were all outpatients that consulted our clinic between October and March. All patients with present or previous hyperparathyroidism were excluded from the study. Blood samples for determination of calcium and phosphate levels in serum were taken on each of 3 consecutive days. A blood sample for determination of 25-OH-D_3 was taken on one of these days. A 24-h urine sample was collected for each of the 3 days for determination of calcium. 25-OH-D_3 was determined by a highly specific and accurate method based on isotope dilution-mass spectrometry.

Summary of results obtained. The male hypercalciuric stone formers (excretion of calcium ≥ 8.1 mmol/24 h; $n = 24$) had a significantly higher mean level of 25-OH-D_3 (28.3 ± 2.3 ng/ml) than had the male normocalciuric stone formers (excretion of calcium ≤ 6.0

mmol/24 h; $n = 16$; 17.6 ± 1.2 ng/ml; $P < 0.001$). Male stone formers with an intermediary excretion of calcium (⩾ 6.1, ⩽ 8.0 mmol/24 h; $n = 10$) had a 25-OH-D_3 level between the levels of the other two groups (21.3 ± 1.2 ng/ml). The female stone formers with normocalciuria ($n = 16$) had a mean level of 25-OH-D_3 (17.6 ± 1.3 ng/ml) identical with that of the male normocalciuric stone formers. The combined small group of female stone formers with high and intermediary excretion of calcium ($n = 4$) had a mean 25-OH-D_3 level significantly higher (26 ± 4 ng/ml) than that of the group of female stone formers with a normal urine excretion of calcium. When the male and female stone formers with high and intermediary excretion of calcium were combined into one group ($n = 38$), the level of 25-OH-D_3 was significantly higher (26.2 ± 1.6 ng/ml) than in the corresponding group ($n = 32$) with a normal calcium excretion (17.6 ± 0.9 ng/ml; $P < 0.001$). When the calcium excretion was plotted against the level of 25-OH-D_3 for each individual patient, only a low correlation was obtained ($r = 0.40$). The serum level of calcium, while within the normal range, was slightly higher in the group of patients with a normal excretion of calcium than in the group of patients with hypercalciuria ($P < 0.05$, males + females). There was a tendency towards lower levels of phosphate in serum in patients with hypercalciuria, but this was not statistically significant ($P > 0.05$). There was no correlation between serum levels of 25-OH-D_3 and serum calcium ($r = 0.06$), or between serum levels of 25-OH-D_3 and serum phosphate ($r = 0.06$).

Conclusions. Since vitamin D_3 is the predominant form of vitamin D, and 25-OH-D_3 is the main circulating metabolite of vitamin D_3, it seems reasonable to believe that the values obtained reflect the total vitamin-D status of the subjects. The restults are in accordance with the contention that the vitamin-D_3 status might be of importance for the development of hypercalciuria in these patients.

155 Reduced Urine Magnesium to Calcium Ratio and Renal Stones in Essential Hypertension

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A significant incidence of renal stones has been reported in essential hypertension (EH) (Ljunghall S et al (1975) Br Med J 4:580). Recently, a consistent calcium renal leak with secondary parathyroid overactivity has been documented in hypertensives (McCarron DA et al (1980) Hypertension 2:162; Strazzullo P et al (1983) Clin Sci 65:137). In addition, magnesium has been reported to reduce calcium oxalate crystal formation in human whole urine (Hallson PC et al (1982) Clin Sci 62:17. In order to investigate whether stone formation in EH may also be associated with abnormalities of urine magnesium excretion, calcium and magnesium urine levels as well as parathyroid activity were evaluated in 60 untreated hypertensives (DBP > 95 mmHg, aged 42 ± 3 years, BMI 26.8 ± 6, 30 males) with normal renal function (creatinine clearance > 80 ml/min) as well as in 60 normal controls (aged 43 ± 2 years, DBP < 85 mmHg, BMI 25.8 ± 0.5, 30 males). Serum total and ionized calcium, magnesium and parathormone were determined as well as urine calcium, magnesium and cyclic AMP. Values of 24-h urine calcium excretion were higher in EH and correlated to blood pressure ($r = 0.26$, $P < 0.05$). There was a correlation between urinary magnesium and calcium excretion both in hypertensives ($r = 0.44$, $P < 0.005$) and in normals ($r = 0.38$, $P < 0.005$), but no differences between the two groups were detected. Compared to normal controls the magnesium to calcium ratio in 24-h urine was significantly reduced in EH (15%) but, surprisingly, the serum magnesium values were significantly higher ($P < 0.01$). Parathyroid activity was found to be increased in EH on the basis of serum PTH and urine cyclic AMP. It is concluded that in patients with EH urinary magnesium excretion is not increased as is urinary calcium. Consequently a reduced Mg:Ca urine ratio may play an additional role in the forming of stones in hypertensives. The increased parathyroid activity in EH, secondary to a tubular defect in calcium reabsorbing, may explain the reduction of magnesium values in pateints with this condition.

156 Proximal Tubule Sodium Handling in Calcium Stone Formers

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A renal proximal tubular defect, with reduced fractional reabsorption of sodium (Na), has been suggested as a possible explanation for idiopathic hypercalciuria (IH). Moreover, daily calcium excretion (UCaV) has been shown to increase in IH following an increase in Na intake. Thus, we evaluated the relationship between Na intake, proximal tubule Na reabsorption and Ca excretion in 15 healthy controls (C) and 32 idiopathic Ca stone formers (CSF) [10 patients were normocalciuric (NC) UCaV > 320 ♂ – 280 ♀ mg/day on a 1 g Ca diet; 22 were hypercalciuric (IH)], before and after an Na dietary supplementation. Proximal Na reabsorption was determined as distal chloride delivery (ClDD) during maximal water diuresis. On a freee Na diet, Na excretion (NaE) and ClDD during free water clearance studies, were lower in C (1.07 ± 0.87 mEq/l GFR and 10.6 ± 4% respectively) than in NC (2.2 ± 0.9, $P < 0.005$, and 14.2 ± 3.3, $P < 0.02$) and IH (2.04 ± 1.1, $P < 0.005$, and 15.4 ± 4.7, $P < 0.001$), without any statistical difference between CSF. NaCl oral supplementation (6 g/day for 7 days) in 8C increased NaE and ClDD to similar levels as in NC and IH (1.94 ± 0.7 and 15.1 ± 6.5) without any significant change in UCaV (from 135 ± 5.5 to to 146 ± 76, P NS) and slightly reduced TmPO4/GFR (from 3.99 ± 0.05 to 3.62 ± 0.7, $P < 0.05$), which still remained higher than in IH on a free Na diet. For any level of Na excretion, the distribution of ClDD fell within the 95% confidence limit of the values observed in C. There were no significant correlations between ClDD and UCaV or TmPO4/GFR either in C or CSF.

Conclusions. 1. There is a reduction in fractional reabsorption of Na in the proximal tubule, not only in IH, but also in NC. 2. This Na reabsorption pattern appears to be related to habitual high Na intake and not to a 'tubular defect'. 3. There is no relationship between proximal tubule function, hypercalciuria and TmPO4/GFR. 4. IH does not seem to be strictly related to tubular Na handling.

157 Critical Role of Oxalate Restriction in Association with Calcium Restriction to Decrease the Probability of Being a Stone Former: Insufficient Effect in Idiopathic Hypercalciuria

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Since simple dietary calcium restriction (CaR) increases oxalate excretion in all subgroups of calcium stone formers (CaSF) and increases the PSF index (probability of being a stone former) of Robertson (Br J Urol 1978, 50:449) in idiopathic hypercaliuria (IH), the PSF was calculated in three groups of idiopathic CaSF [normocalciuric (NC), dietary hypercalciuric (DH), and IH, this latter group being defined by a Ca excretion > 3 mg/kg on a CaRD of 400 mg/day] under four conditions: on a free diet, on a Ca and Ox restricted diet (Ca^-, Ox^-) during 4 days, and after an oxalate load (200 g spinach) while on a Ca-free (Ca^+) and on a calcium restriction (CaR) diet (Ca-).

The table shows that combined Ca and Ox dietary restriction significantly decreases PSF only in NCa and DH. In IH the decrease in PSF is not significant because oxalate exretion significantly increases (33 → 39 mg/day) ($P < 0.02$) when dietary calcium is restricted in spite of concomitant oxalate restriction. The oxalate load significantly increases the PSF in all subgroups only when dietary calcium is restricted.

* $P < 0.02$	PSF (absolute)[a]		ΔPSF with Ox load	
** $P < 0.01$	Free diet	$Ca^- Ox^-$	Ca^+	Ca^-
NCa ($n = 18$)	0.56	0.44*	+0.11	+0.27**
DH ($n = 7$)	0.80	0.48**	+0.06	+0.36**
IH ($n = 17$)	0.77	0.68	+0.02	+0.24**

[a]mean ± SEM of PSF in controls = 0.34 ± 0.04 (Bataille, J Urol, Sept 83).

Conclusions. 1. In all CaSF concomitant oxalate restriction is critical to lessen the oxalate excretion increase induced by a CaR diet. 2. In IH, however, this effect is not sufficient to significantly decrease the PSF, suggesting that additional measures would be necessary.

158 Urinary Isocitrate Excretion in Normal Individuals and in Stone Patients

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Isocitrate, like citrate, was found to form a soluble complex with calcium ions. The complex-forming effect was 41% that of citrate. This was found by mixing solutions containing Ca^{++}, ^{14}C-oxalate and unlabelled oxalate in the presence of increasing amounts of citrate and isocitrate and measuring the radioactivity in the supernatant.

The isocitrate assay was performed using isocitrate dehydrogenase in the presence of $NADP^+$ and $MnSO_4$ at pH 7.5 in triethanolamine buffer. The specificity of the method was good. Citrate, ascorbate, pyruvate, oxalacetate, lactate, oxalsuccinate and acetoacetate did not interfere with the isocitrate assay.

The 24-h urines of 33 healthy adults contained 0.58 ± 0.21 mmol isocitrate. There was no significant difference between men and women; 37 calcium oxalate stone patients excreted 0.54 ± 0.20 mmol, 10 uric acid stone patients 0.68 ± 0.37 mmol and 15 patients with phosphate stones 0.60 ± 0.32 mmol isocitrate in 24 h.

Because of the complex-forming effect of isocitrate, not only citrate but also isocitrate should be determined in urines of stone formers.

159 Sodium Excretion in Normocalciuric and Hypercalciuric Sterile Calcium Urolithiasis

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Dietary sodium has recently been implicated in the pathogenesis of the idiophatic hypercalciuria syndrome (Muldowney FP, Kidney Int 22:292–296, 1982). To clarify the role of dietary sodium in recurrent sterile calcium urolithiasis, we have examined urinary sodium in 93 unselected patients and 57 healthy subjects (C) undergoing a metabolic evaluation by means of an oral calcium loading test (Pak 1975). In contrast to Pak, no Ca- or Na-restricted pretest diet was used. Patients were classified as follows: normocalciuric (NC), $n = 38$; hypercalciuric (HC), $n = 55$ subgrouped as "renal-Ca-leak" ($n = 17$), "renal phosphorous leak" ($n = 11$), "absorptive-HC" ($n = 9$) and "non-classifiable HC" ($n = 18$). Determination of urinary sodium was by flame photometry, urinary calcium and phosphate by SMA 2 autoanalyzer. Statistics: one-tailed *t*-test.

Restults. 1. Sodium excretion rates in 24-h urine and fasting urine were higher in NC and HC patients than in the C group. Likewise, theoretical phosphate threshold (TmPi/GER) and serum phosphate were depressed in both stone-forming groups (NC and HC). 2. No significant differences in sodium excretion could be found between the diagnostic subgroups of HC and between the NC- and HC groups. 3. Correlation between 24-h urine overall sodium (X) and calcium (Y) excretion was weak though statistically significant in the C group ($Y = 0.661\ X + 101.2$ $r = 0.522$, $P < 0.001$) and the HC group ($Y = 0.402\ X + 280.6$, $r = 0.373$, $P < 0.01$) but was absent in the NC group, where relatively high sodium excretion was accompanied by relative low calcium excretion ($Y = 0.083\ X + 160.4$, $r = 0.09$, NS).

	24-h urine		Fasting urine
	Na (mEq/24 h)	Ca (mg/24 h)	Na/Creatinine
C	176.9 ± 69.4	218.4 ± 87.9	0.064 ± 0.039
NC	213.1 ± 132.8[a]	175.8 ± 56.2[b]	0.117 ± 0.058[c]
HC	222.3 ± 96.3[b]	368.0 ± 95.6[c]	0.120 ± 0.077[c]

	Fasting urine		Serum
	Ca/Creatinine	TmPi	PO_4 (mg/dl)
C	0.044 ± 0.031	3.39 ± 0.63	3.74 ± 0.64
NC	0.054 ± 0.028 ns	2.58 ± 0.55[c]	3.02 ± 0.58[c]
HC	0.096 ± 0.051[c]	2.75 ± 0.56[c]	3.25 ± 0.57[c]

[a]$P < 0.05$, [b]$P < 0.005$, [c]$P < 0.0005$ versus control

Conclusions. Our data do not support the hypothesis that idiopathic HC is provoked by high sodium intake. The weak association between calcium and sodium excretion, the slope and y-axis intersection of Ca/Na regression analysis and the uniform sodium excretion in the diagnostic subgroups of HC suggest that mechanisms other than sodium overconsumption are operative in the idiopathic hypercalciuria syndrome. No ready explanation can be given for the altered sodium and phosphate metabolism in normocalciuric patients (NC).

160 The Diagnostic Value of Renal Tubular Reabsorption of Magnesium Calculations (TRMg %) in Calcium-Containing Kidney Stone Formers

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In our previous studies a rather high incidence of hypomagnesemia was found in calcium-containing kidney stone formers. In some hypomagnesemic patients a significant increment in urinary Mg excretion suggested a causal relationship between renal Mg wasting and serum Mg depletion. However, in some patients urinary Mg values did not indicate renal Mg wasting.

Serum and urinary Mg analyses and TRMg % calculations (using the values of serum-ultrafiltrable Mg and urinary Mg analysed by atomic absorption spectrophotometry) were performed in a randomly selected group of 69 calcium-containing kidney stone formers (Table 1). The values found in the whole group of patients (group 2), in the selected group of patients with impaired TRMg (group 3) and in the selected patients with decreased TRMg % and serum Mg values (group 4) were evaluated and compared with the values in healthy individuals (group 1).

No significant changes were found in urinary Mg excretion as evaluated by quantitative urinary Mg analyses. On the contrary, TRMg % calculations indicated significantly lower renal Mg conservation in group 3, the lowest TRMg % values being in hypomagnesemic patients (group 4).

The data indicate that quantitative urinary Mg analyses are of little value in the detection of renal Mg wasting and that TRMg % calculations provide more precise information about renal Mg handling. The data suggest that TRMg % calculations may be very

helpful in clinical practice in the detection of early renal Mg wasting and in the differential diagnosis of Mg deficiency of renal and extra-renal origin.

Table 1. Laboratory findings (M±SD) in 17 healthy individuals and in 69 calcium-containing kidney stone formers

Groups	TRMg %		fS-Mg mmol/l		dU-Mg mmol	
1	95.60	1.46	0.846	0.054	4.100	1.356
2	91.01	13.10	0.799	0.111	4.133	1.304
3	75.85	21.12	0.665	0.123	4.403	1.800
4	69.68	21.98	0.601	0.061	3.963	1.862
Significance of differences (NS = not significant):						
1:2		NS		NS		NS
1:3		$P < 0.02$		$P < 0.001$		NS
1:4		$P < 0.001$		$P < 0.001$		NS

161 Kinetic Study of the Intestinal Calcium Protein Binding in Absorptive Hypercalciuria

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Intestinal calcium hyperabsorption in recurrent renal stone formers has been explained by a phosphate renal leak, followed by an increase of 1,25-hydroxycholecalciferol blood levels and therefore an increase of the calcium-binding protein synthesis. Alternatively, it is proposed that the alteration is primarily intestinal, due to a higher transport capacity of the calcium-binding protein.

Eleven control individuals and 18 calcium hyperabsorptive stone formers were studied. The presence of calcium hyperabsorption was studied with a ^{45}Ca oral load. In both groups the plasma levels of PTH, 1,25-dihydroxycholecalciferol and urinary cAMP were determined. Additionally, a duodenal biopsy was carried out in both groups and the kinetic behaviour of the calcium-binding protein was studied.

The PTH and 1,25-dihydroxycholecalciferol plasma levels and cAMP urinary excretion were not significantly different. The Kd of the calcium-binding protein and the number of calcium binding sites were both higher in the hypercalciuric group. This indicates that kinetic differences really do exist in the behaviour of the intestinal calcium-binding protein, which may explain the higher rate of calcium absorption in the intestines.

162 Dietary Factors as Causes of the So-Called Renal Calcium Leak in Idiopathic Stone Formers

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Idiopathic stone-formers who remain hypercalciuric despite a low calcium (Ca) diet have generally been considered as having a renal leak of calcium, u.e. renal hypercalciuria. However, it appears that besides Ca, other dietary factors such as high sodium (Na), high protein and probably also high carbohydrate intake can generate hypercalciuria. Hence, the incidence of renal hypercalciuria has probably been overestimated in studies in which these dietary aspects have not been taken into account,

Out of 118 male idiopathic stone formers, 41 patients were hypercalciuric ($UCa \cdot \dot{V} > 250$ mg/24 h) on the fifth day on a low Ca diet (max. daily Ca intake 400 mg). On this diet, a positive correlation between $UCa \cdot \dot{V}$ (y) and $UNa \cdot \dot{V}$ (x) had been observed in a control population of 27 male healthy volunteers ($y = 0.414\,x + 83.233$, $r = 0.530$, $P < 0.01$), and the 99.7% confidence limits (predicted y ± 3 SD) for individual values of UCa V corresponding to $UNa \cdot \dot{V}$ values had been calculated. In 21 of the 41 patients who were hypercalciuric on low Ca diet, urinary calcium excretion was in normal proportion to sodium excretion. Only 20 patients had $UCa \cdot \dot{V}$ out of proportion to $UNa \cdot \dot{V}$, as it would be expected for renal hypercalciuria. However, 7 of these patients appeared to have a high daily protein intake, as reflected by hyperuricosuria (> 800 mg/24 h), and 3 patients were obese (body weight > 120% ideal weight), with concomitant fasting hyperinsulinemia (> 20 μU/ml). In addition, reinspection of the IVP's revealed that in one other case, radiological criteria for diagnosis of medullary sponge kidney were present. Hence only 9 patients out of 118 idiopathic stone formers had an excessive urinary calcium excretion on a low Ca intake without detectable cause.

To further evaluate the renal tubular function of idiopathic stone formers and to see whether evidence of tubular defects would be obtained in a particular subgroup of patients, the following measurements have been made in the 2 h fasting morning urine in 74 idiopathic stone formers: fractional excretion of glucose, insulin and bicarbonate (the latter before and after an alkali load), excretion of lysozyme and γ-glutamyl-transpeptidase (γGT). Results showed an even distribution of the tubular defects among idiopathic stone formers without hypercalciuria and those with hypercalciuria of the absorptive, the dietary or the renal type.

Conclusion. True renal hypercalciuria is very rare, and dietary causes such as high Na, protein and probably also carbohydrate intake play a key role in idiopathic hypercalciuria. Some idiopathic stone formers do have signs of renal tubular dysfunction, irrespective of the subgroup to which the patient belongs.

IV. Metabolism

163 Glyoxylate Oxidation and Enzymes of Oxalate Biosynthesis in Thiamine-Deficient Rats

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Glyoxylate (GA), an immediate precursor of oxalate, is formed during metabolism from glycolate, ethylene glycol, glycine, hydroxyproline, etc. It can be converted to (i) oxalate by glycolic acid oxidase (GAO), (ii) to oxalate and glycolate by lactate dehydrogenase (LDH), and (iii) to CO_2 in mitochondria by thiamine cofactor (TPP)-dependent α-ketoglutarate: glyoxylate (αKG:GA) carboligase and glyoxylate oxidation cacle. The TPP-dependent mitochondrial oxidation of GA is expected to be markedly decreased in thiamine deficiency, leading to an increased glyoxylate pool in the body, which is either excreted as such by the kidneys or metabolized to oxalate.

Male Wistar rats (45–50 g BW) were fed a thiamine-deficient (TD) diet for a period of 4 weeks, when they showed marked symptoms of thiamine deficiency, as assessed biochemically by erythrocyte transketolase (ETK) [1]. The pyridoxine status of these animals was ascertained by erythrocyte alanine transaminase (EALT) assay [2]. The oxidation of U–^{14}C-glyocylate to ^{14}C–CO_2 by glyoxylate oxidation cycle and the αKG:GA) carboligase activity was measured [3] in both liver and kidney mitochondria. The specific activities of the three oxalate synthesizing enzymes, viz. GAO, glycolate dehydrogenase (GAD) and LDH, were estimated in the liver homogenates by the methods followed in our laboratory.

Thiamine deficiency significantly decreased ($P < 0.001$) ETK activity as compared to the pair-fed controls (PF) with a stimulation index of 6.5% for PF and 50% for TD animals. The thiamine deficiency caused 35% increase in GAO levels over their PF controls; however, GAD and LDH were unaffected. The oxidation of (U–^{14}C)-glyocylate to ^{14}C–CO_2 was significantly decreased ($P < 0.001$)

in both liver and kidney mitochondria. The activity of αKG:GA carboligase was significantly reduced only in kidney mitochondria ($P < 0.001$) and decreased to a lesser extent in the liver.

The data suggest that thiamine deficiency reduces GA oxidation to CO_2 in both liver and kidney, leading to an accumulation or increased excretion in the urine, and its conversion to oxalate by increased GAO levels.

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164 The Relative Importance of Calcium Phosphate Urinary Inhibitors

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Crystal growth inhibitors are believed to play an important role in the regulation of urinary stone formation. The major inhibitors of calcium phosphate precipitation in urine have been shown to consist of magnesium, citrate and pyrophosphate (PP_i) ions, as well as at least one unknown low molecular weight component. Studies using diluted urines have suggested that PP_i and the unknown component are the most important calcium phosphate inhibitor (Smith and Meyer 1976), whereas an investigation of inhibitory activity at physiological concentrations showed that citrate and magnesium are most important (Bisaz et al. 1978). Each of these investigations, however, used an initial, one-time addition of inhibitor and no attempt was made to maintain the fixed, steady-state concentrations of inhibiting species which are expected to exist in a system under biological control. In this study the inhibition of calcium phosphate crystal growth is investigated at constant inhibitor concentrations so that a more realistic assessment of the relative importance of the three known calcium phosphate urinary inhibitors, magnesium, citrate, and PP_i can be made.

The crystal growth of calcium phosphate was studied by the addition of hydroxyapatite (HAP) crystals to a metastable, supersaturated solution of calcium phosphate at pH 7.40, 37 °C, and a constant ionic strength of 0.15 (NaCl). The rate of crystal growth was monitored by withdrawing aliquots at various times, removing the solid phase, and analyzing the solution phase for calcium and phosphate. The pH was kept constant with a pH-stat. The concentration of inhibitor species was kept constant by measuring their concentration in solution at various stages of the reaction and adding additional inhibitor if necessary. Magnesium concentration was determined by atomic absorption, and citrate and PP_i concentrations were estimated by adding tracer amounts of ^{14}C- and ^{32}P-labeled compounds, respectively, and monitoring the radioactivity remaining in solution.

The results obtained showed that PP_i was strongly adsorbed to HAP crystals and physiological concentrations were rapidly removed from solution, whereas physiological concentrations of citrate and magnesium were not markedly reduced upon the addition of HAP. The inhibition of crystal growth by a one-time addition of a given PP_i concentration was marked by an induction period where no precipitation occurs, followed by a rapid rate of crystal growth which was similar to that obtained by control experiments. Analysis of the solution phase showed that crystal growth began to occur at the point where PP_i is completely removed from solution. In experiments in which a finite, steady-state concentration of PP_i was maintained, crystal growth was never observed under the conditions of this study even at concentrations as low as 0.5 μM. In contrast constant physiological concentrations of citrate and magnesium, although slowing the crystal growth of calcium phosphate, did not completely inhibit the crystallization process.

This study suggests that the means for assessing the importance of urinary inhibitors should be more carefully considered. If urinary inhibitors are to be compared at their normal, steady-state physiological concentrations, then it would appear that species which are strongly adsorbed to crystal surfaces, such as PP_i in the calcium phosphate system, are most important.

165 The Occurrence of Urinary Calcium Phosphate Inhibitors in Plasma and Serum

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The major inhibitors of calcium-phosphate crystallization present in human urine are magensium (Mg), citrate (Cit), pyrophosphate (PP_i), and at least one still unidentified low molecular weight (LMW) species (Smith and Meyer 1976). The source of these species is presumably extrarenal; however, it is unknown whether these species account for all the calcium-phosphate inhibitor activity in plasma or serum. The purpose of this study was to characterize and quantify the serum and plasma inhibitors.

The method used to quantitate the serum and plasma inhibitors was a modification of a procedure already described (Bisaz et al. 1978; Rufenacht and Fleisch 1981). Briefly, an aliquot of each sample is first equilibrated at pH 7.40 and 37 °C with dicalcium-phosphate dihydrate. Other aliquots of the same sample are adjusted to the equilibrium calcium and phosphate concentrations. In this manner all samples have the same supersaturation with respect to hydroxyapatite (HAP) formation. The adjusted samples are then "titrated" by adding successively greater amounts of HAP seed crystals until a measurable rate of crystallization is observed. A quantitative measure of the amount of inhibitor present in a given sample is determined by fitting the rate data to a Langmuir adsorption isotherm. The samples tested were fractions of rat and human plasma and serum obtained by molecular exclusion chromatography.

The LMW inhibitors present in human plasma or serum could be separated into two peaks of inhibitor activity. One peak was due entirely to the presence of Mg whereas the other peak contained Cit, PP_i, and an additional unknown component with chromatographic properties similar to that of an unknown inhibitor present in urine. Although these are the same calcium phosphate inhibitors present in urine, their relative importance is considerably different.

This study shows that activity of the serum and plasma LMW inhibitors decreases in the order Mg > PP_i ≅ unknown > Cit whereas in urine, as determined by a similar methodology (Bisaz et al. 1978), the relative order of importance is Cit > unknown > Mg > PP_i. The LMW inhibitor activity in rat serum and plasma is similar to that of the human except that PP_i is absent in the separated fractions, presumably due to high pyrophosphatase activity. The activity of the unknown LMW component appears to be greater than that found in the human and may consist of more than one species. Surprisingly, however, most of the calcium-phosphate inhibitor activity present in both rat and human plasma and serum occurs in high molecular weight (HMW) fractions with two peaks in activity euluting with 158,000 and 43,000 dalton molecules. The HMW inhibitors are assumed to play no role in kidney calcification; however, they may be important in maintaining the metastability of the circulating fluids. The role of the LMW xomponents would appear to be the regulation of calcification at sites inaccessible to HMW molecules.

166 Effect of Pyruvate on Oxalate-Synthesizing Enzymes in the Liver and Kidney of Glycolate-fed Rats

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Pyruvate or alanine has been shown to lower hyperoxaluria and stone-formation in glycolate-fed rats; however, the mechanism of pyruvate action on oxalate biosynthesis is still not clear. Four-month-old male rats were fed daily (orally) sodium glycolate (50 mg/100 g BW) and/or sodium pyruvate (100 mg/100 g BW) for 7 days, while the control animals received a stock diet. The enzymes, glycolate oxidase (GAO), lactate dehydrogenase (LDH), and glycolate dehydrogenase (GAD) were assayed and expressed as units/mg protein as standardized in our laboratory (Biochem Int 1981 3: 507). Sodium glycolate feeding increased the activities of GAO in the liver and LDH in both the liver and kidney, while the activities of GAD of liver and kidney were decreased. Feeding sodium pyruvate and glycolate resulted in lowered GAO activity, while GAD was further decreased as compared to glycolate-fed rats. LDH activity remained unaffected by pyruvate feeding in comparison with glycolate-fed animals. The probable mechanism of the inhibition of oxalate biosynthesis by pyruvate in glycolate-fed rats is shown in the figure below. It is concluded that glycolate-induced oxalate biosynthesis in rats involves increased activity of liver GAO, while pyruvate feeding inhibits it. Thus, in glycolate-induced hyperoxaluria, GAO plays an important role in oxalate biosynthesis.

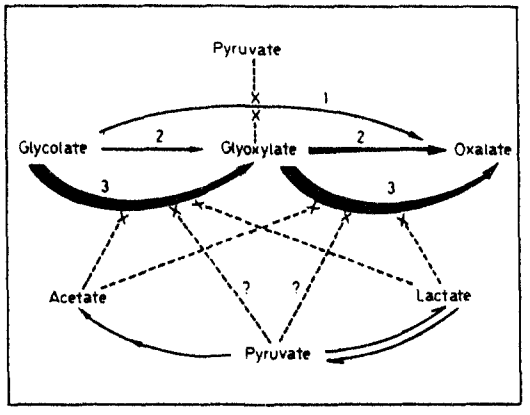

Fig. 1. Mechanism of the inhibition of oxalate biosynthesis by pyruvate in glycolate-fed rats. 1 = GAD; 2 = LDH; 3 = GAO. *Boldface arrows* indicate increased enzyme activity due to glycolate feeding; *dotted lines* indicate inhibition by pyruvate or its metabolites

167 The Effect of a High Intake of Tartaric Acid on Urinary and Plasma Oxalate

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The metabolism of tartyric acid in man has not yet been fully elucidated. In the present study the possibility that oxalic acid may be an end product of tartarate catabolism was investigated. Four healthy subjects, three males and one female, ingested 8 g of L(+) tartaric acid (2 g x 4) daily for 5 days. Three consecutive 24-h urine collections were made by each subject prior to tartarate ingestion and daily during its administration. In addition, two 24-h urine collections were made 1 week postingestion. Blood samples were taken before and during tartarate. Plasma and urinary oxalate, citrate, urate, calcium, magnesium, and the urinary inhibitory activity of calcium oxalate crystal growth were assayed as previously described [1]. Urinary oxalate increased significantly (Paired *t*-test) during and post-tartarate intake compared to controls (Table 1).

Table 1. Urinary oxalate excretion, pre-, during, and post-tartaric acid ingestion (8 g/day). Mean ± SD

Subject	Pre (control)	During	Post
1	16.3 ± 2.45	20.20 ± 3.49	28.20 ± 4.38
2	24.06 ± 5.94	32.18 ± 10.85	47.15 ± 0.21
3	30.45 ± 4.87	37.87 ± 7.79	51.70 ± 6.64
4	24.10 ± 0.71	35.65 ± 12.96	33.70 ± 0.7
		$P < 0.02$	$P < 0.02$

There was an increase in plasma oxalate during tartarate ingestion which was not significant. Urinary urate, calcium, and magnesium were essentially unaltered. Urinary citrate was significantly increased on days 3, 4, and 5 of ingestion ($P < 0.05$, 0.005, and 0.02, respectively); however, the ability of the urine to inhibit calcium oxalate crystal growth was unchanged. These latter findings suggest that citrate is not an important inhibitor of calcium-oxalate crystal growth in urine. The significant increases in urinary oxalate during and post-tartarate ingestion support the metabolism of tartaric acid to oxalate in man, but it is probably a minor pathway. This finding is important, however, because L(+) tartaric acid is present in many fruits and is widely used as an aperient, in baking powder and as a constituent of effervescent drinks. The large increase in urinary oxalate after tartarate ingestion has ceased suggests a possible alteration in the renal handling of oxalic acid during ingestion.

[1] Eur Urol 9:312, 1983

168 Nutrition and Calcium Oxalate Urolithiasis

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Diet has traditionally been viewed as the major source of calcium and oxalate for the formation of urinary tract stones, but in more recent times it has become accepted that approximately 90% of the oxalate appearing in the urine comes from endogenous sources. Two possible dietary sources of endogenous oxalate are refined carbohydrates and animal protein. Using the nutritional approach (with humans, rats and mice), however, we have found it difficult to clarify the relation between dietary carbohydrate (or animal protein) and urinary oxalate excretion.

Using a more metabolic approach, we have shown that the refined carbohydrate, xylitol, can give rise to oxalate by a clearly defined pathway involving fructokinase and aldolase. We have further shown that the experimentally observed, less than 1%, conversion of xylitol to oxalate is biologically significant when considered in models of renal clearance based on a one-compartment kinetic model for oxalate metabolism. Clinically, more than 50 cases of tissue oxalosis have been reported associated with intravenous use of xylitol. It is tempting to consider xylitol as a special case but we have also shown that other carbohydrates, such as fructose, are good precursors in isolated rat hepatocytes. Fructokinase and aldolase would also appear to play a role in oxalate production from these carbohydrates.

Since simple diet studies do not provide clear answers to the problem of nutritionally induced, calcium oxalate urolithiasis, we propose that a more appropriate model should take account of the gastrointestinal absorption of calcium and oxalate, the hepatic production of oxalate, the renal handling of calcium and oxalate and the dietary-induced hormonal milieu. Dietary refined carbohydrates are known to have effects at all these levels.

169 Pyrophosphate Excretion in Various Pathophysiological Groups of Calcium Stone Formers and the Effect of Calcium-Restricted Diet: Significant Decrease in Idiopathic Hypercalciuria Worsened by Calcium Restriction

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To evaluate urinary pyrophosphate as a lithogenic risk factor and its eventual change with the usual calcium restriction, pyrophosphate was measured before and after 4 days of calcium restriction, by an enzymatic method with a centrifugal analyzer, in 24-h urines together with phosphate before and after 4 days of calcium restriction, in 12 controls and in 62 calcium stone formers who were classified according to their calciuria into normocalciuria (NC), dietary hypercalciuria (DH), and idiopathic hypercalciuria (IH) (defined by calciuria on a calcium-restricted diet > 3 mg/kg/day). Plasma phosphate was measured under fasting conditions after calcium restriction.

Mean ± SEM	Free Ca Diet		Ca R Diet		P PO_4 mmol/l
	PO_4 mmol/ day	Pyro mmol/ day	PO_4 mmol/ day	Pyro mmol/ day	
Control $n = 12$	26 ± 1	43 ± 1	22 ± 1[a]	39 ± 5	0.94 ± 0.03
N Ca $n = 27$	30 ± 1	30 ± 0.3	29 ± 1	28 ± 1	0,90 ± 0.02[b]
DH $n = 14$	33 ± 2	29 ± 6	28 ± 1	25 ± 5	0.80 ± 0.05[b]
IH $n = 21$	33 ± 2	27 ± 0.5[b]	29 ± 1[a]	22 ± 3[a]	0.80 ± 0.04[b]

[a] Significance of difference between free and Ca-restricted diet, $P < 0.05$

[b] Significance of difference between controls and Ca stone formers, $P < 0.05$

The table shows that compared to controls, plasma phosphate is significantly lower in all Ca-stone-former groups and that pyrophosphate excretion is significantly lower only in IH. After calcium restriction, there is a significant decrease of phosphate and pyrophosphate urinary excretion in controls as well as in the IH patients group.
Conclusions. (1) Decreased pyrophosphate excretion is a lithogenic risk factor only in idiopathic hypercalciuria; (2) calcium restriction further decreases in IH pyrophosphate excretion, probably because of the simultaneous phosphate restriction due to the prohibition of milk products; (3) the decrease of pyrophosphate excretion with calcium restriction may conteract the beneficial effect of decreasing hypercalciuria and may justify systematic phosphate supplementation with Ca restriction in idiopathic hypercalciuria.

170 A Critical Evaluation of Ascorbate as a Metabolic Precursor of Oxalate

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In man ascorbate is considered to be the main metabolic precursor contributing to the formation of oxalate. Pharmacokinetic studies on the metabolism of ascorbate are therefore of particular interest in urolithiasis research. Under physiological intake, ascorbic acid accounts for 35% to 50% of the daily urinary output of oxalate. This percentage, however, decreases dramatically to about 2% after high-dose intake of vitamin C. This is not the consequence of reduced intestinal absorption of ascorbate or increasing amounts of unchanged ascorbate excreted with urine. From studies measuring the excretion of radioactivity after oral application of (1-^{14}C) ascorbic acid in man, it turns out that after high-dose intake about 50% of the ascorbate is metabolized to carbon dioxide, whereas after physiological intake the metabolic conversion of ascorbate to carbon dioxide is negligibly low. From similar studies in rats and guinea pigs the hypothesis of a presystemic metabolism of ascorbate to carbon dioxide in the intestinal wall could not be confirmed.

Tissue homogenates, as well as cultured intestinal microbial flora, did not cause metabolic degradation on incubation with ascorbic acid to carbon dioxide. It is therefore likely that the observed excretion of carbon dioxide is due to spontaneous non-enzymatical reaction in the liver and eventually other tissues. These results confirm earlier assumptions that the metabolic conversion of ascorbate to oxalate is limited and that with increasing oral intakes of ascorbate the metabolic pathways change to carbon dioxide.

171 On the Protective Role of Intrarenal (Tissue) Glycosaminoglycans in Man – Preliminary Data

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Aims of the Study. From data on man and laboratory animals it has become apparent that, even in healthy subjects, the supersaturation of calcium oxalate in the interstitium of the papilla is excessive. Therefore, it has to be postulated that substances present in the papilla act to prevent solute precipitation under normal conditions. In stone formers lack of these substances, on the other hand, might contribute to stone formation. The object of this paper is to report first data on the intrarenal (tissue) concentrations of glycosaminoglycans (GAGs), a known potent inhibitor of calcium oxalate nucleation.
Methods. Fresh human renal tissue was obtained from healthy and stone-forming kidneys at surgery. Full-thickness sections containing a papilla with adjacent medulla and cortex were excised. Papilla, medulla, and cortex were separately proteolyzed by papain and the GAG fractionated by chromatography on Dowex 1 x 2. The calcium concentrations in papilla, medulla, and cortex were measured by atomic-absorption spectrophotometry. A radioenzymatic procedure was used to determine the corresponding oxalate concentrations. The acid extract was subjected to ion-exchange chromatography and thin-layer chromatography for analysis of various compounds as well as analysis of the GAG content.
Results. We found a steep increase of GAGs from the cortex to the papillary tips, corresponding with that of oxalate and calcium.
Conclusions. It is concluded that GAGs usually protect the papillary tip from calcification. Their possible relation to subepithelial nucleation of calcium containing crystals and to stone formation is discussed.

172 Regulation of Oxalate-Synthesizing Enzymes by Sex Hormones in Weanling Rats

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A few reports imply that sex hormones are significant in oxalate urolithiasis. Therefore, we designed a study in which their role could be elucidated by studying the major enzymes involved in oxalate biosynthesis, viz. glycolate oxidase (GAO), glycolate dehydrogenase (GAD), and lactate dehydrogenase (LDH), Daily injections (0.5 mg in 0.2 ml of sesame oil) of testosterone and β-estradiol were administered IP to six female and six male weanling rats, respectively, for 1 week. The controls received only 0.2 ml sesame oil injections. Supernatants of 10% homogenates of livers and kidneys were assayed for GAO, GAD, and LDH by the methods used in our laboratory (Biochem Int 1981, 3:507). The activities were expressed as units/mg protein. Administration of testosterone led to increased levels of GAO (~56%) and LDH (~17%) in the liver and decreased

levels of LDH (~27%) in the kidney. Conversely, administration of estradiol resulted in decreases of GAO (~30%), GAD (~18%), and LDH (~50%). Kidney LDH, however, remained unaltered. Sex differences were indicated by higher levels of liver GAO and LDH in males, while in kidneys of male rats the LDH activity was lower. The results are tabulated below:

Enzyme units/mg protein	Female rats		Male rats	
	Control	Testosterone	Control	Estradiol
Liver				
GAO	2.43 ± 0.14	3.80 ± 0.15[a]	2.33 ± 0.10	1.67 ± 0.16[a]
GAD	0.15 ± 0.02	0.18 ± 0.03	0.27 ± 0.03[b]	0.13 ± 0.03[a]
LDH	1.81 ± 0.13	2.11 ± 0.17	2.19 ± 0.10[b]	1.89 ± 0.19
Kidney				
LDH	4.36 ± 0.21	3.14 b 0.15[a]	2.25 ± 0.06[b]	2.10 ± 0.06

[a] $P < 0.01$ as compared to respective control animals
[b] $P < 0.01$ as compared to control female animals

One unit GAO is defined as enzyme required for the production of one nanomole of glyoxylate per min at 37 °C; one unit of LDH is defined as enzyme required to produce a change of 0.01 O.D. at 340 nm at 25 °C; one unit of GAD is defined as enzyme required for the production of one nanomole of oxalate per min at 37 °C. The elevation of enzymes of oxalate biosynthesis in liver by testosterone and lowering by estradiol partly explain the sex-linked differences in the formation of oxalate urinary calculi.

173 Effect of Sex Hormones and Glycolate Feeding on Rat Liver Glycolate Oxidase

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Female rats produce less oxalate from its precursors than males, implicating the role of sex hormones in the regulation of oxalate synthesis from its precursors. Interactions of glycolate and sex hormones were studied in male (M) and female (F) rats administered (by gastric intubation) 50 mg/100 g BW of glycolate (G). The hormones testosterone (T)/estradiol (E) were packed in silastic tubing 2 cm in length and surgically inserted SC on the dorsal side of the neck. Each of the following groups comprised six rats: Male rate (M), Male rats fed glycolate (M + G), Male rats administered estradiol (M + E), Male rats administered estradiol and fed glycolate (M + E + G), Female rats (F), Female rats fed glycolate (F + G), Female rats administered testosterone (F + T), Female rats administered testosterone and fed glycolate (F + T + G).

One month after the treatment, glycolate oxidase (GAO) activities were measured according to the method followed in our laboratory (Biochem Int 1981, 3:507). The specific activities were converted to percentages using M + G as 100%. The sequential pattern of the activities in the different groups, M + G (100%) > M (80%) > F + T + G (39%) = M + E + G (40%) > M + E (34%) = F + T (30%) > F (20%) = F + G (22%), indicates that glycolate feeding increases GAO (by ~20%) when only testosterone is present (M), but only marginally raises them (by ~10%), when both hormones are present (M + E, F + T). However, GAO activities are not raised in the presence of estradiol alone (F). Estradiol decreased GAO by 58% in males, irrespective of glycolate feeding (M, M + G). Likewise, no difference in the activities of GAO were noted between M + E and F + T while those of M + E + G were comparable with F + T + G. Glycolate induction of GAO, a peroxisomal enzyme involved in conversion of glycolate to oxalate, occurs in the presence of testosterone and is inhibited by estradiol.

174 Multicompartmental Pharmacokinetics of ^{14}C-Oxalate in Rats

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Oxalate ions play an important role in the pathogenesis of calcium urolithiasis. In rats a high affinity of oxalate for bone tissue was determined by whole-body autoradiography, indicating that bone tissue acts as a deep-storage compartment for oxalate. No pharmacokinetic data are available for this result, however. The following study in rats was therefore designed to determine whether the morphological evidence of the deep compartment could be confirmed pharmacokinetically.

Adult female rats (SIV 50, $n = 12$) were administered 40 μCi ^{14}C-Oxalate (specific activity 74 mCi/mmol) as an IV bolus injection. The ^{14}C-oxalate concentrations in the plasma were determined 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 300 min after the injections. Urine volume, inulin clearance, oxalate excretion, blood pressure (BP), and heart rate (HR) were determined. Data were analyzed according to either a two- or a three-compartment open model, using a least-square nonlinear regression program (NONLIN).

Urine volume, inulin clearance, BP, and HR were practically constant throughout the experiment. The pharmacokinetic calculation showed that the differences between the measured and the calculated results did not exceed 4.4% when using the three compartment model; this model resulted in a statistically significant better fit than the two compartment model. The steady-state volume of oxalate distribution was 876 ml/100 g body weight. The corresponding half lives were $t_{1/2}\,\alpha = 3$ min, $t_{1/2}\beta = 24$ min, $t_{1/2}\gamma = 300$ min.

Thus, oxalate pharmacokinetics in rats can best be explained by the three-compartment open model. The third compartment is bone tissue. The three-compartment pharmacokinetics of oxalate in man has not yet been confirmed, probably because the actual observation time was too short.

V. Physico-Chemistry and Crystal Formation

175 Natural Inhibitors of Formation and Dissolution of Stone Minerals

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Constant composition kinetics methods have been used for studying the mineralization and demineralization of calcium oxalate and phosphate stone minerals at sustained super- and under-saturations in the presence of synthetic inhibitors and natural urinary components. The method is especially sensitive for investigating the influence of urines, fractionated according to the molecular weights of the components. The use of Sephadex columns ranging from P1 to P60 has shown that proteins of high molecular weight (greater than about 50,000) make up an appreciable fraction of the total urinary inhibition. Thus in pure calcium oxalate supersaturated solution ($T(Ca) = T(Ox) = 3.0–5.5 \times 10^{-4}$ mmol l^{-1}), the mineralization rate (typically 0.4 to 15.5 x 10^{-5} mol $min^{-1} m^{-2}$) was reduced by more than 40% even though the dilution factor of the separated urinary component was at least 100X. In the case of hydroxyapatite crystallization, ($T(Ca) = 4.0 \times 10^{-4}$ mol l^{-1}, T(phosphate) = 2.6

$\times 10^{-4}$ mol l^{-1} pH = 7.4, ionic strength 0.1 mol l^{-1} (NaCl) the rate of reaction (4×10^{-8} mol $min^{-1}m^{-1}$) was reduced by similar extents. Of particular interest is the finding that dialyzed serum strongly inhibits the mineralization of calcium oxalate monohydrate at dilutions in excess of 10^4 times. Pre-bladder urines also show appreciable inhibition of both calcium phosphate and calcium oxalate. Analysis of these inhibition data is complicated by morphological changes which may be induced by pregrowing seed crystals before use in the experiments. It appears that changes in the number of growth sites may influence both the extent of inhibition and the kinetics of growth.

The constant composition method has also been developed for studying the dissolution of kidney stones. The rates of reaction can be related to the corresponding demineralization of both the pure synthetic components and mixtures of these salts. In the case of kidney stone dissolution, the use of chelating accelerators and the presence of more then one mineral phase markedly influence the rates of solution.

176 ^{14}C-Calcium Oxalate (CaOx) Crystal Adherence in the Rat Bladder

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Crystal adherence within the urinary tract has been proposed as a mechanism by which a nidus is retained to promote stone formation. These studies examined ^{14}C-CaOx crystal adherence in the rat bladder.

Female rats were anesthetized, the bladders catheterized, and the ureters ligated. Normal saline in controls (C) or 0.1 N HCl in experimental animals (A) was instilled in the bladders for 1 min. ^{14}C-CaOx crystals were introduced into the bladders for 15 min and nonadherent crystals removed by drainage and saline irrigation. The bladders were removed, dreied, solubilized, and the radioactivity counted. Data are expressed as percentage of radioactivity remaining in the bladders following saline washes.

	C (n = 9)	A (n = 18)
% ($\bar{x}$ ± SEM)	0.6 ± 0.2	13.6 ± 2.2

$P < 0.001$

Restoration studies were aimed at restoring antiadherence factors. Chondroitin sulphate (CS), sialiac acid (SA), heparin (H) and pentosan polysulphate (PPS) were introduced into acid washed bladders for 2 min. Crystals were then introduced and the bladders treated as above.

	CS (n = 5)	SA (n = 6)	H (n = 8)	PPS (n = 12)
% ($\bar{x}$ ± SEM)	1.2 ± 0.4[a]	8.6 b 3.6[b]	0.8 ± 0.3[a]	5.5 ± 1.6[a, b]

[a]vs A $P < 0.02$; [b]vs C $P < 0.02$

Alteration studies examined changes in crystal adherence in nonacid washed bladders. Test substances were introduced into intact bladders for 2 min, followed by crystals for 15 min. The bladders were removed and radioactivity counted. Substances studied included 30 mg% calcium (Ca), 10 mg% oxalate (Ox), 800 mg% phosphorous (P) at pH 4,6, and 8 and Tris buffer (T) at the same pH values.

	Ca (n = 10)	Ox (n = 4)
% ($\bar{x}$ ± SEM)	4.9 ± 1.0[a]	0.4 ± 0.2

[a]vs C $P < 0.02$

	pH	4	6	8
% ($\bar{x}$ ± SEM)	P	1.6[a] ± 0.4	2.8 ± 0.6[a]	3.3 ± 0.7[a]
	Tris	0.3 ± 0.1	0.5 ± 0.3	1.2 ± 0.4

[a]vs C $P < 0.02$

The intact rat bladder significantly inhibits crystal adherence, a property removed by acid treatment. This loss of antiadherence can be restored by CS and H and partially restored by PPS but not by SA. In the intact bladder adherence of crystals is increased by calcium and phosphorus but not oxalate. If pH has an effect, it is probably exerted through pH-induced changes in phosphate.

177 Dissolution Kinetics of Calcium Oxalate Calculi

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Following the endoscopic removal of renal calculi, small residual stone fragments may be left in inaccesible calices. Since a nephrostomy tube is already in place, these fragments should, if possible, be dissolved by irrigation techniques. Dissolution of struvite, cystine, and uric acid calculi is usually possible since the solubility of these calculi is highly pH-dependent.

The solubility of calcium oxalate is minimal at physiologic pH. Solubility, however, does increase at low pH levels. To determine the possibility of dissolving small calcium oxalate fragments by irrigation techniques, we have studied in vitro dissolution kinetics of calcium oxalate calculi in acid solution. Rate constants were measured at pH 1, 2, and 3. Dissolution of calcium oxalate is minimal at pH 3, with a rate constant of 0.17 mg/$cm^2 \cdot$ h. The dissolution rate increases sevenfold at pH 1. By computer modeling, a 1-mm calcium-oxalate fragment would require at least 15 days of irrigation at pH 3 to fully dissolve.

Although the tolerance of normal urothelium to acidic irrigating solutions is not fully documented, dissolution of calcium oxalate fragements by irrigation with acidic solutions does not appear to be clinically feasible.

178 Crystal Size Determination by Profile Analysis of X-Ray Diffraction Reflexes

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The angles and intensities of X-ray powder diffraction reflexed contain information related to the crystal structure. Moreover, the profile of diffraction reflexes (breadth and shape) contain information about intracrystalline tensions and crystal size.

Application on in-vivo-grown urinary calculi shows that crystals of uric acid dihydrate are larger than crystals of uric acid siccum. Also, crystals of calcium oxalate dihydrate (weddellite) are larger than crystals of calcium oxalate monohydrate (whewellite).

According to the rules of solid-state chemistry, the findings could be explained in the following manner: First step: formation of the dihydrate crystals. Second step: transformation to the dehydrated forms and smaller crystals

179 A Whole Urine System for Studying Nucleation, Growth and Aggregation of Calcium Oxalate Crystals

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Measurement of the inhibitory effects of diluted urine on calcium oxalate (CaOx) crystal growth and aggregation has contributed greatly to our knowledge of urinary inhibitors. But such effects cannot be regarded as representative of the ability of undiluted urine to inhibit these processes under physiological conditions. The capacity of a urine to resist CaOx precipitation will depend on the free concentrations of reacting ions and inhibitors. Of these, oxalate has been cited as the major determinant [1].

The aim of this project was to develop a system for measuring CaOx crystallisation in whole urine and to determine whether undiluted urines from stone formers and normals differed in their responses to a given oxalate load. Twenty-four-hour urine samples were collected from 35 normal men and 34 men who had passed at least one calcium stone within the previous year. After centrifugation and filtration (0.22 μm) aliquots of each urine sample were treated with sodium oxalate to give final exogenous oxalate concentrations of 0–1.5 mM. These were incubated at 37 °C for 30 min and the number of crystals in each sample was determined using a Coulter Counter. In those aliquots in which crystallisation was detected, crystal number rose linearly in response to increasing oxalate concentration. The point at which this line intersected the abscissa was taken as the minimum amount of oxalate necessary to induce detectable precipitation. Once this value was determined for each urine specimen, that amount of oxalate, plus an additional 30 μmol was added dropwise to 100 ml samples of the urine and the growth of precipitated crystals was followed for 90 min using a Coulter Counter to measure increasing crystal volume.

CaOx monohydrate was the principal crystal type in only two urine samples. In the remainder, classical "envelope" CaOx dihydrate crystals, identical to those found naturally in urine, predominated, either singly or in varying sized aggregates. There was no difference between the stone formers and normals with respect to crystal morphology or the size of crystals and aggregates produced.

The minimum concentration of oxalate necessary to induce precipitation was inversely proportional to the urinary total calcium concentration ($P < 0.0001$) and calcium x oxalate concentration product ($P < 0.0001$), but not to the endogenous oxalate concentration. The stone formers and normals did not differ with respect to urinary calcium and oxalate concentrations, the minimum concentration of oxalate required to produce nucleation, the total volume of crystals precipitated during the incubation period or to the rate of crystal growth.

It was concluded that any difference between the urines of stone formers and normal subjects does not lie in their ability to tolerate a given oxalate load or in the growth and aggregation patterns of crystals precipitated from them.

1 Robertson WG, Peacock M, Heyburn PJ, Marshall DH, Clark PB (1978) Br J Urol 50:449–454

180 Induction and Inhibition of Struvite Bladder Stones in the Rat

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This paper reports a relatively speedy (9-day) method for the in vivo formation of struvite stones and the finding of an approach for the inhibition of stone formation. A mobile, extracorporeal, continuous infusion and irrigation system has been developed for the present investigations.

Twenty rats divided into four groups (five each) were studied under nonsterile conditions. Bladders of rats in Group I were infused with saline and chloramphenicol for 9 days. All five rats were found to have struvite stones in their bladder. All urine samples contained *Pseudomonas* and *Proteus* species (colony count $> 10^5$). The rats in groups II, III, and IV were infused respectively with saline and gentamicin, acetohydroxamic acid (AHA), and keto acid solution. Although *Pseudomonas* and *Proteus* were present in the urine of some of the II, III and IV rats, only one struvite stone (and no other kinds of stones) was found in one of the 15 rats.

The present approach provides a highly successful method (5/5 in Group I) for in vivo struvite stone formation and also reduces the formation period to 9 days. Thus, it enables one to pursue suitable kinetic studies on the dynamics of struvite stone formation. Results also confirm that *Pseudomonas* and/or *Proteus* species are instrumental in the process of struvite formation, since they are strong urea-splitting bacteria converting urea into NH_4^+ and HCO_3^-. The former product is an essential constituent of struvite, ($MgNH_4 \cdot PO_4$). In the inhibition studies, results of Groups II and IV confirm that since gentamicin controls the numbers of *Pseudomonas* and *Proteus* bacteria and AHA inhibits the action of urease, both of which limit NH_4^+, struvite formation is greatly reduced.

It is of clinical significance to note that a keto acid mixture can completely prevent the formation of struvite stones even under nonsterile conditions. Presumably, in the presence of certain encymes and their co-factors in the urine, the keto acid can be aminated to form ketoglutarate and thus reduce the possibility of producing struvite. This result suggests that keto acid irrigation of the bladder could be a therapy for patients having struvite stone disease.

181 Dissolution of Crystalline Calcium Oxalate: In Vivo Studies

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A new animal model for the determination of the relative ability of solvents to dissolve calcium oxalate crystals in the rat has been developed. The keto acid solvent which had been used successfully to dissolve calcium oxalate crystals in vitro (previously reported) was investigated in vivo along with Renacidin[1].

In this model, to induce crystallization of calcium oxalate in the mucosa of the bladder, calcium and oxalate ions were, over a period of 6 h, separately and independently infused into the bladder of the test rat through two pieces of plastic tubing. After formation of mucosal crystals, they were *irrigated* for 6 h with several solutions by continuous infusion through the same tubing. The bladder was then removed from the rat and immersed in 1 N HCl for 16 h. Calcium ion from calcium oxalate crystals was quantitated using a calcium ion electrode.

Four groups of rats (total 32) were studied in this experiment. The average amounts (moles) of Ca^{++} found in the mucosa of each group (8 bladders), after the dissolution of the crystalline calcium oxalate in 1 N HCl, are:

Group	Irrigating solution (4 ml over 6 h)	Moles of Ca^{++} ($\times 10^6$)
I	None	2.03
II	0.9% Saline	1.24
III	24 mM Renacidin	1.14
IV	24 mM Keto acid	0.591

The results suggest that, in vivo, the keto acid mixture has a *stronger potential* than Renacidin for clinical use in dissolution of calcium oxalate stones.

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182 Adsorption of RNA on Aged COM

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In our systems (0.1 M NaCl, 0.01 M Pipes Buffer, MW (RNA) = 35,000 D, pH 6.8, 38 °C), adsorption of RNA on aged COM suppresses seeded crystal growth (initial relative supersaturation = 9.98, $[Ca^{2+}_2] = [C_2O_4^{2-}]$, mixed with magnetic stir bar, crystal surface area = $1.4 \times 10^{+2}$ cm^2 ml^{-1}) a maximum of 95%. The RNA concentration causing 50% of maximum seed-crystal growth inhibition is 1.1×10^{-8} M, whereas in solution-depletion studies, the RNA concentration causing 50% of maximum coverage is 3.8×10^{-6} M. The failure of the two "50%" concentrations to coincide suggests that most growth sites have a higher affinity for RNA than the average COM-RNA adsorption site. A Langmuir estimate of the density of high affinity adsorption sites is $> 4.1 \times 10^{-8}$ M m^{-2}. The inequality is due to the likelihood that one RNA molecule adsorbs at more than one site, but because of relative inflexibility of the RNA random coil, each monomer in the adsorbed molecule does not cover a site.

183 In Vitro Investigations of Stone-Growth Inhibition and Stone Dissolution Dependent on Crystallizing Substances

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Fragments of either an oxalate, uric acid, or a phosphate stone were incubated with urine in the "stone (growth and dissolution) simulator" previously presented by our group. Electrolyte concentrations in urine were changed. The experimental time was 8 days. The following parameters were measured: A) Stone weight pre- and post-experimental. B) Morphology of the stone surface with a scanning electron microscope. C) Calcium, magnesium, phosphate and uric acid in the incubation medium. The results are presented and discussed.

184 Growth and Stability of Magnesium Ammonium Phosphate (Struvite) in Acidic Sterile Urine

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The aim of the study was to demonstrate that struvite, $MgNH_4PO_4 \cdot 6H_2O$, can form and survive in acidic sterile urine. Second, we intend to predict the crystallization of struvite from the urine pH and concentrations in magnesium (Mg), ammonium (NH_4) and phosphate (PO_4).

The voidings of 47 stone formers were systematically analyzed. In the urinary sediments of 8 of them, struvite crystals, spontaneously precipitated, were recognized owing to their typical habits and optical properties. The crystallization of struvite was also obtained in vitro by mixing two urine samples, A and B. In A, Mg was added as aqueous solutions of $MgSO_4$. In B, NH_4 and/or PO_4 were added as NH_4OH, NH_4Cl, $NH_4H_2PO_4$ or NaH_2PO_4. The total dilution of urine was always less than 10%. The initial concentrations in Mg, NH_4 and PO_4 of freshly emitted urine, and the equilibrium values after precipitation, were determined by spectrophotometry. In order to find out the conditions of the struvite crystallization, large ranges of pH (5.0 to 9.6) and large concentrations of Mg, NH_4 and PO_4 (1 to 250 mM) were used.

Among the 8 stone formers, only two suffered infection with urine pH > 7, whereas the pH of the others was in the acidic range. The in vitro experiments proved that struvite can precipitate from urines either at high (basic) pH even if the concentrations in Mg, NH_4 and PO_4 were small, or at low (acidic) pH if these concentrations were large. Actually, the two parameters which must be considered for the struvite precipitation were found to be the pH and the concentration product $CP = \{Mg\} \times \{NH_4\} \times \{PO_4\}$ In a diagram pH versus CP, there is for each CP value, a critical pH above which struvite crystals are formed. If the pH was higher than 7, there was also a possibility for amorphous phases to precipitate, especially when urine was rich in calcium or in uric acid. When pH $\leqslant 6$, newberyite crystals ($MgPHO_4 \cdot 3H_2O$) occurred instead of the struvite ones. In other cases, newberyite was the decomposition product of struvite. This latter phase became unstable and underwent a solution medited phase transition. When NaOH was used to adjust the pH of urine, the amorphous phases formed more easily (at lower pH) and delayed or completely prevented the occurrence of struvite crystals.

The main conclusion is that, contrary to what is commonly admitted, struvite crystals may nucleate and grow in acidic sterile urine. High ammonia contents are not necessary if the concentration product $\{Mg\} \times \{NH_4\} \times \{PO_4\}$ is large enough at a given pH. By measuring these three concentrations and the pH of any urine, it is possible, on the basis of the present study, to predict the risk incurred by the patient to form struvite stones. The results also allow the association of struvite with calcium phosphate in the renal and urinary stones to be understood. Last, the struvite crystals exhibit typical habits which are good indicators of the supersaturation level of urine. The in vitro experiments yield all habits found in vivo.

185 Studies on the Mode of Action of Polyanionic Inhibitors of Calcium Oxalate Crystallization in Urine

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The identification and role of urinary inhibitors of calcium oxalate (CaOx) crystallization have been extensively studied in recent years, but little has been published on their mode of action and on the possible interactions between themselves and other constituents of urine. This study reports on the effect of these inhibitors on the surface properties of CaOx crystals incubated in the presence of artificial urine and with whole urine. The zeta potential (ZP), which is a function of the surface charge on the hydrated crystal, was measured by an electrophoretic procedure. Increasing the individual concentrations of various known polyanionic inhibitors, such as ribonucleic acid (RNA), glycosaminoglycans (GAGS), non-polymerized Tamm-Horsfall mucoprotein (THM) and inorganic pyrophosphate (PP_i), produced a decrease in the ZP from near zero to values between −30 and −40 mV. The order of potency was RNA > GAGS > THM > PP_i. It was found, however, that the activities of these inhibitors were modified in the presence of increasing concentrations of urine, indicating that some factor in whole urine was interfacing with the action of the inhibitors at the crystal-solution interface. Subsequent studies carried out to identify the source of this interference have suggested that it is a macromolecule (possibly uromucoid).

Parrallel studies using a continuous crystallizer to measure the rates of growth and agglomeration of CaOx crystals have confirmed the order of potency of the inhibitors to be RNA > GAGS > THM > PP_i. The effect of various combinations of inhibitors was measured with particular reference to the interaction between uromucoid and the polyanionic inhibitors. The results indicate that the net inhibitory activity observed in the presence of whole urine is a function of a number of inhibitory and anti-inhibitory substances in urine.

186 The Inhibitory Activity of Glycosaminoglycans and Urine

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One theory advanced to explain the purported success of allopurinol in the treatment of calcium oxalate stone formers proposes that the full inhibitory potential of the urinary glycosaminoalycans is not

realised in the presence of colloidal urate. There is evidence that the inhibitory potency of heparin is reduced by its binding to sodium urate. However, this has not been demonstrated with chondroitin sulphate, the principal urinary glycosaminoglycan, and urate has not been shown to affect the inhibitory activity of urine. The aim of this study was therefore to determine whether the known inhibitory effects of chondroitin sulphate, heparin and urine in vitro are reduced by preincubation with solid sodium urate.

The inhibitory effects of heparin and chondroitin sulphate on calcium oxalate crystal growth and aggregation were measured in a seeded crystallisation system [1] after a 2-h preincubation period at 37 °C in the presence and absence of sodium urate (0.5 mg/ml). Crystal growth, expressed as the increase in mean crystal diameter, and aggregation were measured separately using a computer model [2]. Similar experiments were performed with 24-h urine samples (final concentration 1% v/v) collected from ten normal men.

The inhibitory effect of heparin (5×10^{-4} mg/ml) on crystal growth was reduced from 59% in the absence of sodium urate, to 11% after preincubation with this compound. Similarly, its inhibition of crystal aggregation at 5×10^{-5} mg/ml fell from the normal value of 73% to 44% after preincubation with sodium urate. However, the virtual absence of heparin from urine renders these findings of little physiological significance.

Pretreatment with sodium urate reduced the inhibitory effect of chondroitin suphate (5×10^{-3} mg/ml) on crystal growth from 34.5% to 27.1%. Its inhibitory effect on crystal aggregation at a concentration of 5×10^{-5} mg/ml was not altered by pretreatment with sodium urate.

The inhibition of crystal growth by every urine sample was lessened by exposure to sodium urate, the median inhibition without urate being 63.5% and with, 55.9% ($P < 0.005$, Wilcoxon rank- sum test), but the inhibition of crystal aggregation was unaffected.

It was concluded that the observed reduction of the inhibitory effect of urine on crystal growth may be caused by the binding of urate to urinary glycosaminoglycans.

1 Ryall RL, Harnett RM, Marshall VR (1981) Clin Chim Acta 112:349–356

2 Ryall RG, Ryall RL, Marshall VR (1983) Proceedings of the 2nd International Urinary Stone Conference, Singapore (in press)

187 Does the Bladder Mucosa Contribute to Urinary Inhibitory Activity?

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Whilst it is generally agreed that some of the observed inhibitory activity of urine in vitro can be attributed to small molecular weight compounds, the role of high molecular weight substances in stone formation is unclear. The soluble glycosaminoglycans (GAGs) are one group of urinary macromolecules which have been shown to account for some of the inhibitory activity in urine, and which may protect normal individuals from stone formation. However, few studies have been able to demonstrate any difference in the urinary level of these substances between stone formers and normals or in the inhibitory activities of their urines. A failure to demonstrate consistent differences between the urines from the two groups might occur if part of the inhibitory activity of urine were derived from the bladder itself. Under these circumstances the contribution from the bladder might mask any other differences which might be present.

In this study we postulate that a portion of the observed inhibitory activity of urine is derived from the bladder mucosa. Saline (0.15 M) bladder washouts were obtained from 20 normal patients undergoing routine cystoscopy. None of the subjects had a significant probelm such as bladder infection, haematuria or neoplasm. After an initial urine sample was obtained, the bladder was washed of residual urine by filling and emptying twice with sterile 0.15 M NaCl. The bladder was then lavaged via the endoscope for 30 s with sterile normal saline using a 50 ml syringe and the washout collected. To account for urinary contamination in the collected washout, a urine control was prepared by diluting the whole urine sample to the same creatinine concentration as the bladder washout. Both the diluted urine and the washout were then brought to a metastable state by the addition of calcium and oxalate. The inhibitory activities of the samples were then measured in a seeded crystallisation system [1].

In the patients studied the inhibitory activity of the washouts with respect to both crystal growth and crystal aggregation was significantly greater than in the urine controls ($P < 0.01$). Analysis of the washouts showed consistently greater levels of protein than could be accounted for by urinary contamination. In a series of experiments examining the effect of blood, it was found that blood is a potent inhibitor of calcium oxalate crystal growth and aggregation at concentrations as low as 0.005%. However, measurement of haemoglobin in the washouts showed that the protein was not related to trauma.

We conclude from these results that the bladder may be responsible for a significant proportion of the inhibitory activity of urine. However, the factor or factors responsible for these differences still need to be identified.

1 Ryall RL, Harnett RM, Marshall VR (1981) Clin Chim Acta 112:249–356

188 The Oxalate-Tolerance Value, A Reliable Parameter of Possible Urinary Stone Formation

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A new turbidometric method will be presented. This method allows the crystallization kinetics of calcium oxalate in individual urine to be investigated. After pH adjustment a known concentration of sodium oxalate is added at a constant rate to a definite urine probe. At 37 °C urine is stirred electromagnetically. The initiation of calcium oxalate crystallization is measured by a dive photometric cell at 700 nm. Calcium concentration is analyzed by Faas (C_2H_2/N_2O). The measured calcium concentration of individual urine and the consumption of added sodium oxalate until initiation of calcium oxalate crystallization allows the *O*xalate-*T*olerance *V*alue to be calculated:

$$OTV = \frac{\text{Concentration of calcium} \times \text{concentration of added oxalate}}{\text{Solubility product of calcium oxalate}}$$

Our investigations yielded a significant difference between stone formers and non-stone-formers. In this way, the risk of stone formation in individual urine can be estimated.

189 Role of Fluoride in the Formation of Calcium Oxalate Stones

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Fluoridation of drinking water leads to a higher content of fluoride in urinary stones. The aim of this study was to investigate the possible role of fluoride in the formation of calcium oxalate stones.

Methods. 1. The content of fluoride in stones deriving from Basel, Switzerland (fluoride content of drinking water: 1–2 ppm) and from countries without fluoridation were analyzed. 2. Male Wistar rats were treated with 0.8 vol PC ethylene glycol and various concentrations of fluoride. Urine (collected under metabolic ward conditions), kidneys, and one femur were analyzed for calcium and

fluoride content. 3. Crystallization kinetics were studied in solutions adapted to a so-called normal urine (pH = 5.7, temp. 37 °C, ionic strength 0.3, and mean components like normal human urine). To various F contents 39 μmol sodium oxalate was incubated. After 2, 4, and 8 min the solutions were filtered through glass filters (pore size 9–15 μm). Calcium was analyzed by Faas.
Results. 1. Fluoridation leads to a much higher fluoride content in calcium-containing stones. 2. Fluoride inhibits ethylene-glycol-induced calcium-oxalate stone formation or nephrocalcinosis, but evokes a higher fluoride and calcium excretion in urine. 3. Fluoride delays dose- (3–5–10 ppm) and time-dependent initiation of calcium oxalate nucleation, but promotes the formation of smaller crystals.

190 The Effect of Glutamic Acid on the Precipitation of Calcium Oxalate

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Glutamic acid (Glu) is a major constituent of small, molecular, organic matter in human urine and, as such, might be an effective modifier of calcium-oxalate precipitation. To this end, an assesment of the effect of Glu on the kinetics of crystal growth and aggregation of different calcium oxalate hydrates has been attempted.

Precipitation was initiated by mixing calcium chloride solutions with solutions of sodium oxalate to which Glu (1–100 ppm) was added (pH = 6.5 ± 0.2; [NaCl] = 0.3 mol dm^{-3}; T = 298 K). Depending on the manner of mixing, (A) mixtures of calcium oxalate hydrates (mono-, di- and trihydrate) or (B) pure trihydrate precipitated. Changes in the system were followed by Coulter counter or calcium selectrode. By analysis of the kinetic curves, identification of the precipitation process (nucleation, crystal growth or aggregation) prevailing at any given time was possible [1].

In system A, most concentrations of Glu investigated inhibited crystal growth but did not affect the rate of aggregation. The intensity of the effect depended on Glu concentration in an irregular way. Generally, it could be shown that the intensity of the effect increases with increasing Glu concentration. In system B, inhibition of crystal growth was also oberserved, but the intensity of the effect decreased with increasing Glu concentration and initial supersaturation with regard to calcium-oxalate trihydrate.
It can be concluded that Glu retards crystal growth of calcium oxalates. The intensity of the effect depends on the Glu concentration, the solid/solution ratio, and on the form of the hydrate.

1 Füredi-Milhofer H, Škrtić D, Marković M, Komunjer Lj (1981) Urolithiasis. Smith LH et al (eds), pp 401–409. J Cryst Growth (in press)

191 The Conditions of Precipitation of Uric Acid Dihydrate and Sodium Acid Urate

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Uric acid dihydrate ($H_2U \cdot 2H_2O$) is an important constituent of urinary calculi, but the conditions of its precipitation have not been examined and no reliable solubility product has been reported. Sodium acid urate (NaHU) is better known for its occurrence in gouty arthritis, but in a microcrystalline form could initiate calcium oxalate lithiasis. The aim of this study was to define the conditions of precipitation of these solid phases and calculate some of the solubility constants.

Commercial uric acid was dissolved in carbonate-free sodium hydroxide. $H_2U \cdot 2H_2O$ was then precipitated by addition of hydrochloric acid, while NaHU was obtained by the addition of sodium chloride at constant pH 7.5. All systems were aged for 24 h at 25 °C or 35 °C. Precipitates were characterized by light or electron microscopy, X-ray and electron diffraction, and thermogravimetric analysis. Ionic activities were calculated by computer program, using literature values for acid dissociation and coplex stability constants.

In the H_2U–NaOH–HCl–H_2O system, orthorombic $H_2U \cdot 2H_2O$ was the only solid phase obtained which was stable in solution for at least 24 h. From precipitation boundaries expressed in terms of ionic activities the solubility products $K_{sp} = (H^+)(HU^-) = 1.05 \times 10^{-9}$ mol^2 dm^{-6} (25 °C) and 1.58×10^{-9} mol^2 dm^{-6} (35 °C) were obtained. In the H_2U–NaOH–NaCl–H_2O system NaHU · H_2O precipitated at high supersaturations (S > 80) while near the precipitation boundary structurally different microcrystalline forms appeared. The slope of the respective precipitation boundary suggests for this precipitate an approximate Na to HU molar ratio 1:2.

192 Crystal Inhibition: Binding of Heparin and Chondroitin Sulfate to Calcium Oxalate, Sodium Urate and Uric Acid Crystals

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It has been suggested that inhibitors of crystal growth and aggregation act by blocking the growth sites of crystals and thereby prevent or delay crystal growth. We applied the principles used in receptor studies to examine the affinity of various potential inhibitors to calcium oxalate, sodium urate and uric acid crystals.
Methods. ^{3}H-Heparin and ^{3}H-chondroitin sufate were separately added in trace amounts to crystal suspensions, whose amount was chosen to cause an almost complete binding of the tracer. Increasing amounts of non-radioactive inhibitor were then added to compete with the tracer binding. The bound and the free tracer were separated by centrifugation of the crystal solutions, the radioactivity measured, and the affinity and capacity of the crystals to bind the inhibitors were estimated. The ability of a semi-synthetic low-molecular-weight heparin analogue, pentosan polysulfate (SP54), to exert heparin characteristics vis-à-vis calcium oxalate crystals was investigated by substituting heparin with SP54 in the competition experiments. The binding of heparin to sodium urate and uric acid crystals was studied in buffered saline and in a solution metastable with respect to calcium oxalate (RS = 0.6).
Results. The affinity of heparin to calcium oxalate crystals appeared to be higher than that of chondroitin sulfate (CS), but CS's capacity to bind was five times higher. SP54 releaseed ^{3}H-heparin bound to the crystals at a much higher concentration than heparin itself. ^{3}H-heparin did not bind to uric acid crystals under any conditions, whereas it did bind to sodium urate crystals and the affinity was increased substantially (~50-fold) in a metastable calcium oxalate solution, compared with buffered saline. The importance of ionic strength, pH and calcium concentration is being further investigated.
Conclusions. The affinity to bind to calcium oxalate crystals was higher with heparin as a ligand than CS, but the capacity for the latter was larger. Differences in charge density may be the reason. Heparin did not bind to uric acid crystals at all under the conditions tested, but did bind to sodium urate crystals with an affinity that was substantially increased in the presence of a calcium oxalate solution.

193 The Inhibition of Calcium-Oxalate Crystal Growth by Chondroitin Sulfates, Heparin, Pentosan Polysulfate and Tamm-Horsfall Glycoprotein

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It has been suggested that polyanions, in particular proteoglycans and charged glycoproteins, exert the most important inhibition of

calcium oxalate crystal growth (CG) and aggregation in the urine. Vairations in inhibitory activity may be due to different charge densities. The purpose of the present study was to compare the inhibition by polyanions and two new drugs with a potential for prophylactic treatment of renal stone disease.

Methods. The inhibitory activity was assayed by a seeded-crystal growth method where the growth rate was measured through the disappearance of ^{14}C-oxalate from the metastable calcium oxalate solution (RS = 0.6). Increasing amounts of chondroitin sulfates-A, B, C (CS-A, B, C), heparin and a semisynthetic low-molecular-weight heparin analogue pentosan polysulfate (SP54), as well as glycoprotein extracted from urine according to Tamm and Horsfall (T-H), were tested for inhibition of CG. The inhibition of CG by a synthetic trisodium phosphone formate (Foscarnet) was compared with the effect of pyrophosphate.

Results of the polyanions, heparin was the most efficient inhibitor. CS–A and CS–C only exerted 40% of the inhibitory activity of heparin. CS–B was slightly more efficient than CS–A and CS–C. Pentosan polysulfate (SP54) inhibited CG to 80% of the activity of heparin. Assuming a concentration of CS–A and CS–C of 5–10 mg/l in the urine and 20–30 mg/l of SP54, when given orally, SP54 would be 3 times as effective as natural CS in the urine. T–H glycoprotein as an inhibitor was comparable to the effect of 5–10 mg/l CS–A, i.e., the concentration found in urine.

Pyrophosphate was a very strong inhibitor and phosphone formate exerted about 40% of the inhibition of pyrophosphate. In the concentration normally found in urine of pyrophosphate (4–5 mg/l), the inhibition of CG was 3 times more effective than that of 5–10 mg/l of CS.

Conclusion. In vitro heparin was the most potent of the inhibitors tested, and CS only yielded 40% of the inhibition by heparin. In concentrations normally found in urine CS was comparable with T–H glycoprotein and pyrophosphate was 3 times as potent as CS. SP54 exerted 80% of the inhibitiory activity of heparin and the expected urinary concentration of SP54, if given orally, was 3 times as potent as CS in a concentration normally found in urine, regarding inhibition of CG of calcium oxalate.

194 Sephadex G-200 Gel Filtration of Concentrated Urine; The Relation Between Calcium-Oxalate Crystal-Growth Inhibition and Glycosaminoglycan Chromatograms

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This study was undertaken in order to characterize the macromolecular inhibitors of human urine.

Methods. Pooled urines from healthy members of the staff were concentrated with an ultrafiltration procedure using a membrane with a cut-off at 5,000 dalton before it was applied to a Sephadex G-200 gel, primed with 0.3 M saline. After eluation the fractions were analyzed for the inhibition of calcium oxalate crystal growth (ICG) using a seeded crystal procedure. UV chromatograms (E_{280}) were made before and after extraction of proteins. Alcian blue precipitation pre- and postchondroitinase digestion was made to determine the alcian blue precipitable polyanions (ABPP) and the chondroitin sulfate (CS) content of the fractions. The carbazole reaction was applied for measuring total uronic acid (URON).

Results. The maximum ICG peak coincided with the ABPP and CS peaks as well as with the maximum URON peak. The largest UV absorption was also in this fraction, but it is not yet clear whether the UV absorption represents the protein core of the proteoglycans or other charged glycoproteins.

The residual ABPP after digestion with chondroitinase had its maximum closer to V_0, and there was also here a high UV peak, but a lower uronic acid content. The ICG was less than 50% of that of the CS fraction. It probably contained charged glycoproteins of high molecular weight.

A second, smaller ICG peak was found closer to V_t and contained no ABPP and no protein. Middle-molecular-weight inhibitors other than GAGs or proteins is the probalbe explanation.

Conclusion. Urinary macromolecular inhibitors (> 5,000 dalton) had its maximum in the same fractions as urinary GAGs. A second, smaller maximum of inhibition represented a middle-molecular-sized fraction of urine, not containing GAGs or protein.

195 Calcium-Oxalate Crystal Growth: Investigations on Inhibitory Activity of Model Compounds and Urine Samples

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The new constant composition method by Nancollas was used for the experiments, in which seed crystals of calcium oxalate monohydrates (COM) are added to supersaturated solutions of calcium oxalate and to centrifuged urine specimens. The calcium and oxalate ions concentrations are kept constant by the addition of reagent solutions containing these ions, and the rate of addition is controlled by an ion calcium electrode. By this method the following problems were evaluated:

1. Influence of different seed crystals on the kinetics of crystal growth. The results showed that experiments with seed crystals of different size are comparable. The concentration of COM seed crystals was adjusted to yield a constant and predetermined crystal growth rate in pure calcium oxalate solutions.

2. Inhibitory activity of different substances on calcium oxalate crystal growth. The following substances were tested for their influence on crystal growth: δ-carboxyglutamic acid, polyphosphates, polyvinylpyrrolidon, polyacrylate, etc. Highest inhibitory activity is exhibited by polymers with negatively charged endgroups (COO^-, SO_3^-). The monomer δ-carboxyglutamic acid showed no inhibitory effect.

3. Inhibitory potential in the urine of recurrent calcium-oxalate stone-formers compared to non-stone-formers. Urine is normally supersaturated with respect to calcium oxalate. Stone formation may be the result of a lack of inhibitors which normally prevent nucleation and crystal growth. Substances only inhibiting crystal growth might be detected by our method. The investigation showed no difference between the inhibitory potential in the urine of stone formers and non-stone forming persons. Yet a significantly higher calcium activity was found by calcium selective electrode in the urine of stone formers, although total calcium concentrations were not significantly different.

A major disadvantage of all methods for the study of crystal growth kinetics is the fact that diluted urine (about 1:100) has to be used. The question whether this dilution does any harm to the inhibitory substances in urine. One possibility to solve this problem might be to remove all calcium ions from urine by ion exchange while retaining the inhibitory activity. At the moment different ion exchangers are being tested. These results will also be presented.

196 A Critical Viewpoint of Urinary Inhibitory Activity

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An imbalance between promoting and inhibiting factors has been repeatedly suggested as one of the main determinants of idiopathic calcium-oxalate stone disease. However, the importance of each factor has been assayed only in artificial solutions, and the possibility of interferences between them has never been taken into account. In this study we have followed a different approach, seeking a relationship between the inhibitory activity (IA) of the whole urine with the concentrations of each of 17 urinary constituents; pH, Ca, PO_4, Ox, K, Na, Mg, urate, citrate, pyrophosphate, glucosaminoglycans (GAGs), creatinine, urea, Tamm-Horsfall glycoprotein (TH), cAMP,

osmolarity, and volume. The investigation was carried on 24-h urine specimens of 35 controls and 27 recurrent idiopathic calcium-oxalate stone formers. The 17 parameters taken into account are capable of explaining 71% of the whole IA. Eight of them (in order of importance: K, TH, creatinine, Na, Ca, GAGs, and cAMP) account for 67% of the IA. Among the known inhibitors, GAGs, pirophosphate, Mg and citrate account for only 7.7% of the IA. Setting the 8 statistically significant variables in order according to their standardized angular coefficient β, i.e., according to their potential for modifying the IA, the most important ion is calcium with a coefficient of -0.31, while of the inhibitors GAGs occupies the fourth (0.26) and pyrophosphate the sixth (0.19) place. From the above data it is concluded that to label a substance as "stone promoter" or "inhibitor" is an oversiemplification and that the so-called inhibitors play only a marginal role in crystal growth. However, it cannot be excluded that these substances may have a greater importance in crystal aggregation.

197 Further Studies on a Possible Lithogenetic Role Played by Uric Acid in Calcium-Oxalate Stone Disease

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The object of this study was to ascertain the potential of proteoglycans (GAGs) to bind uric acid and to check the chance that molecular or colloidal urate directly promotes CaOx crystallization. To solve the first problem scalar quantities of GAGs were added to a uric solution containing ^{14}C-urate as a tracer. After incubation and dialysis, the amount of ^{14}C uric acid bound to GAGs was counted. To verify the second hypothesis, in a metastable solution for CaOx, the effect of the addition of growing amounts of uric acid on CaOx crystallization was followed. The ability of GAGs to bind uric acid was shown to be very weak. Furthermore, uric acid in both molecular and colloidal form was unable to promote CaOx crystallization directly.

These "in vitro" observations, together with the impossibility of demonstrating binding "in vivo" between urate and GAGs, as well as the doubts existing about the real change urate crystals promote an epitactic precipitation of CaOx "in vivo", altogether seem to deny that uric acid plays a direct or indirect lithogenetic role on CaOx.

198 Determination of Stone-Forming Risks by Measuring Crystallization Inhibitor Activity in Urine with a Gel Model

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The influence of different substances and native urine on calcium oxalate crystallization can be studied with an easily manageable gel model. A microslide is uniformly coated with 3 ml of 1% agar. A punch hole pattern, produced with a special device, is loaded with starter solutions (calcium chloride and ammonium oxalate) and test substances or urine. In preliminary studies it was demonstrated that the density and breadth of the resulting crystal streak are good indicators for the crystallization-inhibiting activity of the test substances. The extinction of the crystal zone is measured photometrically and a so-called inhibitor index is derived by a simple mathematical operation. The classic inhibitors citrate and magnesium have been proved effective in reducing crystal formation when tested in physiological concentrations. In testing native urine samples a positive correlation was found between inhibiting activity and specific gravity. This influence of ionic strength was eliminated by enrichment of the gel with 130 mmol/l sodium chloride. In further studies the urine samples of healthy volunteers were examined after oral loading with oxalate, magnesium and alkalyzating agents. The results were compared with those under normal diet. Correlations between calcium, magnesium, oxalate, citrate, sodium, phosphate, uric acid and the inhibitor index were performed. In order to check the clinical relevance of the method, urine samples of recurrent urinary stone formers were examined and the results compared with the values of healthy persons. The results show that with this simple gel model, good determination of the risk of stone formation is possible, if one looks at the disposition to produce crystals in urine as the leading cause for urinary calculi and one notes, that an increase in stone constituents like calcium and oxalate in urine means an increased risk of stone formation.

199 N-Sulpho-2-Amino Tricarballylate, a New Analogue of Phosphocitrate: Metabolic Studies and Inhibitory Effects on Renal Calcification

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To be used therapeutically, a calcification inhibitor should be well absorbed when given orally, potently effective with minimal or no side-effects, target-specific and rapidly cleared. Few anticalcifying drugs satisfy these criteria. Diphosphonates, proven potent inhibitors, have their value reduced by restricted absorption and known secondary changes to bone metabolism. Phosphocitrate (PC), however, is a naturally occurring compound with potent anticalcifying activity and no documented side-effects [1, 2]. Its susceptibility to enzyme hydrolysis, however has led us to develop a more stable PC analogue, namely N-sulpho-2-amino tricarballylate (SAT) [3]. Some characteristics of this enzyme-resistant sulphamate analogue are described.

Stability in vivo has now been confirmed by giving ^{35}S-SAT intravenously to rats and recovering it unchanged from tissues, blood and urine. Apart from bone which retained 1% of the initial dose for at least 3 days, few counts were present in tissues after 24 h. Oral administration of SAT indicated 70% absorption with 80% of the absorbed SAT in urine within 24 h. SAT is a strong inhibitor of calcification in vitro and although not as good as PC, its activity nevertheless is comparable to pyrophosphate. Comparison of the inhibitory effects of SAT and PC on calcification in vivo was made using two models:

A. Rats (100 g) received 6.7 μmol of inhibitor intraperitoneally 1 h prior to a sodium oxalate challenge (7 mg). The rats were killed 4 h later and the extent of the calcium oxalate deposition determined by kidney calcium and oxalate analysis. Results indicated SAT reduced by 25% kidney calcium relative to control whereas PC at this level had no effect. Higher concentrations of SAT (50 μmol) resulted in only a 30% reduction.

B. Rats (50 g) received 1.0 ml of 10% calcium gluconate intraperitoneally daily for 9 days and the inhibitor (60 μmol/kg) was also given intraperitoneally 1 h prior to calcium gluconate. Kidney calcium was determined 24 h later. PC was very effective in preventing hydroxyapatite formation as induced by chronic calcium gluconate administration. Kidney calcium was reduced by 50% whereas SAT was ineffective at the same concentration.

In conclusion, SAT is well absorbed, resistant to enzymic degradation and rapidly cleared to the urine. In the short term, there was no evidence of toxicity in rats given large doses. The data suggest that SAT may be more effective in preventing calcium oxalate nephrolithiasis than PC, whereas with the deposition of hydroxyapatite, the reverse action of the inhibitors seemed to occur. The responses with SAT encourage the continuing search for other analogues, thus extending knowledge of structure-activity relationships of calcification inhibitors.

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200 Inhibitors of Precipitation of Stone-Forming Urinary Constituents: Are the Established Inhibitors Effective in Preventing Stone Formation and Growth or is a Moss-Covered Stone Inevitable?

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Although the idea of controlling stone formation and growth through inhibitor action was proposed several decades ago, a single compound has not yet been discovered which could become a likely panacea for all the varying types of stone patients. Information continues to flow, however, in respect to the many possible candidates present as normal urinary constituents. It is apparent that both small ions and macromolecules can significantly alter parameters involved in nucleation, growth, aggregation and disaggregation of crystals. A difficulty lies in interpreting the contribution of each urinary inhibitor and being able to translate data from purely in vitro physicochemical studies to events occurring in a natural physiological state.

Nevertheless, useful new information concerning the likely mechanisms of action of the various urinary constituents is being derived from in vitro studies. In addition, important advances are occurring in the field of synthetic inhibitors such as the diphosphonate class of compounds. PC, a powerful natural inhibitor of apatite formation, calcium oxalate crystal formation and ectopic calcification, together with analogues of PC, are also being closely examined. Another interesting facet is the possibility of useful compounds present in plant extracts. From knowledge of the structure-activity relationships of synthetic and natural compounds, an inhibitor compound with maximum effect and perhaps selective site inhibition may be an attainable goal in the near future.

201 Macromolecules in Whole Urine that Promote Calcium Oxalate and Phosphate Crystal Formation

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Aims. 1. To study quantitatively the macromolecules that promote calcium oxalate and phosphate precipitation in whole urine. 2. To find out whether there is a second promoter of crystal formation apart from Tamm-Horsfall (T-H) mucoprotein.

Methods. Crystal-free urine samples were evaporated at 37 °C on a rotary evaporator to 1,250 mosmol/kg at pH 5.3 or 6.8 for calcium oxalate and calcium phosphate studies respectively. ^{14}C-oxalate was used to compare calcium oxalate and measurements of cold calcium for calcium phosphate in the precipitates. 0.3% SDS was added to urine to reduce aggregation of T-H mucoprotein. Mixtures of whole urine and ultrafiltrates were used to study the relationship between concentration of macromolecules and crystals formed. Fractions of macromolecules that trigger crystal formation were studied by double ultrafiltration using molecular weight cut-offs at 10,000, 100,000, 300,000 daltons before evaporation.

Results. Removal of macromolecules from urine by ultrafiltration prior to evaporation reduced the calcium phosphate precipitated to 25%. After addition of T-H protein to the ultrafiltrate, the precipitate rose, but only 40% of what was found in whole urine. The increase in calcium oxalate and calcium phosphate crystal formation produced by the macromolecules was directly proportional to their concentration.

Addition of 0.3% SDS to whole urine prior to evaporation reduced the calcium oxalate precipitated to 56 (SD 20.1)% and calcium phosphate precipitated to 48% (SD 14.5) of the whole urine values.

Calcium oxalate crystal formation was reduced to 27.0% of whole urine value after ultrafiltration with 300,000 molecular weight cut-off, to 14.6% with 100,000 molecular weight cut-off, and to 13.2% with 10,000 molecular weight cut-off. Similar results were obtained with calcium phosphate studies.

Conclusions. 1. T-H protein is a strong promoter of calcium-phosphate crystal formation in whole urine, but there is a second promoter with less activity, and with a molecular weight between 100,000 and 300,000 daltons. 2. The promoting effect of macromolecules in whole urine is directly proportional to their concentrations. 3. The reduction in molecular weight of T-H protein by addition of SDS reduces its ability to promote calcium oxalate and phosphate crystal formation, confirming that it is the aggregated form which triggers crystal formation.

202 Equations Defining Urinary Crystallization Conditions with Respect to Stone-Forming Calcium Salts

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Aim. Equations which allow the degree of urinary saturation to be calculated with respect to stone-forming calcium salts and the inhibitory capacity on crystal growth to be predicted on the basis of chemical urine analyses alone without the necessity of performing complex and time-consuming physicochemical tests.

Methods. Seventy urine samples were collected from healthy controls, normocalcemic calcium stone formers and patients with primary HPT. The concentrations of calcium (Ca), phosphate (P) or oxalate (Ox), respectively, were measured in urine before and after saturation with respect to calcium oxalate monohydrate (COM) and brushite, and the critical supersaturation to induce the growth of small amounts of COM and hydroxy apatite was determined.

Results and Conclusions. The correlation between Ca and Ox in saturated urine with respect to COM permits the calculation of a thermodynamic solubility product of COM, which is in the order of values obtained in artifical solutions, and allows the calculation of the degree of urinary saturation by a single analysis of Ca and Ox in urine. 2. The degree of urinary saturation with respect to brushite can be calculated from urinary (Ca) and (P) at a given pH. 3. The correlation between the critical Ca x P concentration product for hydroxy apatite growth at a given pH and the corresponding pyrophosphate concentration (PPi) allows urinary inhibitor capacity towards the growth of small amounts of hydroxy apatite from urinary PPi to be predicted. 4. In contrast to urinary saturation with respect to COM, which mainly depends on Ox, the inhibitory capacity towards COM has turned out to be as sensitive to variations of Ca as variations of Ox, a finding that must be taken into account in stone therapies.

203 Crystallization Characteristics of Synthetic Urine in a Fast Evaporator

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In an attempt to gain insight into some of the physico-chemical factors governing crystalluria and urolithiasis, we have carried out a series of crystallization experiments using a standard reference artifical urine [1] in a fast evaporator system similar to that described by Hallson and Rose [2]. Urines were initially refrigerated but allowed to equilibrate at 37 °C prior to evaporation. Precipitates formed in the experiments were filtered using 0.45 μm pore size Teflon filters, washed with distilled water, weighed and then subjected to qualitative and semi-qualitative analysis by X-ray powder diffraction.

In the first set of experiments (series 1) the pH was varied from 3.0 to 9.5 using concentrated HCl or NH_4OH. These experiments were then repeated (series 2) with urine solutions that had not been previously refrigerated but had been allowed to stand on the laboratory bench for 4 days prior to evaporation. In series 3, which covered the pH range 5 to 7, the urine was again refrigerated prior

to experimentation, but its composition was varied slightly by the inclusion of additional components (e.g. uric acid, urea, creatinine) so that their roles in determining the crystallization characteristics might be investigated.

Calcium oxalate trihydrate (COT) precipitated over almost the entire acid range in series 1. At the lower pH values the monohydrate (COM) occurred as well, but always as the minor component, while the dihydrate (COD) was not observed at all. Brushite (BRU) was formed in the pH range 5 to 9, occurring mainly with apatite (APA). COT was not formed at all in Series 2. When creatinine was included in the urine (Series 3), neither COT nor BRU was precipitated.

These results again suggest [3] that COT might be a thermodynamically unstable precursor of COM. BRU, on the other hand, might be regarded as having some precursor role in APA formation. These hypotheses are epitaxially feasible in that the lattice networks of the respective species are compatible and lie within the tolerance "misfit" criteria permitted by Lonsdale. If indeed the precursor mechanisms are operative in vivo it might prove clinically worthwhile inhibiting COT and BRU. With this in mind, creatinine levels in stone- and non-stone-formers should possibly be more fully investigated.

Several other noteworthy results were obtained. The relative ratio of individual constituents in each mixture as well as the total mass of precipitate formed in each experiment were found to be pH dependent. The most "potent" pH in terms of the number of constituents precipitated occurred at approximately 5.5 where BRU, COM, COT and APA were identified. In series 3 uric acid dihydrate, COD and even NaCl were formed under certain conditions. Discussion of these and other results will be presented at the Symposium. In the meantime our studies using the fast evaporator continue.

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204 Hyperuricosuria and Calcium-Oxalate Stone Formation

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The effect of uric acid on the urine's ability to retard in vitro precipitation of calcium oxalate was tested utilizing the Discrimating Index Test (DI).

The DI was determined in 26 hyperuricosuric calcium-oxalate stone-formers and in 12 hyperuricosuric non-stone-formers. The distribution of DI values in these patients was compared to that of 86 idiopathic calcium oxalate stones formers and 146 normal healthy adults previously studied.

It was found that hyperuricosuric calcium-oxalate stone-formers differed significantly in their distribution of DI values from the hyperuricosuric non-stone-formers. In addition, the values of DI were very similar in the hyperuricosuric and in the idiopathic calcium oxalate stone-formers. The distribution of DI values in the hyperuricosuric non-stone-formers were very similar to those of the 146 normal healthy adults. These findings led us to believe that hyperuricosuria per se does not affect the urine's ability to retard calcium-oxalate precipitation.

In a second part of this study the concentration of uric acid in the urine was reduced by incubating the specimens with seed crystals of sodium urate. It was found that in spite of the decrease in uric acid levels, no significant change occurred in the DI.

In the third part of this study patients were treated with allopurinol. It was found that in spite of a significant decrease in uric acid levels, no significant change occurred in the DI. In addition, in some patients, allopurinol seemed to reduce the urine's ability to retard calcium oxalate precipitation. In hyperuricosuric non-stone-formers treatment with allopurinol seemed to cause a shift of the distribution curve of DI values toward that of the calcium-oxalate stone-formers.

When hyperuricosuric calcium-oxalate stone-formers were treated with orthophosphates, a significant decrease in the DI occurred. This was similar to the effect of orthophosphates on the DI of idiopathic calcium oxalate stone formers perviously described.

We conclude that uric acid does not seem to play a significant role in the phases of nucleation and crystal growth of calcium oxalate – those phases reflected in the DI test. Hyperuricosuric calcium-oxalate stone-formers seem to suffer from the same yet undefined etiology as other calcium-oxalate stone-formers. Our results seem to suggest the need for a long-term clinical trial comparing the efficacy of orthophosphates, allopurinol, or a combination of the two in preventing stone recurrence.

205 Urinary Inhibitors of Hydroxyapatite Crystal Growth: A Constant Composition Approach

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In the calcium-oxalate system, the inhibitor pyrophosphate has been shown to be pH dependent over the urine pH range, increasing in activity 100-fold with increasing pH from 5 to 7. In order to examine the effect of pH on the inhibitors of hydroxyapatite (HAP) crystal growth, we employed the constant composition system described by Nancollas et al. (Science 200:1059, 1978). This technique allows HAP crystal growth to be studied at constant supersaturation over a pH range without the formation of other calcium-phosphate phases. The computer program EQUIL was used to model test solutions to maintain the same supersaturation of HAP at different inhibitor concentrations and at different pH. One inhibitor unit (that concentration of material giving rise to a 50% reduction in growth rate from the control system) was calculated using the Langmuir adsorption isotherm.

Examination of pooled urine specimens from normal male subjects showed that increasing the pH of the assay system, increased the total inhibitor activity by a factor 3. The results are as follows:

	Pool 1	Pool 2	Pool 3
pH 7.40	143 ± 16	149 ± 7	169 ± 10
pH 6.60	80 ± 12	105 ± 8	85 ± 10
pH 5.80	55 ± 9	46 ± 6	69 ± 10

We studied the known major ionic inhibitors, pyrophosphate, citrate, and magnesium, in order to determine the respective contributions to total inhibition in the urine assay. One inhibitor unit (concentration of ion), for each of these species is:

	Pyrophosphate	Citrate	Magnesium
pH 7.40	$4.7 \pm 0.3 \times 10^{-7}$	$9.2 \pm 1.8 \times 10^{-5}$	$1.1 \pm 0.05 \times 10^{-4}$
pH 6.60	$3.1 \pm 0.8 \times 10^{-7}$	$8.6 \pm 0.5 \times 10^{-5}$	$3.7 \pm 0.4 \times 10^{-4}$
pH 5.80	$2.6 \pm 0.7 \times 10^{-7}$	$3.9 \pm 0.7 \times 10^{-5}$	$11.5 \pm 1.8 \times 10^{-4}$

This indicates that both pyrophosphate and citrate increase in inhibitor activity with a decrease in pH; magnesium decreases in activity with decreasing pH. This is contrasted to the situation with the urine assay. In this situation, if pyrophosphate and citrate are the major components to total urine inhibition, then it would be anticipated that the urine assay would also increase with decreasing pH.

These results suggest that other modulators of HAP crystal growth – either inhibitors or promoters – are active in this system over the physiological pH range of urine.

206 Bladder Contribution to Calcium Oxalate Crystal-Growth Inhibition of Normally Voided Urine

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Experimental data showing that the formation product of a calcium-oxalate (CaOX)-supersaturated solution increases when the bladder is used as a physiologic test tube, and that the bladder is coated with glycosaminoglycans, suggest that the bladder may have an influence on CaOx crystal formation observed in urine. It was the purpose of this study to investigate the possible difference in CaOx crystal growth inhibition that exists between normally voided urine (bladder urine) and urine collected from ureterostomies (kidney urine) in a dog model.

Nine female dogs, fed the same diet, had urine collections before and on day 10 after bilateral ureterostomies. Normal postoperative renal function and absence of dilatation of the upper urinary tract were determined by serum creatinine (1 ± 0.15 mg% preoperatively, 0.97 ± 0.16 mg% postoperatively) and intravenous pyelograms. Parameters measured in urine included creatinine, calcium, CaOx crystal growth inhibition (using a CaOx seeded growth system) and estimation of glycosaminoglycans by measurement of the alcian blue precipitable material (ABPM).

Inhibition concentration, and ABPM concentrations expressed as concentrations per mg of creatinine excreted were signifcantly lower in kidney urine than in bladder urine (see Table 1). A positive correlation ($r = 0.85$, $P < 0.05$) was observed between the kidney-bladder CaOx inhibition concentration variation and the kidney-bladder ABPM concentration variations in each dog.

Table 1

	Kidney urine Mean ± SE	Bladder urine Mean ± SE	Mean change ± SE
CaOx crystal-growth inhibition (units/mg creat.)	0.07 ± 0.01	0.14 ± 0.03	0.07[a] ± 0.02
ABPM x 10^{-2} (mg/mg creat)	0.87 ± 0.09	1.58 ± 0.30	0.07[a] ± 0.25

[a] Significant change based on a paired t-test $P < 0.05$)

We conclude that urine collected from the bladder contains more CaOx inhibition than urine collected from the ureters. The difference observed may be explained by the increase in GAG concentration observed in the bladder urine. However, substances that precipitate with alcian blue include RNA. RNA is a potent inhibitor of CaOx crystal growth and may contribute to the inhibition added to urine by the bladder. Measurements of CaOx crystal growth inhibitors in normally voided urine from patients may over estimate the actual inhibition present at the level of the kidney.

207 Effect of Additives and Whole Urine on Calcium-Oxalate Dihydrate Formation

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After adding ammonium oxalate to urine, calcium oxalate dihydrate crystals (COD) may be obtained almost exclusively. With a simple inorganic calcium-oxalate (CaOx)-supersaturated solution, only a few COD crystals are observed in the same conditions. The purpose of the study was to determine the factors normally present in urine that favor the stabilization of COD. An assay was developed to study COD formation in urine and inorganic solutions in the presence of additives.

Crystals resulting from the incubation of 25 ml of the solution to test with 1 ml 0.05 M ammonium oxalate were separated from the liquid phase by vacuum filtration, collected from the 0.2 μm Nuclepore filter and analyzed by infrared spectrophotometry. Given reference spectra of mixtures of COD and calcium oxalate monohydrate (COM) crystals of known proportions, the percentage of COD over COM in the sample could be estimated (confidence interval < 5%). In a standard calcium oxalate supersaturated solution, the following additives were studied at pH 6.5: pyrophosphate, citrate, magnesium, RNA from yeast, chondroitin sulfate, and heparin. (Corrections of the standard solution were made for complexation in the citrate and magnesium experiments.) RNA-citrate, RNA-pyrophosphate mixtures were studied. The effect of addition of urine, in various amounts, was determined. The effect of pH was studied with different additives and urine.

The addition of pyrophosphate (1 to 8 x 10^{-5} M), sodium citrate (10^{-4} to 2 x 10^{-3} M), RNA (5 x 10^{-9} to 5 x 10^{-7} M), and heparin (2 x 10^{-9} to 2 x 10^{-7} M) to the inorganic solution led to an increase in the amount of COD that was linearly correlated to the concentration used at pH 6.5 ($r > 0.09$, $P < 0.01$ for each additive). Magnesium and chondroitin sulfate had no effect. When urine was added to the inorganic solution, the proportion of COD observed was linearly correlated to the proportion of urine present in the mixture ($r = 0.98$; $P < 0.001$). RNA-citrate, and citrate-pyrophosphate mixtures showed additivity of their effects. pH variations (4.5 to 7.5) induced a proportional linear increase in COD formed with citrate, and pyrophosphate (respectively $r = 0.86$, $r = 0.97$, $P < 0.01$), as well as with whole urine ($r = 0.81$; $P < 0.01$).

Pyrophosphate, citrate, RNA, and heparin are known CaOx crystal-growth inhibitors. Pyrophosphate, citrate, and RNA had their effect in the system described at concentrations likely to be found in urine. Similar to the effect of these substance on CaOx crystal growth inhibition, the effect of these substances on COD formation is proportional to their concentration, pH dependent, and additivity is found. The phase-stabilizing effect on COD induced by these inhibitors may reflect the CaOx crystal-growth inhibition present in solution. Whole urine can be tested. This system may provide a much-needed assay of CaOx inhibitory activity in undiluted urine.

208 Effects of Human Urine on Aggregation of Calcium Oxalate Crystals: Normal Persons Versus Stone-Formers

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We utilized our continuous crystallization system in series with a Couette agglomerator to analyze the effects of addition of normal or stone-former urines to a defined synthetic urine that is supersaturated with calcium (6 mM) and oxalate (0.6 mM). Comparisons made were: 1. Synthetic urine only versus normal (N1.) and stone-forming (S.F.) 5% v/v added urines. 2. S.F. versus N1. urine, age and sex pairs (5 each). 3. S.F. versus N1. total group comparisons.

In addition to measurement of continuous crystallization parameters (growth G, nucleation B° and total mass M_T), we also measured changes in particle numbers and average size occurring in the agglomerator. (Analysis of agglomerator kinetics will be discussed in a separate communication to this symposium.) Our purpose here is to report the effects of addition of human urine to the system. This resulted in consistent inhibition of G by about 1 μm/min and enhancement of B° by 30,000 no · ml^{-1} · min^{-1} and production of greater M_T by 2–4 mg/l. Average agglomerate size in the agglomerator decreased, as did total number of agglomerates. These changes occurred with any addition of human urine. No significant difference between N1. and S.F. urines could be seen in simple crystallization (not agglomeration) parameters. Stone-formers' urine did, however,, consistently result in a significantly larger number of agglomerated particles over 15 μm than did normal urine experiments (S.F. x = 592, N1. = 563, $P < 0.02$) and S.F. experiments revealed more

agglomerated particles by a slight but consistent margin of 5%. These direct observations confirm previous reports of increased production of large CaOx crystals by stone-formers as a probable component in the genesis of human urolithiasis, and demonstrate that all human urine has powerful anti-agglomerating capability, which is slightly deficient in stone-formers.

209 Epitaxial Growth of Calcium Oxalate on Uric Acid

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The study was undertaken to determine whether epitaxial growth of calcium oxalate does or does not occur on crystals of uric acid or sodium urate during "real-time" periods of about 10 min.

An experimental system of calcium-oxalate metastable solutions was defined and monitored by specific calcium electrode. The metastable condition was ascertained by constancy of the electrode measurement during the specified period and lack of visible turbidity. Seed crystals of either uric acid or sodium urate were introduced into the solution and the decrease of calcium ion concentration was noted. The seed crystals were separated and inspected by SEM. The presence of calcium on the surface of the seed crystal was measured by analysis of induced X-ray radiation (LINK). Control experiments included introduction of uric acid seeds into calcium solutions in the absence of oxalate and introduction of crystals with non-compatible crystal structure to calcium-oxalate solutions.

It has been found that calcium oxalate is deposited on sodium urate in a time interval compatible with the urine residence in the kidney. Parallelly, no deposition on uric acid seeds was observed. These results, which are in excellent agreement with the conclusions of previous studies in literature, form a challenging contrast with clinical facts: (a) uric acid and not sodium urate is found in niddi of calcium oxalate stones; (b) no crystals of sodium urate were ever found in warm fresh urine. Secondly, crystallographic considerations can serve to predict epitaxial growth of calcium oxalate on uric acid.

In light of this cevelation, the above-described experiments with uric acid were modified by addition of 4–5 ppm glutamic acid. Sidnificant decrease in calcium concentration occurred and calcium was found on the surface of uric acid crystals.

It appears that glutamic acid adsorbs on uric acid crystals and attracts calcium ions, thus helping realize the potential of epitaxial growth of calcium oxalate on uric acid in real time. Presumably, other structurally compatible compunds in urine can fulfill the same function.

210 The Relative Inhibitory Potential of Urinary Macromolecular Fractions on CaOx Precipitation

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Ultrafiltration membranes of 10,000Δ, 1,000Δ and 500Δ were used to remove urinary macromolecules from urine of normal and stone formers. The filtrated urines were examined for their residual inhibitory potential for calcium-oxalate precipitation by the discrimination method of Sarig et al. (DI test). The filtrate test results are complementary to the information gained by analyses of retentates obtained in successive ultrafiltration. The method has an inherent advantage because the manipulation of solids retained on membranes may inadvertently modify their inhibitory potential.

A non-continuous gradation of molecular weights with at least two distinct groups of inhibitors was found in 20 normals urines. The first group had a molecular weight above 10,000Δ while that of the second group of inhibitors was in the range of 500–1,000Δ. The mean of the DI. values increased dramatically from the normal range (< 0.6) to the stone-former range (> 1.1) ($P > 0.001$) after the 500Δ filtration. It is worth noticing that some of the normal urines, even after the 500Δ filtration, still had a reasonable residuary degree of inhibition power. This inhibition power is related to inorganic compounds which are found in the urines.

In the urine specimens of seven stone formers, the inhibitors with molecular weight in the range of 500–1,000Δ were less potent than those in normal urines and the inhibitors with MW above 1,000Δ were almost absent. The stone formers' urine specimens exerted a moderate degree of inhibition before the 10,000Δ filtration and they had almost no inhibitory power after the 500Δ filtration.

From all the observations it appears that the inhibitors with MW of 500–1,000Δ are significantly more active than these of MW above 10,000Δ.

Our results support the following suggestions: (1) human urine contains various types and species of inhibitors toward the precipitation of calcium oxalate with a wide range of MW; (2) inhibitors of high MW (above 10,000Δ cotoff) and very low MW (under 500Δ cutoff) are only slightly active in the urine of stone formers; (3) the inhibitory effect induced by the 500–1,000Δ fraction seems to be the relatively most significant both in healthy and stone-former populations; (4) the overall inhibitory effect is more likely to be synergistic than connected with a specific substance or groups of substances.

211 Study of Inhibitors and Promotors of Crystallization in Vitro in Silica Gel Media

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Aims. A clear picture of the therapeutic utility of the various inhibitors of crystallization is yet to emerge. In this study we tried to grow various crystals seen in human urine, namely whewellite, brushite, struvite, octocalcium phosphate, and newberite in silica gel media. The effect of inhibitors and promotors of crystallization was studied by adding these agents to the crystal growth medium.

Methods employed. The silica gel medium was prepared in test tubes by incorporating a nucleating substance in the medium. Once gelation occurred, the other nucleating solute was poured on top of the gel. Taking one as control, the experimental set up was reproduced by adding the test agent along with the top layer of nucleating agent. Crystal growth occurred ranging in duration from a few hours to several weeks. The agents tested for inhibitory/promoting activity included citric acid, magnesium, pyrophosphate EDTA, tartaric acid, glucoronic acid and other indigenous extracts.

Summary of results. 0.25 M tartaric acid produced total inhibition of crystal growth. Citric acid, EDTA, and plantain stem extract showed evidence of inhibitory activity in a descending order. Pyrophosphates, magnesium, and glucoronic acid did not show any significant effect.

Uric acid promoted crystallization. Changes in pH showed that struvite and newberite grow better at pH 7, brushite and octocalcium phosphate best at pH 6.

Conclusions. Growth of crystal in silica gel medium is identified as a useful in vitro technique for studying inhibition and promoting crystallization. Tartaric acid is seen a having maximum inhibitory capacity. It is hoped that future studies with this technique will make definite inroads into the problem of the chemoprophylaxis of urinary stones.

212 Effect of Silicate on in Vitro Mineralizing Capacity of Inorganic Medium and Stone-Formers Urine

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Excess intake of some trace elements may be one ot the associated factors in the genesis of urinary calculi disease. This possibility was investigated by measuring the effect of some trace elemtnes on the in vitro mineralizing capacity of an inorganic medium, and on urine using bovine achilles tendon. Some observations on the effect of silicon are reported in this paper.

Different levels of silicon (0–200 ppm as Na_2SiO_3, $5H_2O$) were added to inorganic mineralizing media (with calcium x phosphorus products ranging from 10–75). Calcium uptake by tendon collagen from the medium was measured. The presence of silicate beyond 5 ppm resulted in a dramatic increase in calcium uptake, from media with a product above 20. The higher the Ca x P product of the medium, the more pronounced the effect of silicate. Urine of normals showed very low mineralizing capacity. On the other hand, the mineralizing propensity of stone formers' urine was very high. Silicon concentration in the majority of stone formers urine was higher than that of normal urine. In the case of stone formers, a positive correlation between the concentration of urinary silicate and its capacity for in vitro mineralization was observed. Addition of silicate to normal urine enhanced its mineralizing capacity. These in vitro observations suggest the possible involvement of silicate in the formation of urinary calculi.

213 Inhibitors of Calcification from Dolichos biflours

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Kulthe (*Dolichos biflorus*) seeds and their extract is taken by kidney stone patients in India with the hope that they will get rid of the stones without undergoing surgery. To test its efficacy, a cold water extract (obtained by soaking 20 g seeds overnight in 200 ml glass-distilled water) was assayed for its effect on the homogeneous as well as the heterogeneous precipitation of calcium phosphate/oxalate. When 1.5 ml of this extract was used, the homogeneous calcium phosphate precipitation was inhibited by nearly 50% and calcium oxalate precipitation by nearly 35%. In collagen-induced uptake of Ca^{2+} and HPO_4^{2-}, 0.1 ml of the extract caused 25–30% inhibition of the ion uptake. The inhibitory factor(s) was found to be dialyzable. The inhibitory potency was unaffected by treatment of the extract with activated charcoal and with 10% TCA. The data suggest that *Kulthe* extract may be beneficial to patients suffering from urolithiasis. The chemical nature of the inhibitor is under investigation.

214 A Scanning Electron Microscope (SEM) Study of the Bladder Mucosa in Paediatric Patients with Idiopathic Calculus Disease

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The object of the study was to find out the role of the bladder mucosa in the genesis of idiopathic vesical calculus. Multiple biops specimens of the bladder mucosa in 9 patients, aged between 6 months and 7 years, were obtained during cystolithotomies. The specimens were fixed in 2.5% glutaraldehyde ih 0.1 M phosphate buffer at pH 7.4, then dehydrated, dried, and gold spluttered. Scanning with the Hitachi Mini SEM showed attachment of the crystals to the mucosa in a back-to-back arrangement. Some exophytic growths from the crystalline beds were evident. Buttressing matrix was observed stretching from the mucosa to the crystals. The crystals were identified to be uric acid. Calcium oxalate dihydrate and monohydrate could be seen. The crystalline aggregates varied in size from 0.5 M to 15 M. An examination of the centrifuged early morning and intraoperative urinary specimens showed the crystal aggregates to be from 1 M to 100 M.

The new observation suggests that attachment of the crystals, chiefly uric acid, to the mucosa is a important initiating process in vesical stone formation. A critical period is gained for the crystal aggregates to grow to a size before breaking off to become free particles, some of which may be too large to pass through the small urethra (8F–10F). The predominatly uric-acid encrustations may have therapeutic implications.

215 Improvements of the Gel Crystallization Method (GCM): Kinetic Investigation of Calcium-Oxalate Growth by Microphotometry

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Microphotometry was applied to the determination of optical kinetic parameters of the growth of calcium oxalate crystals in gels. The new instrumentation consists of an inverted microscope photometer equipped with a special scanning stage for microtiter plates (96 wells/plate). An on-line desk computer is used to control the instrument as well as for evaluating photometric measuring results. Crystal growth of calcium oxalate can be followed up by bright- and darkfiled measurement and in polarized light. A camera attached to the microscope allows the performance of photomicrography for the evaluation of crystal-size distributions within the gel.

The method is suitable for large-scale determinations of optical crystallization kinetics (up to 400 single kinetic curves per hour) and can be applied to clinical routine measurements. The measuring device is described in detail. Kinetic determinations were carried out with artificial solutions in the presence and absence of crystallization inhibitors as well as with native urines.

The results are presented and discussed with respect to the influence of different factors and urinary constituents on the optical signal and their importance to calcium-oxalate stone formation.

216 The Effect of Sulfopentosan Sodium on Crystallization of Calcium Oxalate

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Several authors have demonstrated a decreased inhibition of calcium oxalate (CaOx) crystal growth and aggregation in urine from stone formers. Glycosaminoglycans are thought to be of importance for the inhibiting activity. However, no reliable therapeutic regimens have been described for correction of a low crystallization inhibition.

Aims. These experiments were undertaken in order to study the in vitro effects of sulfopentosan sodium (ELMIRON) on CaOx crystallization. Sulfopentosan sodium (SPS) is a small glycosaminoglycan-like substance, which is thought to be excreted in urine following oral administration.

Methods. The rate of crystal growth in a seeded metastably supersaturated system was followed after addition of SPS in different concentrations. A solution of SPS was also highly supersaturated with calcium chloride and sodium oxalate, and the crystallization followed without seed crystals. Finally, the rate of crystallization and the crystal size ratio were studied in supersaturated urine containing SPS.

Results. SPS in concentrations as low as 39 μg/l inhibited crystal growth in the metastable crystallization system. In the highly supersaturated system, SPS resulted in a decreased rate of crystal growth. SPS in concentrations above 50 mg/l modified the crystallization in supersaturated urine. Crystal-size ratio decreased when urine was supersaturated with respect to CaOx, but this change in crystal size distribution appeared to be reatarded by SPS.

Conclusion. If future research shows that orally administered SPS is excreted to a significant extent with urine, the results obtained

indicate a possible method to increase the inhibiting potential of urine in calcium-oxalate stone formers.

VI. Methods of Analysis

217 The Effect of Ascorbic Acid on Urine Oxalate Measurement

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Urinary oxalate measurement is important in the diagnosis and treatment of patients with primary hyperoxaluria and oxalate kidney stone disease. Excreted oxalate is derived from several metabolic precursors, including ascorbic acid. Much attention has recently been focused on the relationship between ascorbic acid ingestion and oxalate excretion, but the possibility that a chemical conversion of ascorbic acid to oxalate may occur during the urine analysis procedure has not been fully investigated. A wide variety of techniques is currently used to measure urinary oxalate. These subject the urine to markedly differing assay conditions. This study was undertaken to investigate the effect of ascorbic acid on the measurement of urinary oxalate, with particular emphasis on the influence of pH.

Varying levels of ascorbic acid (1–15 mmol/l) were added to acidified 24-h urine collections (pH < 1.5). Oxalate was assayed by gas-chromatography of dimethyl oxalate. Calcium chloride and ethanol were used to precipitate oxalate from urine overnight and recoveries obtained were greater than 90%. After drying the precipitate, oxalate was methylated with boron trifluoridemethanol [1]. The precipitation step was carried out at pH values of 5, 6, 7, and 8 ± 0.1 in the presence of the added ascorbic acid. At pH 5, 15 mmol/l of ascorbic acid caused less than 10% increase in the measured level of oxalate above the basal value of 0.2 mmol/l, and at pH 6, the increase amounted to less than 15%. However, when precipitation was performed at pH 7, the same concentration of ascorbic acid resulted in an increase in the measured oxalate level of more than 200%. The effect was even more pronounced at pH 8, with increases ranging from 20% at 1 mmol/l ascorbic acid to more than 300% at 15 mmol/l.

The chemical conversion of ascorbic acid to oxalate at alkaline pH was found to be very rapid. Urines with and without added ascorbic acid were alkalinised to pH 9 for short periods of time (1, 5, 30 min), immediately readjusted to pH 5, then precipitated overnight. The addition of ascorbic acid resulted in marked increases in the measured level of oxalate compared with the untreated urines. When urine samples containing 1 mmol/l of added ascorbic acid were exposed to pH 9 for only 1 min, an increase of 50% in the measured level of oxalate was found. This rose to more than 200% after an exposure of 30 min. The increases in measured oxalate were correspondingly greater in the presence of higher levels of added ascorbic acid.

Since urine ascorbic acid levels can be as high as 15 mmol/l, it was concluded from these results that accurate determinations of urinary oxalate in the presence of ascorbic acid can only be guaranteed by using analytical methods which do not incorporate steps carried out at pH values > 6, or by ensuring the complete absence of ascorbic acid from the urine sample.

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218 Spontaneous in Vitro Generation of Oxalate from L-Ascorbate in Some Assays for Urinary Oxalate

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Aims. Erroneously high levels of oxalate were found in summertime urine samples when a commercial enzymatic kit (Sigma Chemical Co. Kit no. 590 – A) was used to measure urinary oxalate. Ascorbate can be oxidised to oxalate and we therefore studied this conversion in the conditions used in some of the assays for urinary oxalate.
Methods. Aqueous solutions of L-ascorbate and urine samples with and without added L-ascorbate were processed by an alumina extraction step as used in the Sigma oxalate kit for separating urinary oxalate. Oxalate levels in the alkali extracts were measured by four different assays: 1. A routine enzymatic oxalate decarboxylase assay. 2. Sigma oxalate kit assay. 3. A newly developed automated oxalate assay using immobilised oxalate oxidase. 4. Dionex Ion Chromatograph.

L-Ascorbate levels in the samples were measured by titration with 2, 6 dichlorophenolindophenol. Similarly aqueous solutions of ascorbate were assayed for oxalate after these samples had been kept at room temperature, pH 2.0–13.0, for different periods of time (0–60 min). Aqueous solutions of ascorbate and urine with added ascorbate were mixed with charcoal at pH 12.5 and the oxalate and ascorbate levels measured before and after charcoal treatment.

Finally, aqueous solutions of ascorbate (0.50–3.0 mmol/l) were diluted with double-deionised water and assayed for oxalate using Dionex Ion Chromatograph by standard procedures recommended by Dionex.
Results. Conversion of L-ascorbate to oxalate was found to be 83% and 63% complete in aqueous solutions and urine respectively when the alumina separation procedure was used. L-Ascorbate is progressively converted to oxalate at pH > 10.5 and the rate of this conversion is enhanced by activated charcoal. L-Ascorbate is apparently converted to oxalate in the Dionex Ion Chromatograph, which uses alkaline (pH 10.6) elution of anions adsorbed onto the anion exchange resin.
Conclusions. Because of the high efficiency of conversion of L-ascorbate to oxalate demonstrated here physiological amounts of L-ascorbate to be expected in urine from subjects on a normal diet will give serious interference in oxalate assays in which this conversion can occur. The Sigma and Dionex methods are therefore unsatisfactory. Methods for oxalate in urine using pH over 10 or alumina extraction or charcoal treatment at alkaline pH should be abandoned unless the conversion of L-ascorbate can be overcome.

It is necessary to check many of the oxalate assays for interference by ascorbate and also reconsider the evidence for in vivo conversion of ascorbate to oxalate since this might have been in vitro instead.

219 Enzymatic Assay of Oxalate Using Oxalate Oxidase Isolated from Sorghum Leaves

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Estimation of oxalate in biological fluids using plant and moss oxalate oxidase have met with limited success due to its inhibition by sodium ions normally present in these fluids [1, 2]. The aim of the present study was to isolate oxalate oxidase from the leaves of *Sorghum vulgare* (which is unaffected by sodium ions under normal physiological concentrations) and to employ this for assaying oxalate in urine and other biological fluids.

The enzyme was isolated from the leaves of 10-day-old sorghum seedlings, according to the method of Chiriboga [3]. The supernatant (15,000 g) was partially purified by 30–65% ammonium sulfate saturation, and the pellet obtained at 10,000 g was dissolved in 0.05 M sodium succinate buffer, pH 5.0. The enzyme thus prepared was stable for 2 weeks at 0–4 °C.

The assay mixture, consisting of 80 μmol sodium succinate (pH 5.0), 1 μmol sodium oxalate, and 0.7 mg enzyme in a total volume

of 2.0 ml, was incubated at 45 °C for 10 min, and the H_2O_2 generated during the enzymatic reaction was measured using a colour reaction with 4-amino-phenazone and peroxidase system [4]. Preliminary characterization of the enzyme revealed a pH optimum of 5 and a temperature optimum at 45 °C. The enzyme activity remained linear up to 10 min and the apparent Km for oxalate was 2.4×10^{-5} M. The enzyme was induced by Fe^{2+} and FAD and inhibited by sulfhydryl agents (viz. iodoacetate, PCMB, and NEM).

This simple, rapid, and sensitive method was employed for direct estimation of oxalate in urine. The assay system was the same as described above, except that the substrate (oxalate) was substituted by 0.1 ml diluted urine. This method was sensitive to oxalate concentrations as low as 5 μmol/l and the percentage recovery was 99.2 ± 2.

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220 Determination of Urinary Oxalate by Reversed-Phase Ion-Pair "High-Performance" Liquid Chromatography

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Most reported studies on oxalic acid in urine are insufficiently precise to satisfactorily measure the low concentrations of oxalate normally present in human urine. Published methods are based on preliminary concentration of oxalate by either direct precipitation, solvent extraction, or ion-exchange chromatography, followed by colorimetry of oxalic acid. Enzymatic methods tent to underestimate oxalate by 10 to 20%. Recently a method involving high-performance liquid chromatography (HPLC) was presented (Hughes et al (1982) Anal Biochem. 119:1). This method, however, included both derivatization and gradient elution. For many years we have used the tedious and time-consuming method of Hodgkinson and Williams (Clin Chim Acta 26:127, 1972) in our laboratory.

Aim of the study. To develop a simple, rapid and precise method for determination of urinary oxalate.

Method. Our method is based on a reversed-phase ion-pair HPLC technique, which has been described in detail by Larsson et al. in (Clin Chem 28:2272, 1982). In this liquid-chromatographic method interfering compounds in the urinary samples were eliminated before chromatography by passage through a preparative C_{18} mini-column (Sep-pak cartridge, Waters Associates). In the reversed-phase system tetrabutyl ammonium was included as a counter ion to enhance the retention of oxalate. The pH of the mobile phase was kept low (2.00) to avoid precipitation of calcium oxalate.

Results. One important feature of the method was preparative treatment of urinary samples with Sep-pak C_{18} cartridges to eliminate interfering substances. The relation between peak height and oxalic acid concentration was slightly sigmoid but linear between 150 and 750 μmol/l, which includes most normal and hyperoxaluric specimens. Overall analytical recovery was 90.3 to 104.6%, with a mean of 95.9%. Ascorbic acid gave a negative interference of 10%, but no interference was found for glucose and citric acid. A comparison between results by the present HPLC method and our colorimetric method showed a within-assay standard deviation, calculated with analysis of variance from duplicate determinations on urinary samples sent to our laboratory for routine analysis of oxalate, of 12.7 and 4.2 μmol/l for the colorimetric and HPLC procedures respectively, corresponding to a CV of 5.1 and 1.7% at 250 μmol/l. The between-assay precision of the colorimetric and HPLC procedures was estimated from duplicate analyses of a pooled urine and the mean value was 398 (SD 30.6) μmol/l (CV 7.7%) and 372 (SD 12.2) μmol/l (CV 3.3%) for the respective procedures.

Conclusion. The described method for determination of urinary oxalate is rapid and precise and readily adapted for routine clinical use.

221 A Comparison of Three Methods for Measuring Urinary Oxalate

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This study was designed to compare three different techniques for measuring oxalate in urine: (a) the chemical method of Hodgkinson and Williams, (b) the ion-chromatographic method of Robertson et al. and (c) the enzymatic method involving oxalate oxidase using the kit and instructions supplied by the Sigma Chemical Co.

Twenty-four-hour urine samples were collected and analysed for oxalate according to the authors' instructions. The chemical and ion-chromatographic methods compared well but the enzymatic method, as described by Sigma, initially underestimated oxalate by 20 to 50%. Amendment of the Sigma procedure involving acidification of the original urine to pH 1 rather than pH 3, as suggested by the manufacturers, increased the recovery of oxalate almost to the values obtained by the other techniques.

The relative merits of the three methods are compared and discussed with reference to analysis time, accuracy, precision and cost.

222 On the Preservation of Urines for the Determination of Citrate

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It is known that bacteria use citrate as their C-source and many growth media contain citrate. Since many urines contain bacteria, the citrate concentration may decrease during the collection of urines or in the time between the collection and the citrate determination.

The citrate concentration of urines, kept for 2 days at 20 °C, decreased from 1.42 ± 0.76 mmol/l to 0.73 ± 0.82 mmol/l (n = 16). In contrast, urinary oxalate was stable when urines were stored for 2 days at 20 °. This fall of urinary citrate was caused by bacteria, for the citrate content of urines which were heated 10 min at 100 ° and stored for 2 days at 20 ° did not change (3.01 ± 1.28 mmol/l at time 0 and 2.99 ± 1.28 mmol/l 2 days later). The citrate concentration of urines which were filtered through a bacteria filter decreased only by 9.7 ± 2.5% in 2 days. Citrate was determined by the citrate lyase method of Welshman and McCambridge.

To prevent the growth of bacteria, the following preservatives were tested: ethyl mercurithiosalicylate (0.1 mg/ml urine), sodium azide (3 mg/ml urine), penicillin G and streptomycin sulfate (1,000 IU/ml each), thymol (saturated), and benzyltributylamine (5 mg/ml urine). First of all, it was tested whether these substances inhibit the enzymes used for the citrate determination (citrate lyase and malate dehydrogenase) and the oxalate determination (oxalate decarboxylase and formiate dehydrogenase). The citrate determination was not influenced by these substances, whereas less oxalate was found in urines which contained thymol and sodium azide.

Twenty-one urine specimens were stored with and without preservatives. The citrate concentration in urines without additives decreased by 70.9 ± 28.6%, in the presence of thymol by 14.2 ± 16.2%, in the presence of ethyl mercurithiosalicylate by 15.8 ± 21.0%, in the presence of penicillin G/streptomycin sulfate by 4.9 ± 8.9%, and in the presence of sodium azide by 4.5 ± 7.8%. The 24-h urines of 20 healthy men contained 2.20 ± 0.76 mmol citrate and

of 17 healthy women 2.75 ± 0.96 mmol citrate. These urines were collected over penicillin/streptomycin sulfate (110 mg/190 mg).

Our findings show that a mixture of penicillin G and streptomycin sulfate is best suited for the preservation of urines for the determination of citrate and oxalate. The hitherto used thymol is less suited for this purpose.

223 Human Plasma Oxalate Concentration Re-Examined

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A long-standing discrepancy has existed between the plasma oxalate concentration measured by in vitro chemical/enzymatic (~10 μmol/l) versus in vivo isotopic dilution (~1 μmol/l) methods; however, in recent reports, the gap between the two measurements is narrowing. The invasive and indirect nature of the in vivo method necessitates, for clinical use, the validation of an accurate in vitro chemical assay for blood oxalate. With this aim in mind, the radioenzymatic isotope dilution assay (REIDA) for plasma oxalate concentration has been abbreviated by a rapid and direct precipitation of calcium oxalate from ice-cold plasma ultrafiltrates initiated within 1 h of phlebotomy. The abbreviated assay of plasma pooled from fasting normal males yielded a value of ~4.0 μmol/l, an estimate consistent with recently reported mean values of 3.0 μmol/l and 2.3 μmol/l by enzymatic methods and 2.8 μmol/l by a gas chromatographic technique, and significantly lower than 10.0 μmol/l reported by earlier chemical/enzymatic methods. Evidence is provided that the lower REIDA estimate of plasma oxalate concentration results primarily from avoiding the time-dependent, apparently nonenzymatic in vitro conversion of an untrafilterable substance(s) to oxalate, and less dramatically, from avoiding organic chemical contamination through the use of "fire cleaned" glassware. Using the abbreviated REIDA, no difference in fasting plasma oxalate concentration was observed between age-matched, apparently healthy black and white males. Plasma oxalate concentration was unaffected by collecting blood in the presence of inhibitors of the conversion of glyoxalate to oxalate. Substrate destruction (enzymatic) experiments demonstrated that the REIDA assay measures oxalate exclusively. These data indicate that the higher estimates of plasma oxalate concentration from in vitro assays were erroneous, resulting at least partly from the rapid conversion of blood substances to oxalate after phlebotomy. Precautions to minimize this conversion are suggested.

224 Combined Enzymatic Degradation with Chondroitinases and Alcian Blue Precipitation in Determination of Urinary Chondroitin Sulfates

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The alcian blue precipitation method is not specific for urinary glycosaminoglycans, but also precipitates polyanionic urinary glycoproteins and ribonucleic acids. The purpose of this study was a further development of the method to measure glycosaminoglycans specifically.

Materials and methods. We combined the alcian blue precipitation method (ABPM) with enzymatic degradation, using chondroitinase-AC and chondroitinase-ABC to determine urinary chondroitin sulfates. Nitrous acid oxidation was used in an attempt to determine urinary heparan sulfate. The difference in precipitation pre- and post-degradation yielded a quantitation of chondroitin sulfate-AC (CS-AC) and CS-B (dermatan sulfate). The linearity and specific precipitability of heparin, CS-A, CS-B, DS-C, and a semi-synthetic low-molecular-weight heparin analogue pentosan polysulfate (SP54) were determined.

The method was applied to 24-h urines from 15 healthy controls, 25 renal stone patients, 7 patients with acromegaly, as well as to urine samples from 11 children under 7 years of age.

Results. The alcian blue precipitation was linear for CS-A, CS-B, CS-C, heaprin and SP54. The specific precipitability was slightly higher for heparin than CS and 50% higher of SP54 than of heparin. The choncroitinases only degraded CSPs and not heparin or SP54. Nitrous acid only oxidised heparin and heparan sulfate (HS) but not CS or SP54. HS could not be detected in urine by this procedure; neither could CS-B (dermatan sulfate).

The total urinary ABP-polyanions (ABPP) in controls was 24.3 ± 5.2 mg/24 h and the urinary CS-A, C was 7.5 ± 2.7 mg/24 h, higher in males than in females ($P < 0.05$). The ABPP was lower in stone formers ($P < 0.06$), but the CS-A, C were *not* different. Patients with acromegaly had a higher CS-A, C (10.3 ± 3.9) than the controls ($P < 0.01$) and the excretion was related to the activity of the disease. The highest values were found in children, with ABPP 48 ± 22 mg/l and CS-A, C 22.4 ± 12.4 mg/l ($P < 0.001$).

Conclusion. The described procedure offers a specific and sensitive method of measuring CS in small quantities of urine without preceding concentration of the urines. The urinary CS was not different in stone formers and controls, whereas the total ABPP was lower in stone formers. The basis of the difference in ABPP remains to be identified.

225 The Measurement of Oxalate and Glycollate with Immobilized Enzyme Systems

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Oxalate oxidase (1.2.3.4) and glycollate oxidase (1.1.3.1) have been bound to nylon tubing and used in continuous flow systems to measure oxalate and glycollate. Conditions for immobilizing oxalate oxidase have been described previously [1, 2] and, because of the presence of a potent oxalate oxidase inhibitor in urine, oxalate must be precipitated before assay.

To immobilize glycollate oxidase excessive amounts of the cofactor, flavin mononucleotide (FMN), must be removed from the preparations. It was found that the immobilized enzyme was unstable and lost up to 30% of its activity per day. This inactivation could not be prevented by using FMN, albumin, glycerol or dithiothreitol, either alone or in combination with each other. Attempts at stabilizing the enzyme by crosslinking the subunits with either glutaraldehyde or dimethyl suberimidate also proved unsuccessful. The bound enzyme could be stabilized in phosphate buffer containing 0.5 mM FMN and 0.66 M ammonium sulphate.

As with the oxalate oxidase system, the hydrogen peroxide formed by the glycollate oxidase reaction is detected using a colour reaction with peroxidase, 3-methyl-2-benzothiazolium hydrazine and N,N-dimethylalanine. Glycollate oxidase can also catalyse the oxidation of lactic acid to hydrogen peroxide and, even though the rate is slower than that for glycollate, the concentration of lactate in the sample must be determined and a correction made. Lactate was measured using lactate dehydrogenase in either a discrete assay or continuous flow system.

These two systems are currently being developed to measure oxalate and its precursor, glycollate, in urine. Although oxalate excretion is a major factor in renal stone formation, it does not vary greatly in normal subjects. It was considered that the measurement of both oxalate and glycollate levels would give a better indication of the predisposition to stone formation. Therefore, we have collected urines from normal subjects who have been on various dietary regimens and the oxalate and glycollate results from these studies will be discussed.

1 Bais R, Potezny N, Edwards JB, Rofe AM, Conyers RAJ (1980) Anal Chem 52:508–511

2 Potezny N, Bais R, O'Loughlin PD, Edwards JB, Rofe AM, Conyers RAJ (1983) Clin Chem 29:16–20

226 The Value of Oxalate Determination by High-Performance Liquid Chromatography in Clinical Practice

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Aims of the study. The analytical dilemma of oxalate is well recognized. Numerous attempts using various aproaches have been undertaken to find a cheap, reliable, reproducible and fast method. We have added the advantages of high-performance liquid chromatography (HPLC) and report our experience with respect to the usefulness of oxalate determination with this technique, as obtained in our stone clinic where approximately 3,000 stone formers are followed.
Methods. Oxalic acid was extracted from urine with 3-α-butyl phosphate and converted into the fluorescent derivative by esterification with 9-anthryldiazomethane (ADAM). The reaction mixture containing the oxalic acid derivative can be directly chromatographed on HPLC, using octadecylsilane reverse-phase column monitoring with a fluorophotometric detector. A linear relationship was observed in the range of 1–100 μg/ml of standard oxalic acid dissolved in saline. The method has been published in detail (Analyt Biochem 128:549–464, 1983: Specific and Rapid Assay of Urinary Oxalic Acid Using High-Performance Liquid Chromatography by S. Imaoka, Y. Funae, T. Sugimoto, N. Hayahara and M. Maekawa.
Summary of results. Adults excrete 23.8 ± 9.0 mg (mean ± SD) of oxalic acid per day. The data obtained from normal individuals, hyperoxaluric, and nonhyperoxaluric stone formers, as well as oxalate determinations in smaller urine fractions (circadian rhythm), are presented and compare favorably with results obtained with different standard methods (i.e., enzymatic, gaschromatography, isotachophoresis).
Conclusions. This method has proven valuable for routine measurements of urinary oxalic acid, as it is accurate, simple and specific, and has the distinct advantage of being fast.

VII. Matrix

227 A Microscopic Study of the Matrix of Some Calcium Oxalate Renal Stones

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Normally, over 95% of the weight of urinary stones is crystalline material, making it impossible to study the matrix, i.e., the noncrystalline component of stones, by conventional microscopic means without losing information about the crystal-matrix interface. We have developed a procedure whereby decalcified crystals are represented as crystal ghosts and stones retain their architectural integrity.

Freshly removed clacium-oxalate renal stones were fixed in half-strength Karnovsky's fixative and then sectioned with a diamond wafering saw. Approximately 0.2- to 1-mm-thick sections were collected over water and then embedded in 1% aqueous solution of bactoagar. Once the agar hardened, some sections were transferred to Karnovsky's fixative and others to buffered formalin. Following this primary fixation, sections were washed in water and decalcified in 0.25 M EDTA, pH 7.2, washed in several changes of water, and then transferred to their fixative solutions. They were processed as any other biological sample for microscopic studies: Karnovsky's fixed sections for transmission electron microscopy (TEM); buffered formalin fixed sections for light microscopy (LM). For TEM they were embedded in Spurr's plastic and for LM in paraffin. Before TEM examination ultrathin sections were stained with aqueous uranyl acetate, followed by Reynold's lead citrate. Paraffin sections were examined after staining with hematoxylin and eosin, colloidal iron, alcian blue, pH 0.5 and 2.5, vonKossa, alizarin red, and periodic acid-Schiff. A part of every fixed stone was critical point dried and processed for scanning electron microscopy (SEM).

Crystals were replaced by crystal ghosts, i.e., spaces delimited by an electron dense layer. Noncrystalline matrix material was ubiquitously present in the intercrystalline spaces and was composed of fibrillar and amorphous components. In paraffin sections most of the noncrystalline material was colloidal iron, alcian blue, pH 2.5, PAS and hematoxylin positive; and alizarin red and vonKossa negative. The stones with concentric laminations showed layers of calcium oxalate crystals, alternating with layers of matrix material in which some red cells and cells of epithelial origin were also embedded.

The microscopic structure and staining properties of the matrix suggest that it is made of cellular debris and nonsulfated complex carbohydrates. This study has shown that the EDTA-insoluble part of the matrix can be studied by conventional microscopic means and can give information about the nature and precise location of matrix in the stones.

228 Childhood Urolithiasis in Iran: A Study of Urinary Calculi Using X-Ray Diffraction, Polarizing Microscopy, Chemical Analyses

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Out of a total of 148 patients with urolithiasis that underwent surgery in the Children's University Hospital of Tehran between 1970 and 1982, 160 calculi originating from 121 children were studied using three different methods: 98 children (65% male) had stones in the upper urinary tract, 19 (74% male) in the bladder and urethra, and 4 both in the upper and lower tract. Since there were insufficient studies on the metabolic disorders of some children, we could establish in only 30% of the cases obvious reasons such as metabolic disorders and malformations to be responsible for children with kidney and ureter calculi shows a bimodal distribution with weak maxima at 5 and 11 years. Besides whewellite concrements, which often occur in compact sperulithic and pure form, the most frequent phase assemblage consists of whewellite and ammonium acid urate. Thin sections examined under the microscope prove that the urate preferably forms the core of the concrement, while whewellite is enriched in the outer rims. Typically, infection stones composed of apatite and struvite make up less than 20% of the cases. Calculi in the lower urinary tract more frequently show uric acid in addition to whewellite-urate concrements. No pure whewellite concrements were found in the bladder or urethra. It is striking that in our stone analyses, six had cystine and three xanthin.

The relatively high incidence of childhood urolithiasis in Iran, with calculi frequently containing ammonium acid urate, must be interpreted as the consequence of malnutrition in an industrializing country. In contrast to reports made 12 years previously from other less-developed parts of Iran, and other countries such as Thailand, India, Iraq and Egypt, not bladder stones but upper urinary tract calculi prevail, which might indicate that 'endemic urolithiasis' is fading in Iran. A comparison of stone analyses with other Asian countries (Turkey, Thailand, India) indicates the same distribution of different components.

229 Alternating Crystallisation – A Proposed Mechanism for Lamellar Structure Formation in Renal Stones

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Leduc [1] in 1928 proposed that stratified precipitations (Liesegang layers) of calcium salts in gelatin were formed by "equipotential lines of diffusion force". Based on Leduc's theory, Lichtwitz [2] in 1944 proposed that all concretions were formed by incrustation of preformed organic matrices and their lamellar structures were Liesegang layers. In contrast, Schade [3] in 1928 proposed that concentric laminations were due to alternate depositions of colloid and crystalloid. In 1961, Finlayson and Vermuelen [4], having successfully produced concretions with laminations artificially, proposed that matrix was a non-essential concomitant resulting from protein adsorption and crystalline surface and stone formation was governed by crystallisation phenomena rather than by organic matrix.

Although recently Cheng et al. [5] observed in "matric stones" some focal calcification of organic matrix by incrustation, the Liesegang layering mechanism of Leduc-Lichtwitz for lamellar structure formation in renal calculi is probably incorrect. In particular, it fails to explain the commonly observed structure of alternating layers of calcium apatite and non-apatite (e.g. struvite, $MgNH_4PO_4 \cdot 6H_2O$) crystals [6].

An alternating crystallisation mechanism is proposed here to explain the lamellar structure: Under high supersaturation, calcium apatite forms only very fine crystallites spontaneously [7]. When co-precipitating with an organic matrix, these fine crystallites form densely packed fibres and membranous layers which become the denser layers of the lamellar structure. Assuming slow repletion of Ca^{++} ions, perhaps due to a local stasis, spontaneous mucleation calcium apatite will soon cease. However, the ambient urine can be still very much supersaturated with respect to other crystals. e.g. struvite, because concentrations of other ions, e.g. Mg^{++}, NH_4^+, have not been depleted. The non-apatite crystals are usually larger and polyhedral. Although co-precipitated also with organic matrix, they are more dispersed, forming layers less dense than the calcium apatite layers. Again, the degree of supersaturation with respect to these non-apatite crystals will be greatly reduced after their precipitations. In the meantime, the Ca^{++} ions would have been repleted to a level high enough for a new round of nucleation of calcium apatite crystallites. In turn, another round of non-apatite crystallisation will follow. The concentration of any common ion, e.g. PO_4^{3-} in apatite/struvite stones, is assumed to be always sufficiently high. This alternating crystallisation mechanism can probably explain the lamellar structure formation in most renal stones.

1 Colloid Chem 2:59–79, 1928
2 Ibid 5:1063–1082, 1944
3 Ibid 2:803–844, 1928
4 J. Urol 86:355–363, 1961
5 Scan Electron Microsc, in press
6 Scan Electron Microsc III, 163–168, 1981
7 Calc Tissue Int 35:596–601, 1983

VIII. Stone Morphology

230 A New Method for Quantitative Wet Chemical Analysis of Urinary Calculi and the Principles for Calculation of Results and Mass Recovery by an Algorithm

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We describe a simple method for quantitative chemical analysis of urinary calculi requiring no specialized equipment. Pulverized calculi are dried over silica gel at room temperature and dissolved in concentrated nitric acid, which was found to be the only effective agent for complete dissolution of calculi. Calcium, magnesium, ammonium, and phosphate are then determined by conventional methods, whereas oxalate, uric acid, cystine, and protein are determined by specifically developed methods. Oxalate is then determined by a method based on the quenching action of oxalate on the fluorescence of a zirconium-flavonol complex. Uric acid, when treated with nitric acid, is stoichiometrically converted to alloxan, which is determined fluorimetrically with 1,2-phenylenediamine. Similarly, cystine is oxidized by nitric acid to sulfate, which is determined turbidimetrically as barium sulfate. Protein is determined spectrophotometrically by the xanthoprotein reaction. The total mass recovery of authentic calculi was 92.2 ± 6.7 (SD) per cent. The method permits analysis of calculi as small as 1.0 mg and if cystine and protein are omitted calculi, down to 0.5 mg could be analysed. Internal quality control is performed with specially designed control samples.

To facilitate the interpretation for the treating physician, the results are recalculated and expressed as chemical compounds usually occurring in urolithiasis (whewellite, struvite, apatite, brushite, ammonium urate, uric acid, cystine, and protein). Total mass recovery is also calculated and a low recovery makes reanalysis or more specialized analyses (e.g. X-ray diffraction) necessary. For the above calculations we used a microcomputer (ABC 80) and an algorithm, which will be presented.

231 Urinary Calculi in Children – Epidemiological and Mineralogical Aspects

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In central laboratories for urinary stone analysis in the GDR, 2134 urinary calculi were analyzed, which were callcuted from January 1970 to September 1983 from children up to 15 years of age: 1,034 calculi were passed spontaneously; 1,020 were removed by open surgery and 80 transurethrally; 856 stones were from girls and 1,278 from boys. Calcium oxalate calculi were found most frequently; 706 weddellite and 675 whewellite calculi were analyzed; the main component in 239 calculi was struvite and in 156 calculi carbonate apatite; uric acid/uric acid dihydrate was only found in 53 concrements; cystine was the main component in 14. Detailed representation of frequency peaks, related to different kinds of calculi dependent on age, sex and kind of calculi removal was performed. Furthermore, calculi were divided according to localization in the urinary tract. Results were compared with a great complete urinary calculi statistical analysis.

Mineralogical texture was examined in 100 calcium oxalate calculi from children. The texture of calculi was characterized by four basic texture types, which we have classified earlier. For texture examination by polarization microscopy grain preparations and thin sections from calculi were used. The frequency that this basic type of texture occurs in calculi from children was compared with the texture distribution of 1,000 calcium oxalate calculi from a representative population.

232 Clinico Chemical Study of Urinary Stones in Jeddah. I. Uric Acid and Urate Stones

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One hundred urinary stones were subjected to quantitative wet chemical analysis, atomic absorption (AA), nuclear magnetic resonance (NMR), infrared and ultraviolet visible spectroscopy techniques for the determination of uric acid and urate percentage. The nature of the urate salts present was also characterized. The above findings were correlated with the clinical and biochemical data in order to understand better the nature of the disease.

233 Composition and Structure of Infected Stones

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We herein deal with the results from a study of the composition and structure of 18 infected stones, which were studied by polarized microscopy, scanning electron microscopy, and energy dispersive analysis of X-ray (EDAX).

Fourteen of the 18 stones in this study were bladder stones. All stones we examined contained magnisium ammonium phosphate in variable quantity. Sixteen stones contained ammonium urate (in 10 of 16, ammonium urate was the main composition). Fourteen stones contained apatite. The shapes of the above-mentioned three compositions are described.

Stones of this group can be divided into two types. One is formed from infection and the other is accompanied by infection after stone formation. The compositions of the former are mainly magnesium ammonium phosphate and ammonium urate, but those of the latter are more complicated: the nucleus usually contains whewellite or uric acid, although magnesium ammonium phosphate and ammonium urate occur less frequently. The relationship between magnesium ammonium phosphate and ammonium urate is rather close. All but two crystal structure stones are of oolite structure without radial striation.

In general, the formation of magnesium ammonium phosphate and calcium phosphate in infected stone is due to the large amount of ammonia which results from the hydrolysis of urea by urease that is produced by bacteria in urine and alkalized urine. In our study we discovered that ammonium urate was one of the main compositions of infected stones and its formation was also related to the action of urease. Therefore, competitive inhibition of urease has a certain effect on the prevention and treatment of infected stones.

234 Stone Analysis – in the Doctor's Office or in a Specialized Laboratory?

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Although it is an accepted that every urinary stone has to be analyzed if an effective urinary stone prophylaxis is to be prescribed, there are objective and subjective reasons which condition different opinions about the method of analysis. X-ray diffraction and IR spectroscopy are rather expensive, need mailing and the results last longer, but they are more accurate than the cheaper and quicker chemical analysis. A total of 100 urinary calculi, either spontaneously passed or obtained by surgery or loop extraction, were analyzed by chemical analysis (Temmler stone analyzing set), X-ray diffraction (Prof. Gebhardt, Bonn, FRG) and IR spectroscopy (Urinary Stone Laboratory Borstel, FRG). The results were compared: 1. There are differences that do not depend on the method. 2. The limitations of the chemical method are clearly shown when its results are compared with those of the other two methods. 3. Only with regard to curtailing expense does the chemical analysis have a place in the doctor's office. 4. The chemical and other physico-chemical methods compare well, if one disregards differentiation of the hydrate forms of calcium oxalate and urate, the rarely occurring phosphates, and quantification of the components in mixed stones.

Therefore, for practical purposes it follwos: 1. The first urinary stone of each patient should be chemically analyzed in the doctor's office. 2. Recurrent stones should be examined chemically, only if there is enough material to perform another analysis in the event of controversial chemical results. 3. That means that the chemical method gives satisfactory results in about 50% of all calculi, while other methods are necessary in mixed stones, recurrent stones, if exact analysis is required, if the stones result from metabolic disorders, or in children calculi.

235 Results of the Combined Phase and Texture Analyses of 1,028 Urinary Concrements

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In the last four years, 1,028 urinary concrements have been analyzed using scanning electron microscopy (SEM) with attached energy dispersive X-ray microanalysis (EDX) for texture studies and subsequent X-ray diffraction for semiquantitative phase analysis. Of these stones, 437 (42.5%) came from patients treated in our clinic and 591 (57.5%) were sent in by other hospitals or urologists in the surroundings of Münster; 570 (55.4%) stones were removed by operative methods (surgery, extraction, or lithotrypsy), while the other 458 (44.6%) were passed spontaneously. The ratio of males to females was 1.8:1.

There were 717 (69.7%) Ca stones (mixed Ca-oxalate/phosphate stones), 163 (15.9%) infection-induced stones (containing struvite or ammonium hydrogen urate), 113 (11.0%) uric-acid-containing stones and 17 (1.7%) cystine-containing concrements. Eighteen calculi could not be assigned to one of the main classes because they very seldom contained compounds or had a more complex composition. Nearly all of the concrements could be further subdivided into one of the textural groups or subgroups [1]. The high content of Ca-phosphate-containing oxalate-rich stones (more than 70% [2]) and the abundant appearance of this phase within or near the central core could again be confirmed. Further results and statistical evaluations will be presented concerning age distribution, sex ratios, or stone localization compared to specific stone compositions.

1 Leusmann DB (1983) Texture analysis of urinary concrements By SEM. 2nd International Urinary Stone Conference, 6–9 February 1983 at Singapore (in press)
2 Leusmann DB (1981) Erste zusammenfassende Ergebnisse der kombinierten Phasen- und Gefügeanalyse von Harnsteinen mittels Röntgenbeugung und Rasterelektronenmikroskopie. Fortschr Urol Nephrol 17:275–305

236 Scanning Electron Microscopy Studies on Infection-Induced Urinary Concrements

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The aim of this study was to compare bacteriological findings of urine with cultivations of bacteria from stone fragments and optic realization of the germs by scanning electron microscopy. Likewise, the cause of occasional appearances, commonly interpreted as bacterial prints, was also investigated.

Thus, since April 1983, during all operations on presumed infection-induced stones, one fragment of the stone was separated for bacterial cultivation under sterile conditions, and another part was put into alcohol for SEM studies after preparation by the critical-

point-drying method. The main part of the stone was crystallographically analyzed by X-ray diffraction.

Excellent visualization of the germs was possible in only a few cases, which contrasts with the urine and cultivation results. The depiction of *Proteus mirabilis* was especially difficult, whereas *Escherichia coli* and *Candida albicans*, for example, could easily be prepared. Only in concrements containing *E. coli* could the described prints be detected, and in cases where *E. coli* itself was found, these germs were mineralized by a more or less amorphous Ca-phosphate, obviously a precurser of apatite. Further results are discussed and compared with the findings of one concrement found in a human colon colonized by *E. coli*.

237 Analysis of Urinary Calculi by Inductively Coupled Plasma-Atomic Emission Spectroscopy: New Insight into Stone Structure

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Several advantages associated with inductively coupled plasma-atomic emission spectroscopy (ICP–AES) prompted us to embark on a study to investigate, develop and apply this relatively new technique in the routine analysis of urinary calculi, in the hope of identifying compositional features that might be missed by other currently used methods in this field. Beneficial aspects of ICP–AES include, amongst others, freedom from interelement interference, linear calibration graphs over several orders of magnitude, multi-element capability and speed. Urinary calculi from the Cape Town area were first subjected to X-ray powder diffraction analysis using both film and goniometer procedures so as to qualitatively detect the crystalline phases present. Microchemical analysis for C, H and N in each stone was also performed. Thereafter, a routine procedure for the preparation of solutions suitable for ICP–AES analyses was developed. Crushed stone fragments were pre-digested in Erlenmeyer flasks using concentrated nitric acid and the remaining organic matter was oxidized using a refluxing mixture of conc. nitric and perchloric acids. To overcome systematic errors in the analysis results the acid contents of the samples and reference standards were matched prior to analysis for calcium, magnesium and phosphorus.

Of the 36 stones analysed thus far, 21 belong to the struvite/apatite (STR/APA) group, 11 to the uric acid/calcium oxalate monohydrate (UA/COM) group and 4 to the calcium oxalate (COM/COD) group.

Calcium was detected in all stones. In the STR/APA group the amount of APA varied between 2 and 56 wt-%. A third component, calcium oxalate, was identified in 5 stones from this group. The relative amounts of each phase in the two-component stones were independently calculated from the Ca and Mg figures and yielded values which agreed within 2 to 3%. It was also possible to calculate (A. Hodgkinson et al., Proc Renal Stone Res Symp, 1968, p 113) the amount of oragnic matrix present by assuming that excess N (i.e. N unaccounted for after stoichiometric calculations involving its presence in STR) was associated with such deposits. This varied but never exceeded 6% in 13 of the calculi. In the remaining stones of this group, the excess N was exceptionally high, indicating either an abnormally high matrix content or, more likely, the presence of an additional, undetected organic phase (e.g. ammonium urate).

In the UA/COM group, UA always occurred as the major component. Uric acid dihydrate (UAD) was also detected in 3 stones from this group. With regard to the 4 COM/COD stones, small amounts of P, corresponding to APA concentrations between 1 and 12% were detected.

Our results illustrate the value of using highly sensitive instrumentation for the detection of very small constituent concentrations which would otherwise go undetected by routine analytical procedures. The existence of the so-called pure stone is again brought into doubt while conversely, the repeated association of STR and APA, of UA and COM and of oxalate and APA, in this and other studies, tends to lend support to the hypothesis that stone formation is governed by a heterogeneous nucleation mechanism. Our results also provide further evidence in support of a COM/UA epitaxial relationship as well as a role, possibly as a "cementing" agent, for APA. Additional studies involving trace element determination (e.g. Si, Al, Fe, Zn) are currently being investigated by us. The aim now is to develop a method whereby both major and trace elements can be quantitized simultaneously, thereby reducing sample consumption.

238 Struvite Stone Analysis by IR-Spectrophotometry in Adults and Children

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We analyzed 200 stones in children by microdissection and IR spectrophotometry: 50% contained struvite (MAP). We also analyzed 2,000 stones in adults, with the same protocol; only 16.6% contained struvite.

Of the children with struvite lithiasis, 60% were under 5, 31% were between 6 and 13; 75% (of the total of 200) were boys; in adults, 75% were females, of whom 70% were between 25 and 55 years of age.

MAP stones are always mixed, mostly with carbonate apatite (CA) and ammonium urate (Am ur); see below. Am ur also occurs more frequently in stones in children (46% than in adults (9.7%).

The distribution of the constituents and their main associations are:

	Children (200)		
	Pure	Mixed	Total
Ca ox	6.7	54.3	61
Ca ox–ap	0	20	20
Ca ph+	0.6	81	81.6
MAP	0.6	49.5	50.1
MAP–CA	0	9.5	9.5
MAP–Ca ox–CA	0	4.5	4.5
MAP–Ca ox–CA–Am ur	0	12.8	12.8
MAP–CA–Am ur	0	19.6	19.6
Am ur	0	46	46
Na ur	0	0	0
Ur ac	0	7	7
Cy	1.7	0	1.7
Miscellaneous			11
	9.6%	90.4%	

The struvite stones are often of the staghorn type and in this form we find struvite in 62% in adults and in 80% in children. The nucleus was isolated by microdissection in 85% of the staghorn stones; it contained MAP in 48% of the adults and 60% of the children, proving that MAP and other ammonium ions were present at the inception of the nucleation.

Urine must be supersaturated for crystallization to occur and, with respect to struvite, increased levels of both alkainity and ammonia must be present. This only occurs in the presence of urease-producing bacteria that split urea and increase urinary levels of ammonia, ammonium hydroxyde, bicarbonate, carbonate and alkalinity. Thus the normal negative correlation between urinary pH and ammonium concentration is reversed. Urinary infection by

	Adults (2,000)		
	Total	Mixed	Pure
Ca ox	82.7	63.2	19.5
Ca ox–ap	41.9	41.9	0
Ca ph+	75.1	74.7	0.4
MAP	16.6	16.6	0
MAP–CA	2.9	2.9	0
MAP–Ca ox–CA	6.1	6.1	0
MAP–Ca ox–CA–Am ur	2.5	2.5	0
MAP–CA–Am ur	2.5	2.5	0
Am ur	9.7	9.7	0
Na ur	0.5	0.5	0
Ur ac	16.6	11.2	5.4
Cy	1.7	0	1.7
Miscellaneous	6		
		73%	27%

these germs is found in 60–75% of the cases, children and adults, and *Proteus* is isolated in 50–60%.

In conclusion, struvite stones occur more frequently in boys under 5 and in adult females, often have a staghorn aspect, few clinical symptoms and are linked with urinary infection in 75% of the cases (*Proteus* ++). There are few relapses after complete extraction and urinary disinfection.

239 Hardness Testing of Urinary Calculi

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With the introduction of electrohydraulic lithotripsy and extracorporeal shock-wave lithotripsy, a description of physical properties of urinary calculi has become important. The success of each of these techniques depends on both the compressive and tensile strength of a calculus.

We tested several types of urinary calculi to determine if differences in hardness existed. Hardness testing was performed with a Tukon tester with the degree of hardness expressed in Knoop values. Calcium oxalate and brushite were the hardest calculi tested (Knoop values 80–117). Cystine calculi were found to be very soft (Knoop values 22–29). Uric acid and struvite calculi were of intermediate hardness (Knoop values 21–50). In order to compare these numbers to published values of compressive strength, three struvite calculi were tested for compressive strength with an Instrom device. Compressive strength ranged from 25–76 kg/cm^2.

Urinary calculi differ in their degree of hardness. This may help to explain why lithotripsy procedures are more effective with certain types of calculi.

240 Matrix-Mineral Configuration in Whewellite Kidney Stones: Microchemical Analysis

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It has been suspected for a while that the two major components of whewellite kidney stones, the matrix and the mineral phases, exhibit extensive interaction. The matrix of protein has been shwon to be continuous [1], and the mineral (calcium oxalate monohydrate – COM) to occur in discrete packets of platelets [2]. However, it has not been established whether the two phases interpenetrated down to the level of individual crystal platelets. High-resolution transmission electron microscopy (TEM) images strongly indicate that this is so, but accurate techniques of phase identification have so far been very difficult to apply to whewellite at the ultrastructural levels required. The aim of this study was to determine the spatial distribution of phases in whewellite kidney stones at such ultrafine levels.

We have successfully used X-ray photoelectron spectroscopy (XPS) to confirm the visual images of TEM in this regard. In this method, energy-analyzing electrons ejected from the specimen surface by a beam of X-rays provides an accurate means of identifying the atomic species present and their chemical (bond) environment. Their relative abundance is computed from the XPS spectra, using known photoionization cross sections [3]. The technique easily discriminates between the phases of whewellite kidney stones; the way it was employed here ensures that the spatial distribution of those phases is revealed if TEM information is correct.

XPS runs were made on broken or crushed samples of both air-dried and critical-point-dried whewellite kidney stones. The results showed dramatic increases in apparent matrix proportion over what is to be expected from bulk composition. The fracture process clearly exposes the matrix preferentially. This indicates that the matrix provides a continuous fracture path and confirms the TEM result: that ultrafine layers of matrix sandwich the crystal platelets of the mineral phase.

1 Vermeulen CW, Lyons ES (1968) Am J Med 45:648
2 Ogbuji LU, Finlayson B (1981) Invest Urol 19:182
3 Scofield JJ (1976) J Electr Spectrosc 8:457

IX. Theoretical Models Related to Urolithiasis

241 Calculations of Complex Chemical Equilibria in Urine: Estimate of Stone-Formation Risks and Derivation of Prophylactic Measures

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In a computer simulation study, normalized concentrations of complex chemically acting constituents of human urine were varied to follow their effect on the relative activity products of calcium oxalate, calcium phosphates, and uric acid.

For this purpose, an improved BASIC computer program was applied which is described elsewhere (Achilles, this symposium). The different instructive diagrams obtained by the calculations may be used to estimate the influence of single and simultaneous variations of urinary constituents on the "thermodynamic" risk of stone formation.

On the other hand, possible therapeutic (prophylactic) measures can be derived from the curves or from special calculations. The results show, e.g., in the case of calcium oxalate formation, that dilution of urine (simulating fluid intake) and decrease of oxalate are most effective in reducing the corresponding activity product (AP). By comparable relative changes of calcium and oxalate in opposite directions, AP may be increased (Ox↑, Ca↓) or decreased (Ca↑, Ox↓).

With respect to these results, computer simulations of complex chemical equilibria in urine may be a useful tool in evaluating stone formation risk factors as well as in the management of urolithiasis.

242 A Computer Program for the Calculation of Activity Products and Solubilities of Stone-Forming Constituents in Urine

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A BASIC computer program is described which allows the calculation of equilibrium concentrations and activity products of urinary constituents from analytical data (pH, total concentrations) and thermodynamic equilibrium constants taken from literature. The algorithm of Perrin and Sayce was used to iterate analytical and calculated total concentrations. Activity coefficients were calculated from ionic strength by the Davies equation, using an additional iterative method.

More than 30 equilibrium reactions between ligands, protons and metal ions were taken into account, reflecting the main complex chemical interactions in human urine. Solubilities of calcium oxalate, calcium phosphates, and uric acid at equilibrium conditions can be estimated by another method of mathematical approximation. The program has been tested for different systems (artifical solutions as well as urines). The fomula of Achilles and Cumme for a simple estimate of relative activity products of calcium oxalate has been improved and extended.

243 A Comparison of Tiselius Risk Index (RI) and Relative Saturation (RS) in Calcium Oxalate (CaOx) Stone Formers and Normals

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In 1982, Tiselius reported a method for routine clinical evaluation of patients with recurrent calcium oxalate stone disease. By using a computerized calculation program, adapted from Finlayson and Robertson, a simplified estimate of the ion activity product for calcium oxalate in the urine was derived:

$$*RI = \frac{(Ca/Cr)^{0.71}}{(Mg/Cr)^{0.14}} \times \frac{(Ox/Cr)^{1.0}}{(Cit/Cr)^{0.1}}$$

The derived equation of risk index (RI) was formulated to obtain a comprehensive mathematical expression of the principal biochemical substances shown to be of importance for calcium oxalate crystallization. This equation is based on the 24-h excretion values of calcium (Ca), oxalate (Ox), magnesium (Mg), citrate (Cit), and creatinine (Cr), using literature values accepted as normal. Although the Tiselius program showed volume to be an important risk factor, a volume factor was not included in this equation because he felt patients collecting 24-h urine samples tended to drink more than they usually do, so that concentration values obtained from 24-h urine samples would be misleading. Creatinine was included to compensate for errors in urine collection. The exponents indicated the weighted value obtained when that substance was the only variable changed in the mathematical program. Using different extreme combinations of high and low values for oxalate, calcium, magnesium, and citrate, he found this activity product index to correlate well (0.997) with the calculated activity product of CaOx when urine volume is kept at 1,500 ml/day and creatinine kept at 0.015 mol/day.

We studied 20 CaOx stone-forming patients, comparing their CaOx Tiselius risk index with their relative saturation on high and low dairy diets. Patients were grouped according to methods outlined by Pak, Type I ($n = 4$), having a calcium excretion greater than 200 mg/24-h urine on restricted dietary calcium intake (~400 mg/day), increasing to > 200 mg/24-h urine on high calcium (~1200 mg/day intake. Type II ($n = 7$) less than 200 mg/24-h urine on restricted calcium and increasing to greater than 200 mg/24-h urine on high calcium intake. Renal leak ($n = 4$) greater than 200 mg/24-h urine on restricted calcium intake with no change on high calcium intake, and normocal ciuric patients who had less than 200 mg/24-h urine on restricted and high (Ca) intake. Oxalate excretion, as observed by others, varied inversely with dietary calcium intake in each group, being significant ($P < 0.01$, $n = 20$) when groups were combined. We also measured RS and RI in 24-h urine of fasting normal white males on free diet and in fasting normal males during water-induced diuresis.

In no case were we able to demonstrate the strong *linear* relationship between RS and RI predicted by Tiselius ($r = 0.997$). Weak, but statistically significant correlations were observed between RS (y) and RI (x) in 24-h urines of patients on low dairy (power, $y = 0.05\ x^{0.79}$, $r = 0.62$, $P < 0.01$, $n = 20$) and high dairy intake (parabolic, $y = 2.76 + 0.025-0.0004$, $r = 0.54$, $P < 0.05$, $n = 20$), with normals (power, $y = 0.04\ x^{0.87}$, $r = 0.73$, $P < 0.05$, $n = 9$) and in urine from fasting normals during diuresis (power, $y = 0.0026\ x^{1.1}$, $r = 0.63$, $P < 0.01$, $n = 20$).

We feel that the reason for the discrepancy between our experimentally observed nonlinear relationships between RS and RI and the linear one calculated by Tiselius is that the latter calculation depends upon a constant ratio between urine volume and creatinine, a condition which does not pertain to man.

244 A Model for Predicting Stone Recurrence

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It would be of great value to know which stone-forming subjects are at high risk for recurrence and which are likely to remain in remission without treatment. We have studied the predictive power of a number of demographic, clinical and laboratory variables, using several statistical programs (SAS), including 1) logistic regression, 2) stepwise discriminating analysis and 3) discriminant classification analysis.

Our patient population consisted of 168 recurrent and active calcium oxalate stone-forming subjects. All had been asked to participate in our double-blind prospective studies of various stone prophylactic regimens. 32 had refused to take no treatment at all. 134 accepted the study design but were placed on ineffective medication (58 took a placebo, 41 took 650 mg of magnesium hydroxide daily and 35 took 1,300 mg of magnesium hydroxide daily). Recurrence rates were comparable in each group and for the purpose of this analysis, all subjects were pooled.

The recurrence rate in this group was 0.206 stones per patient per year. After two years of follow-up, 40% of subjects have formed new stones. Using the variables from this data base, we have developed models which give optimum separation of the recurrences versus the remissions.

Late Abstract (Topic IV: Metabolism)

245 Activity of Adenine Phosphoribosyltransferase (APRT) in Patients with Renal Failure and Urolithiasis

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In order to investigate the frequency of APRT deficiency in renal disease, we measured APRT activity in haemolysate in three groups of patients. These groups were: 139 patients with renal failure on dialysis (no transfusions), 118 patients with nephrolithiasis, and a control group of 524 unselected patients of the Medical Policlinic. APRT activity was measured microradiochemically using the method of Kelley et al. (1967).

In all groups APRT activity was distributed normally (Chi-Square Test). The enzyme activity ($\bar{x} \pm SD$) was 28.9 ± 5.7 nmol/mg protein/h in the control group (Banholzer et al., 1982), 44.6 ± 17.1 nmol/mg protein/h for dialysis patients, and 20.8 ± 6.1 nmol/mg protein/h in the urolithiasis group. Four patients of the control group, no dialysis patient, and one patient with urolithiasis showed an activity below the 2 SD limit particular to their group. One way analysis of variance revealed that the differences between the groups were highly significant ($p < 0.001$).

Within the urolithiasis group no significant difference was found between not-identified stones ($n = 39$), uric acid stones ($n = 16$), cystine stones ($n = 3$), and calcium stones ($n = 60$). The different metabolic subgroups of calciumurolithiasis (Scholz et al. 1980) also did not exhibit any significant differences.

The results show that complete APRT deficiency is not common even in patients with renal failure or urolithiasis. Presumably due to their younger erythrocyte population (Becher et al. 1980) dialysis patients exhibit a considerably raised APRT activity and thus need their own standard. The lower activity in stone patients cannot be traced to any (homo- or heterozygous) individuals nor to specific stone types. It is suggested that low APRT activity might be an unspecific risk factor for urolithiasis by allowing 2,8-dihydroxy-adenine to be produced under certain dietary conditions.

Kelley et al (1967) Proc Natl Acad Sci 57:1735
Banholzer et al (1982) J Clin Chem Clin Biochem 20:345
Scholz et al (1980) Urologe A 19:202
Becher et al (1980) Klin Wochenschr 58:1243