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Abstracts of the 6th International Symposium on Urolithiasis and Related Clinical Research

Vancouver, Canada, July 24–28, 1988

Conference Co-Chairman: R. A. L. Sutton, D. M., Urolithiasis Symposium, Department of Medicine, 3rd Floor – 910 West 10th Avenue, Vancouver, B.C., Canada, V5Z 1M9

These international symposia are held every 4 years with the objective of bringing together renal and metabolic physicians, physiologists, biochemists, urological surgeons and other physicians and scientists interested in kidney stone disease. Authors of more than 300 abstracts were invited to submit them under one of six categories:

A. Metabolism and Biochemistry

B. Physiology

C. Physical Chemistry – Inhibitors

D. Medical Management

E. Urological and Radiological Management

F. Case Reports

In this volume the abstracts are similarly divided into these six categories, with prefix letters A–F as indicated above. The final programme of the meeting will include eight major symposia and four oral free communicative sessions (the latter selected from these abstracts). The remaining abstracts will be presented either in general poster sessions, or in smaller theme poster sessions which will include discussion of the posters led by a chairman.

As would be expected, in view of recent developments in the prevention and treatment of urolithiasis, there will be major emphasis on the underlying mechanisms of stone formation, the role of inhibitors in pathogenesis and treatment, and the latest developments in urological management including indications, complications and new technological advances in extracorporeal shock wave lithotripsy.

We anticipate an exciting and informative Urolithiasis Symposium in Vancouver in July 1988.

J. H. Dirks, M.D.
Chairman

R. A. L. Sutton, D.M.
Co-Chairman

A. Metabolism and Biochemistry

A1. Microdetermination of Urinary Constituents by Vertical-Lightpath Photometry in Microplates

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Automated photometric measurements in microtiter plates have been used for years in our laboratory in order to determine kinetic parameters of crystal growth in gels. We have now applied vertical-lightpath photometry to quantify various soluble constituents of urine and serum that are relevant with respect to diagnosis and basic research on urinary stone formation.

Method: Reagents and samples were handled by a computer-controlled pipetting station (Tecan 505, Zinsser Analytik, FRG) with IBM PC corresponding to programs specifically established for the methods under consideration (microliter range). Measurements were carried out in 96-well microtiter plates using the microreader MR 600 (Dynatech) with IBM PC-XT. Measuring wavelength: 340–700 nm (filters). Programs for control of the photometer, acquisition, and evaluation of measuring data were written in BASIC.

Results: The following enzymatic assays (Boehringer, Mannheim, FRG) were adapted to the special conditions of microdetermination: (1) citrate, (2) isocitrate, (3) uric acid, (4) creatinine, and (5) oxalate. In general, measuring volumes were about 300 μl per well for all tests. Mean nonprecision within series was 2%–3% in the normal range. The agreement of results obtained from corresponding micro- and macrotests was good ($r > 0.980$). About 85% of reagents could be used again and manual work was reduced drastically.

Conclusions: The analytical principle described here is characterized by efficiency, flexibility, and economy and may be recommended to laboratories concerned with the diagnosis of urinary stone formation.

A2. Chemical Composition of Renal Stones in Mosul

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One hundred forty-six renal stones were analyzed to identify the chemical structure of the stones. The wet chemical method was used. More than 95% of the stones were of the mixed type, the most common of which was mixed oxalate. Ammonium urate was more common in renal stones than uric acid. Only 4.1% of the stones were composed of pure oxalate; pure phosphate stones were uncommon; apatite (calcium phosphate) stones were by far the most common. Individual types of stones affect certain age groups of patients more than others. In general, the disease affects males more than females. It is concluded that the chemical nature of the stone could be of value in the management of the disease.

A3. Tartaric Acid Ingestion and Urinary Stone Inhibition in Rats

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Studies were undertaken in rats to ascertain whether or not feeding of tartaric acid (L+) inhibits urinary calculi formation. Forty-eight male weanling rats were equally divided into two groups and fed ad libitum a potentially calculogenic diet. This diet provided 10% protein (casein), 80% starch, adequate vitamins and minerals, and contained a high concentration of calcium (9 g/kg). One group served as the control. The rats in the experimental group were fed 50 mg tartaric acid (L+) per day, along with the above diet. After 16 weeks, 24-h urine was collected for two consecutive days from all the rats. Fresh urine samples were examined for crystalluria and pH. Urinary creatinine, phosphorus, calcium, magnesium, citrate, oxalic acid, and tartarate were estimated. In addition, the inhibitory activity of urine towards calcium oxalate crystal growth was measured in an in vitro system. Subsequently, the rats were killed and examined for presence of urinary calculi. All calculi were weighted and analyzed. Urine of the control group of rats contained very dense, large crystals of calcium oxalate (> 20 μm). In contrast, these crystals were very few and much smaller (between 2 and 5 μm) in the urine of rats fed with tartaric acid. Tartaric acid resulted in a significant fall in urinary oxalate and a rise in urinary phosphorus, citrate, and tartarate. The in vitro activity of urine towards calcium oxalate crystal growth was significantly enhanced by tartaric acid feeding. A high incidence (90%) of calcium-oxalate urinary was observed in the control group. Tartaric acid resulted in a drastic reduction in this incidence (40%). Besides, the calculi developed in the experimental group were strikingly smaller (8.0 ± SE 3.8 mg) than those in control rats (76.3 ± SE 2.23 mg). In the tartaric group a significant inverse correlation between the weight of the calculi and urinary phosphorus ($r = 0.93$) was observed. This study, the first of its kind in animals, demonstrates unequivocally, that tartaric acid (L+) helps to reduce both the incidence and size of urinary calculi.

A4. The Effect of Feeding Tamarind to Men on the Lithogenic Properties of Urine

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An investigation was conducted on the effect of feeding tamarind to normal men, i.e., the important lithogenic properties of urine. Tamarind is a fruit rich in tartaric acid. The metabolic study was well controlled and used four normal young adult men on normal vegetarian diets. During the first 7 days of this study (pretamarind period), moderate calcium oxalate crystalluria was induced in these subjects by the inclusion of common dietary ingredients rich in oxalate in the diets. Subsequently, aqueous extract of tamarind (10 g/day) was included in the diets for another week (posttamarind period). Intake of water was equalized for all the subjects and kept constant during the entire study. Throughout the experimental period, morning samples of urine were collected every day. Crystalluria in these samples was immediately examined under a high-power microscope. The sizes of these crystals were determined with the help of millipore filters of different pore sizes. At the end of each period, urine was collected over toluene for 2 consecutive days. Volume and pH of urine were recorded. Creatinine, oxalate, calcium, magnesium, phosphorus, citrate, and tartaric acid were estimated in the urine. The capacity of urine to inhibit the growth of calcium oxalate crystals was tested in an in vitro system. Urine volume was not influenced by tamarind feeding. Inclusion of tamarind extract very promptly brought about the following striking changes in the pattern of calcium-oxalate crystalluria: (1) complete disappearance of massive aggregates of calcium oxalate crystals (within 3 days); (2) a fall in the density of crystalluria (within 5 days); (3) a reduction in crystal size from 15–20 μm to less than 5 μm (within 5 days); (4) the complete absence of crystalluria by the end of 7 days. The post-tamarind period was also associated with a significant rise in urinary citrate, phosphorus, and tartaric acid, and a fall in oxalate. Feeding of tamarind also enhanced the inhibitory activity of urine toward calcium oxalate crystal growth. These changes in urine properties were similar than those observed by us in rats fed tartaric acid. This human study offers very convincing evidence of the probable efficacy of tartaric acid as an inhibitor of calcium-oxalate stone formation – even in man. Our results thus confirm the suggestions of earlier investigators.

A5. The Calciuretic Effect of Methionine in Humans

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Sulphur-containing amino acids are thought to be involved in protein-induced hypercalciuria. The effect of a high methionine diet on urinary calcium was investigated in six male stone-formers, six normal males and six post-menopausal females. These subjects were studied on a basal diet for 1 week. During the subsequent week, the same diet was supplemented with 16.8 mmol of DL-methionine. The analysis of 24-h urine samples collected during the last 3 days of each study period showed that methionine increased urinary calcium significantly. Although the mechanism by which methionine increased urinary calcium is not clearly established, a decrease in urinary pH and in the maximal tubular reabsorption of calcium (TmCa) suggest that an increase in urinary calcium might be due to reduced tubular reabsorption of calcium.

A6. Normal Values of Lithogenic and Inhibitory Substances in the Urine of Healthy Children

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So far there have been no sufficiently reliable reports on normal values for urolithiasis parameters in childhood. In 473 healthy children (320 boys and 153 girls) up to 13 years old, the usual lithogenic inhibitory substances were determined in 24-h collected urine. Tables of normal values for concentration (mmol/l), excretion (mmol/24 h), excretion (mmol/24 h/m^2 body surface), excretion (mmol/24 h/kg body weight), and relative supersaturation for calcium oxalate were drawn up. The results were mutually compared separately for various age groups and sex and in relation to the normal values in adults. The following analytical parameters were investigated: urine volume, pH value, sodium, potassium, calcium, magnesium, phosphorus, chloride, sulfate, uric acid, oxalic acid, citric acid, and creatinine. As an example, the results on calcium excretion in the 24-h urine are specified the *calcium excretion* in mmol/24 h/m^2 body surface increases continuously from 1.52 ± 0.46 in male and 0.817 ± 0.46 in female infants to 2.87 ± 0.50 in the male and 1.35 ± 0.29 in the female children of 4–6 years of age. Adult values are only reached from the 13th year of life (boys 2.61 ± 0.68; girls 1.136 ± 0.41). On average, the calcium excretion in boys is about one-third higher than in girls in each age group. This difference between sexes is very pronounced, the values for excretion in boys being four times higher than in girls, e.g., in the group of 7–9 year olds. The difference is significant. The smallest difference between boys and girls is present in the group of babies (up to the end of the first year of life), with the boys having excretion values rather less than twice as high as the girls. The *calcium/creatinine quotient* falls to an equal extent with increasing age in both sexes. Calculation of the relative supersaturation of calcium oxalate revealed a significantly higher value in boys (6.72 ± 0.89) than in girls (4.46 ± 0.63). This is a possible explanation for the lower incidence of calculus in girls compared to boys reported in the literature. If the age groups are considered singly, the relative supersaturation of calcium oxalate was highest in the group of 7–9 year olds in boys and in the group of 10–12 year olds in girls. Investigations of the other parameters also show that it is necessary to establish different normal urinary values in children than in adults and to differentiate between sexes.

A7. Decreased Erythrocyte Glycosaminoglycan Content in Idiopathic Calcium-Oxalate Nephrolithiasis

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In red cells (RBC) of idiopathic stone-formers (ISF) we have recently described an abnormal oxalate self-exchange, which seems to depend on the altered status of membrane protein phosphorylation. Since glycosaminoglycans (GAGs) are known as RBC proteinkinase inhibitors, a fall in their concentration might be postulated as responsible of the increased phosphorylation. The above consideration led us to investigate RBC GAG content (according to Whiteman), together with membrane protein phosphorylation rate (by endogenous phosphorylation in the presence of γ^{32}P ATP) in 14 ISF and in 20 healthy controls. Furthermore, we also investigated four pairs of brothers: one of these brothers was a stone-former with an abnormal RBC oxalate self-exchange and the other brother was stone-free and showed normal RBC self-exchange values. In comparison with controls, we found a lower RBC GAGs content (132.8 ± 24.06 SD μg/mg protein versus 198.4 ± 52.29; $t = 4.37$; $p < 0.001$) and an increased membrane protein phosphorylation rate in ISF (83,461 ± 6,846 SD cpm/mg protein versus 64,265 ± 4,860; $t = 9.6$; $p < 0.001$) with a significant negative correlation between GAG content and protein phosphorylation ($r = 0.71$; $p < 0.01$). Among the brothers an abnormally low GAG content associated with an increase in phosphorylation was found only in stone-forming subjects. The data obtained seem to suggest: (1) a lower than normal RBC GAG content might play a role in the cellular abnormalities present in ISF; (2) the alteration in GAG metabolism is most probably inheritable; (3) if this anomaly is also present in urothelium, it might have a very important impact on calcium-oxalate crystallization.

A8. Stone Type and Urine Composition in the Middle East with Particular Reference to Saudi Arabia

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A modified method for the quantitative analysis of urinary calculi was developed using Fourier Transform infra-red (FTIR) spectrophotometry. Using this technique, over 400 stones were analysed from patients attending the King Faisal Specialist Hospital. The data showed that "pure" calcium oxalate (CaOx) stones accounted for 45% of all calculi analyzed. "Mixed" CaOx/calcium phosphate (CaP) stones constituted a further 27%, uric acid (UA) or urate (U)-containing stones 22%, infection stones 4%, cystine stones 1% and the remainder consisted of either 2,8-dihydroxyadenine, silica or artefacts. The relatively high proportions of "pure" CaOx stones and UA – or U-containing stones, compared with corresponding data from the West, are consistent with the general composition of urine in the population of the region. Studies on the latter showed that hypercalciuria (>8 mmol Ca/day) is relatively rare, mild hyperoxaluria (>0.46 mmol/Ox/day) is extremely common, hyperuricosuria (>4 mmol UA/day) is common, and that urine volume and pH are both lower than in the West and lower than in Western expatriates living and working in Saudi Arabia. The incidence of urinary tract infection in woman is also lower than in the West. Taken together, these observations account for the high incidence of CaOx- and UA- or U-containing stones and the relatively low occurrence of CaP-containing stones and infection stones in the population of Saudi Arabia.

A9. Lower Vertebral Mineral Density and Greater Bone Resorption in Calcium Stone-Formers (SF) with Idiopathic Hypercalciuria (IHC)

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Although primary intestinal hyperabsorption is considered to be the most prevalent mechanism of IHC by PAK, calcium balances have been found negative in two-thirds of the cases, suggesting a loss of bone mineral. To test this hypothesis, vertebral CT densitometry was performed in 50 SF, together with the parameters of the table. The results are given as ΔL3, i.e., the difference between the observed density of L3 (expressed in g/cm^3 of K2HPO4) and the theoretical value derived from the equation normally relating this density to age and sex. The patients were classified as normocalciuria (NC) if their calciuria was <0.1 mmol/kg/day on a free diet, dietary hypercalciuria (DHC) if their UCaV was >0.1 mmol/kg/d on a free diet and <0.07 mmol/kg/d on 400 mg of calcium and IHC if their UCaV was >0.07 mmol/kg/d on 400 mg of calcium. Natriuresis, body weight and age were comparable in the three groups as were their plasma concentrations of calcium, phosphate, PTH 1-84, 250HD and gla-protein, which were normal. Vertebral density was lower in IHC than in NC or DHC and below normal in NC. Total hydroxyprolinuria was significantly higher on a 0.4 g Ca diet and fasting in IHC than in NC and DHC. A negative correlation was found in the NC group between ΔL3 and fasting Ca/Cr, but not in the other groups.

Conclusions: (1) Compared with NC or DHC, IHC is associated with lower vertebral density and higher hydroxyprolinuria, but not with higher PTH, which suggests that the primary event leading to hypercalciuria is not intestinal hyperabsorption or renal leak but bone hyperresorption; (2) even in NCSF vertebral density is below normal and related to fasting Ca/Cr. This suggests that SFs are in chronic negative calcium balance and that a calcium-restricted diet should not be used as a therapeutical measure for them except for those with DHC.

Ca Diet	Parameters (mean ± SD sex ratio F/M)	Controls	Idiopathic calcium stone-formers		
			NC 6/9	DHC 5/11	IHC 5/14
0.4 g	UCa/UCr mmol/mmol	0.28 ± 0.1	0.28 ± 0.1	0.34 ± 0.1	0.64 ± 0.2^{b2c2}
	TOPH/UCr μmol/mmol		26 ± 6	24 ± 6	31 ± 10^{b1c1}
Fasting	UCa/UCr mmol/mmol	0.17 ± 0.02	0.21 ± 0.12	0.22 ± 0.08	0.47 ± 0.28^{b2c2}
	ΔUCa/UCr post 1 g Ca load	0.39 ± 0.2	0.46 ± 0.26	0.55 ± 0.33	0.49 ± 0.18
	TOHP/UCr μmol/mmol	24 ± 4	26 ± 5	25 ± 6	31 ± 14^{b1c1}
	PCa mmol/l	2.39 ± 0.09			
	PPO4 mmol/l	1.05 ± 0.12	0.97 ± 0.1	0.98 ± 0.2	0.98 ± 0.1
	PPTH 1-84 pg/ml	10–65	35 ± 10	31 ± 14	34 ± 26
	P250HD ng/ml	10–50	10 ± 4	14 ± 6	12 ± 6
	PGLA prot. ng/ml	2.5–9.5	8 ± 3	9 ± 2	9 ± 3
	ΔL3 g/cm^3	> –25	–30 ± 31	–11 ± 29	–41 ± 78^{b1c2}
	Age – years		47 ± 14	43 ± 11	46 ± 1
	Body weight (kg)		73 ± 15	73 ± 10	73 ± 14

Comparison: NC vs DHC: $^{a1} = P < 0.05$; $^{a2} = P < 0.01$; NC vs IHC: $^{b1} = P < 0.05$; $^{b2} = P < 0.01$ and DHC vs IHC: $^{c1} = P < 0.05$; $^{c2} = P < 0.01$

A10. Jejuno Ileal Bypass (JIB) in the Rat – Failure to Produce Enteric Hyperoxaluria or Urolithiasis?

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JIB has been practiced for years in the surgical treatment of morbid obesity in man. However, JIB-induced body-weight reduction is associated with calcium oxalate urolithiasis, and "enteric hyperoxaluria" is considered the underlying cause [1, 2]. Because the frequency of this combination varied greatly among reports, we developed JIB (2 cm behind ligamentum Treitz – 5 cm cephalad of valvula Bauhin) in the male Sprague-Dawley rat (initial body weight 283 ± 5 g); normal lab chow and deionized water were fed for 42 days postoperatively. Urine was collected on 4 preterminal days, and the mean excretion (per 100 g body weight) of stone-forming constituents and small-molecular inhibitors was assessed; in blood, renal tissue, and feces, additional bone variables reflecting the state of mineral metabolism were monitored (to be demonstrated)

Variables	SHAM; $n = 10$	JIB; $n = 13$
Oxalate (mg)	0.15 ± 0.01	0.11 ± 0.01^a
Calcium (mg)	0.27 ± 0.06	1.03 ± 0.15^b
Magnesium (mg)	0.94 ± 0.15	1.39 ± 0.10^a
Citrate (mg)	3.61 ± 0.57	6.15 ± 0.49^a
Pyrophosphate (μg)	1.35 ± 0.18	2.38 ± 0.28^a
pH	7.50 ± 0.1	6.51 ± 0.09^b

$^a P < 0.01$; $^b P < 0.001$ versus Sham (t-test)

Results: Urinary concretions were not present in any of the JIB rats; no signs of secondary hyperparathyroidism were detected (normal serum calcium, PTH, urinary cAMP). Urine composition was markedly changed by JIB (table; $\bar{x}$ ± SEM): pH and oxalate decreased, but calcium, magnesium, citrate, pyrophosphate increased; urate and ammonium were unchanged.

Conclusions: (1) In contrast to humans, JIB in the rat fails to result in hyperoxaluria and urolithiasis; (2) the combination of de-

creased oxalate and increased inhibitors in JIB rats suggests that supersaturation of urine with calcium oxalate is below the formation product of this phase; (3) after JIB, hypercalciuria in the presence of hypooxaluria may reflect dissociation of intestinal transport of these ions or some yet unknown event.

References: 1. Earnest DL (1977) Am J Clin Nutr 30:72 – 2. Scholz D et al. (1982) Klin Wochenschr 60:803

A11. Reliability of a Single 24-h Urine Test for the Detection of Abnormal Stone-Forming Risk Factors

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A comprehensive evaluation of patients with recurrent nephrolithiasis based on multiple urine collections can uncover a specific abnormality in greater than 90% of patients. In order to assess the value of a single urine collection, we compared data from one random 24-h urine versus another random 24-h urine sample from over 1,100 patients evaluated in our stone clinic over the past 10 years. We found that 82.5% of patients who had hypercalciuria (>200 mg/day) in the first urine sample also had this abnormality in the second sample. Similarly, recurrence in the second sample of hypocitraturia (<320 mg/day) was 73.9%, hyperuricosuria (>600 mg/day) 68.9%, hypomagnesuria (<50 mg/day) 32.6%, hyperoxaluria (>44 mg/day) 43.5%, low urine volume (<1,1000 ml/day) 48.7%, and low urine pH (<5.5) 54.3%. We conclude that a single 24-h urine collection will accurately identify patients with hypercalciuria. In addition, one random sample is a relatively good predictor of other important risk factors for recurrent stone formation, including hypocitraturia and hyperuricosuria. For more reliable diagnosis of gouty diathesis, low urine volume, hyperoxaluria and hypomagnesuria, serial urine collections may be required.

A12. The Inhibition of Experimental Nephrocalcinosis with "Parthenolide": An Extract of Feverfew

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Feverfew has long been used in folk medicine as a remedy for arthritis and migraine. The methylene lactone, parthenolide, a constituent of feverfew was shown to be ten times more potent than indomethacin in inhibiting the synthesis of prostaglandin E2 and prostaglandin F2 using the rat fundic strip preparation. Our previous studies have shown that urinary calcium excretion can be reduced and experimental nephrocalcinosis prevented by prostaglandin synthetase inhibitors. The aim of this experiment was to investigate the effect of parthenolide on renal parenchymal calcification and calcium excretion in an experimental animal model.

Method: Nephrocalcinosis was induced in a group of rats (n = 15) by intraperitoneal (i.p.) injection of 10% calcium gluconate for 10 days. Another group of rats (n = 13) were treated with oral parthenolide (2 mg/kg) for 4 days prior to receiving i.p. calcium gluconate and parthenolide was continued during the 10-day course of i.p. calcium gluconate administration. A third group of rats (n = 15) were treated with oral indomethacin. Urinary calcium excretion (24 h) was measured before the treatment and on days 3 7 and 13. The animals were killed and the kidneys studied for localisation of nephrocalcinosis with microradiography and histology and quantitative calcium concentration by energy dispersive analysis of X-rays (EDAX) and wet chemical spectroscopic analysis.

Results: Calcification in the kidneys was markedly reduced on microradiography and histology with parthenolide and indomethacin. Calcium concentration measured by EDAX and chemical analysis was significantly less ($P < 0.005$) than in the rats given i.p. calcium gluconate alone. Urine calcium excretion (24 h) was reduced on day 3 on parthenolide and did not rise above pretreatment levels when calcium gluconate was commenced, unlike the significant increase ($P < 0.001$) in urine calcium excretion, which occurred in rats given calcium gluconate alone. We are investigating the therapeutic potential of this interesting herbal compound in the treatment of hypercalciuria and urolithiasis.

A13. Epidemiological Investigation of Nephrolithiasis Within the Region of Sap Kosovo in Yugoslavia

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The relatively high percentage of nephrolithiasis morbidity leads to the conclusion that certain specific risk factors do exist in the region of Sap Kosovo. In order to determine clinical-epidemiological characteristics, 875 stone-formers were studied. According to sex, a higher prevalence in males (63.42%) versus females (37.57%) was found. Familial nephrolithiasis was found to be 14.62%. The recurrence of calculi were discovered in 42.08%. The proven nephrolithiasis morbidity up to the age of 19 was 18.28%, which is considerably high. The average age was 33.69 ± 14.60. The frequency of nephrolithiasis according to profession, groups of inhabitants, period of migration, and formation of stones showed no significant differences. When evaluating the quality of the food in this area, it was established that the predominant food was cereals. Protein is about 60% plant origin and only 25% animal origin. Special attention was paid to the composition of stones. The core, shell or stone in its entirety was analyzed semiquantitatively by the IR spectrometric method. The sets of data of 193 patients were stored in a computer and analyzed: 68.40% stones with a core were found. When the stones were classified as to the main components, the cores consisted of 34.86% ammonium hydrogenurate, 43.41% oxalate, 16.43% phosphate, 5.25% others, and the shells of 9.33% ammonium hydrogenurate, 68.66% oxalate, 18.65% phosphate, and 3.33% others. On the basis of the aforementioned data it may be concluded that the calculi composed of ammonium hydrogenurate alone or together with other crystals are more representative here than in other parts of Yugoslavia, Europe and the USA, which is mainly due to economic and social conditions and to nutritional habits.

A14. Hydrochlorothiazide, Hypocalciuria due to PGE_2 Inhibition?

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Increased intestinal calcium absorption seems to be the main feature of idiopathic hypercalciuria. Renal 1α-hydroxylase, the critical enzyme in vitamin D activation, is stimulated by PGE_2. Indeed, PGE_2 has been proposed as a possible pathogenetic factor in renal hypercalciuria. On the other hand, thiazide diuretics have been demonstrated to reduce 1.25 vitamin D plasma level in idiopathic renal hypercalciuria. The above considerations led us to set up a study to assess whether the hypocalsiuric action of hydrochlorothiazide (HCTZ) was related to PGE_2 in the same way. To this end, ten hypercalciuric patients were treated for 15 days with 50 mg/day of HCTZ, and urinary PGE_2 was evaluated before and after treatment by radioimmunoassay after silica gel column chro-

matography. As suspected, HCTZ promoted a significant fall in urinary Ca^{++} (374.58 ± 29.73 mg/day vs 202.05 ± 8.82, $t = 5.2$, $P < 0.001$) associated with a decline in PGE_2 urinary excretion (624.53 ± 52.63 ng/day vs 481.4 ± 49.07, $t = 3.37$, $P < 0.01$). Furthermore, both urinary calcium and urinary PGE_2 were directly correlated ($r = 0.613$, $P < 0.01$), and there was also a direct correlation between the decrease in urinary calcium by HCTZ and that of PGE_2 ($r = 0.671$, $P < 0.05$). It is suggested that the hypocalciuric action exerted by HCTZ might be due, at least partly, to PGE_2 inhibition, leading to 1α-hydroxylase activity blockade. Moreover, since PGE_2 seems to antagonize the effect of parathyroid hormone (PTH) in renal tubules, HCTZ, by lowering the availability of PGE_2, could remove prostaglandin desensitization to PTH in renal tubules, promoting an increase in calcium reabsorption.

A15. Oxalate Exchange in Red Blood Cells of Calcium Oxalate Stone-Formers – A Pharmacologic Study

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Some time ago we reported a defect of oxalate self-exchange in red blood cells (RBC) of idiopathic calcium oxalate (CaOx) stone-formers. Such a defect could be corrected by anion carrier inhibitors such as stilbenes and was associated with an increase in the phosphorylation status of band III protein. These observations pointed toward a hyperfunction of the kinase(s) devoted to phosphorylation of the anion carrier and led us to investigate the in vitro effect on oxalate self-exchange of calmodulin inhibition (Trifluoperazine, 100 μM) and protein kinase C (Diacylglicerol, 10 μM), as well as cAMP stimulation (Forskolin, 25 μM). Calmodulin inhibition promoted a 80% decrease in the oxalate transmembrane flux rate, while protein kinase C produced a 10-fold and cAMP activation an 8-fold increase in the exchange process. These data suggest that either calmodulin inhibition or protein kinase C and cAMP activation are important in controlling the functional status of the anion carrier. The impact on oxalate transport exerted by all three intracellular messangers can be explained by taking the possibility into account that, to be active, band III protein needs more than one phosphorylation step – most probably under hierarchic control where protein kinase C seems to play the major role.

A16. Bone Mineral Content and Recurrent Calcium Nephrolithiasis with Idiopathic Hypercalciuria

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Hypercalciuria has been associated with decreased bone mineral content (BMC), but this finding is still under debate. We studied 12 male patients (44.4 ± 13.2 years) affected by recurrent Ca-nephrolithiasis (RCN) and idiopathic hypercalciuria (IH) for a 1-year period; we compared their data with those of 12 matched subjects. Mean urinary Ca-excretion was 372.3 ± 127.8 mg/day, and the patients, as a whole, had a mean occurrence of 10.2 ± 11.2 calculi/person. All patients had undergone conservative treatment using low calcium and low oxalate diets, with no specific pharmacological therapy. At the beginning of the study, the hypercalciuric patients had a mean average areal density (AAD) of the lumbar spine, measured by dual photon absorptiometry, of 0.876 ± 0.150 g/cm^2 which, compared to the value in the controls (0.990 ± 0.134 g/cm^2), resulted in an 11.56% decrease (P = NS). The AAD decrease did not correlate with either age or with urinary Ca-excretion. However, compared to controls, AAD reduction was more pronounced in 6 patients over 45 years of age (16.54%), as opposed to 6 patients below 45 years (6.59%). At the end of the 1-year study, mean urinary Ca-excretion in the IH patients was 347.7 ± 92.3 mg/day, while the mean AAD was 0.831 ± 0.161 g/cm^2 ($P < 0.005$ compared to the beginning of the study) with a demineralization rate of 4.1% a year. In contrast, the AAD value in the control group was virtually unchanged (0.989 ± 0.131 g/cm^2). No relationship was found between demineralization rate/year and age, initial AAD value, or number of calculi in the IH group. We conclude that IH and RCN also induce a moderate decrease in BMC in the male population and that this reduction is not related to the degree of hypercalciuria. Moreover, in the absence of metabolic control of the disease, BMC appears to progressively decrease.

A17. Deficit of Inhibitors and Renal Lithiasis

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In calcium lithiasis, inhibitors play a significant role in reducing the crystallization processes. Some urinary inhibitors, i.e., magnesium, pyrophosphate, citrate and glycosaminoglycans, were determined in a group of patients with calcium oxalate lithiasis and in a control group in order to detect any possible differences between the two groups. Inhibitor analysis and other metabolic parameters (calcium, oxalate, uric acid, and ortophosphate) were evaluated by established methods. The results obtained showed that there was no difference between the two groups with respect to magnesium, pyrophosphate or glycosaminoglycan excretion, but there was when the results were expressed as a concentration. However, for citrate, there was a significant difference in concentration and excretion. Thus, the detection of relatively low levels of magnesium, pyrophosphate, and glycosaminoglycans was therefore of no fundamental diagnostic value in our series of patients – either in explaining the occurrence of stones or in predicting the likelyhood of further episodes – but citrate seems to play an important role. The results induced us to consider the relationship between inhibitors and certain urinary lithogenic parameters. The relationship between ortophosphate versus pyrophosphate, uric acid versus glycosaminoglycans, and citrate versus calcium were studied. The relationship between citrate and calcium was particularly interesting. It was found that stone-forming patients with hypocitraturia have hypercalciuria. Thus, it is interesting to note the importance of citrate in preventing the risk of lithiasis in the group studied. However, in studies carried out by other authors, the results seem to be different. This can be explained when we recognize that the special characteristics of a particular region can determine a number of factors according to which a substance can present a particularly intense inhibitory action; this action is less important when these factors vary. For these reasons, a risk formula was proposed, taking into account the parameters that can be significant predisposing factors towards calcium oxalate stone formation in the Balearic population: F = (Ca) diuresis/(citrate). This relationship allows excelent discrimination within 84.5%.

A18. A New Method for Glycolate Determination in Plasma and Urine

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A new enzymic method was developed for the determination of glycolate in human plasma and urine. Urine (50 μl), after treatment with activated charcoal to remove interfering substances, and plasma

ultrafiltrate (100 µl) are used for analysis. The concentration of glycolate in the ultrafiltrate is the same as that in plasma, as determined by ^{14}C-glycolate internal standard. The glycolate concentration of each sample is measured with chromotropic acid after incubation with glycolate oxidase and a separate aliquot unreacted with enzyme. After color development, the samples are read at 570 nm on a spectrophotometer and glycolate values calculated as follows: Absorbance at 570 nm (unreacted – reacted with glycolate oxidase) = ΔE due to glycolate. An aqueous standard curve for glycolate is run with each sample batch. Glycolate oxidase also acts on lactate-producing pyruvate; however, neither compound contributes to color development and can therefore be ignored. Glycolate values for normal plasma (n = 23) and urine (n = 13) were 0.17 ± 0.06 mm/l and 0.62 ± 0.36 mm/24 h, mean ± SD, respectively. These values show good agreement with previously published results. Recoveries of glycolate added to urine at three different concentrations (0.20–0.53 mm/l) were 94.4 ± 4.8% mean ± SD. A within-run for plasma ultrafiltrate gave a coefficient of variation (CV) of 9.5% for a glycolate concentration of 0.19 mm/l; between-run CV was 10.8%. The sensitivity of the assay is 3.3 nmol. The assay is simple, reproducible, and requires only small volumes. Enzymes required for the assay are commercially available. We conclude that the enzyme assay developed for plasma and urinary glycolate is accurate, precise, and simpler than previous methods; it does not require the simultaneous measurement of lactate, and gives comparable results.

A19. Hydroxycarboxylate Malabsorption and Calcium Oxalate Nephrolithiasis

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The aim of this study was to define more clearly the role of citrate and other hydroxycarboxylic acids in recurrent calcium oxalate nephrolithiasis since recent studies have indicated that this stone-forming group malabsorbs and hypoexcretes ascorbate and citrate. Also, the malabsorption of ascorbate and resultant increased oxalogenesis in the gut from this vitamin was potentiated by citrate [1, 2]. Oral load studies using calcium carbonate and oxalate in stone patients and normals indicated that citrate increases the uptake and urinary excretion of both calcium and oxalate. Also, 22 recurrent calcium oxalate stone formers maintained in hospital for a week on low citrate (50 mg/day), low ascorbate diets (45 mg/d) had normo-oxaluria (oxalate <0.5 mmol/d) in 82 of 88 collections (93.2%). In contrast, whilst on at-home diets, in which the citrate intake exceeded 6 g/d and ascorbate was about 200 mg/d, 32 of 61 urine collections (52.4%) gave normo-oxaluria. By comparison, 3 patients with recurrent calcium oxalate stones resulting from jejunoileal bypass surgery had urinary oxalates ranging from 0.60 to 1.23 mmol/d on the controlled hospital diet, indicating a significant oxalate and oxalate precursor content of this diet. The hospital and home diets were similar in terms of animal and vegetable protein (80–100 g total/d) fat (100 g/d), calcium (1,000 mg/d) and caloric (2,000–2,500 calories/d) content. These findings taken together suggest a possible aetiological role for malabsorbed citrate and other structurally related hydroxycarboxylic acids in calcium oxalate nephrolithiasis since they increase calcium and oxalate absorption and excretion, increase ascorbate-induced oxalogenesis, and result in decreased excretion of these organic acid inhibitors of stone formation into urine. The results also indicate that low-citrate diets may prove beneficial in the treatment of recurrent calcium oxalate stone-formers by normalizing urinary oxalate excretion.

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A20. Urinary Calculi and Urinary Tract Infection

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The site and composition of stones related to bacterial identification in urine were studied. A total of 427 patients (374 adults, 87.6% and 53 children, 12.4%) with urolithiasis were treated between March 1975 and April 1986. Males predominated at a ratio of 3.4:1; 227 patients (53%) gave a history of at least one episode of UTI; 114 patients (90 adults and 24 children; 27%) had a positive urine culture (MSU). Fifty-two stones were passed spontaneously and the rest were removed surgically. A biochemical analysis was conducted on 225 stones (from 199 adults and 26 children) and were associated with infected urine in 52 and 14 cases, respectively. Stones with infected urine occurred more frequently in the upper urinary tract in both age groups (113, 50%), and there was a higher incidence of UTI in children (45.3%) compared to adults (26%). Forty-three percent of stones in children were composed of uric acid/urate compared to 21% in adults and 21% of phosphate compared to 19% in adults. *E. coli* was the most common organism cultured in urine in both age groups. *Proteus, Pseudomonas, Klebsiella* spp., *Staphylococcus* spp. and *Enterobacter* occurred more often in adults (47, 50.2%) compared to children (9.37%). Thorough evaluation of the associated urinary infection and recognition of the character and composition of stones aid in proper management and treatment.

A21. Laxative Abuse as a Cause for Ammoniun Urate Renal Calculi

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Nine patients with laxative abuse and predominantly ammonium urate renal calculi underwent metabolic studies in order to identify common chemical abnormalities and determine the pathophysiology of this rare type of stone. All individuals had a serum chemistry profile, urine culture, 24-h urine chemistries performed on their usual diet, and stone analysis. All subjects had pure or predominantly ammonium urate renal calculi. Urine cultures were sterile and none of the individuals had chronic urinary tract infection. Twenty-four hour urine studies demonstrated marked reductions in volume (902 cm^3), sodium (28 mEq), citrate (116 mg), and potassium (21 mEq). Significant elevation in ammonium urate supersaturation was found when compared to control subjects when studied by the computer model EQUIL 2. All patients were female and seven had one or more urine specimens positive for phenolphtalein. The remaining two individuals and four of the other seven patients were known laxative abusers. Gastrointestinal loss of fluid and electrolytes allowed for chronic extracellular volume depletion. Intracellular acidosis was present as judged by low urine citrate and potassium. As a consequence, increased ammonia is secreted into the urine to buffer the hydrogen ion. Urate, which would normally combine with sodium and potassium, cannot because of the paucity of these ions and instead is linked with ammonium. The fact that the ion product for ammonium urate is significantly increased compared to controls reflects the stated pathophysiologic changes. Laxative abuse should be suspected whenever a female patient with an ammonium urate renal calculus in sterile urine is discovered.

A22. Urine and Blood Biochemistry – Stone Patients Versus Controls in India

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Precise biochemical diagnosis is essential for effective therapy and prophylaxis of urinary stone disease. An urine and blood biochemistry study is a simple and effective means of making this diagnosis. However, one needs to know the significance of the individual values in relation to those of the control population in the specific region. This paper studies the biochemistry of urine and blood of 512 proved urinary stone patients and 100 age- and sex-matched controls. Early morning urine (EMU) and random samples were collected from all individuals to study sediments; 24-h urine samples were collected in con-HCl; blood samples were collected. Calcium, phosphorus, uric acid, creatinine, magnesium and citric acid were estimated. Crystalluria was seen in 62% of stone patients as against 12% of the controls. The basic biochemical observations and the statistical significance of difference are as shown below.

Parameter	Stone mean (SE)	Non-stone mean (SE)	*P*-Value
S. Calcium (mg%)	10.08 (0.2)	10.87 (0.17)	$P < 0.001$
S. Phosphorus (mg%)	3.99 (0.16)	4.82 (0.22)	$P < 0.001$
S. Uric acid (mg%)	7.6 (0.35)	5.81 (0.39)	$P < 0.001$
S. Creatinine (mg%)	1.98 (0.17)	3.35 (0.48)	$P < 0.001$
S. Magnesium (mg%)	2.78 (0.32)	2.14 (0.12)	$P < 0.1$
U. Calcium (mg/day)	199.45 (4.2)	165.87 (4.34)	$P < 0.001$
U. Phosphorus (mg/day)	705.97 (51.94)	884.76 (51.22)	$P < 0.001$
U. Uric acid (mg/day)	488.0 (33.53)	435.4 (18.85)	$P < 0.001$
U. Creatinine (mg/day)	2.18 (0.1)	1.18 (0.2)	$P < 0.001$
U. Magnesium (mg/day)	96.34 (7.96)	94.28 (7.15)	NS
U. Citric acid (mg/day)	131.0 (21.16)	164.84 (14.09)	$P < 0.001$

SE = Standard error; NS = not significant. The significance of the various findings are discussed

A23. A Specific Calcium Sensor/Receptor Function in the Renal Proximal Tubule

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Monoclonal antiparathyroid antibodies, reacting with structures involved in the sensing or gating of Ca^{2+} in parathyroid cells, have recently been developed. Using immunohistochemistry, it has previously been shown that the plasma membrane staining of normal parathyroid cells is less pronounced in parathyroid adenomas. The antibodies also inhibit a rise in cytoplasmic ionized calcium in response to an increased extracellular calcium concentration (Juhlin et al., BBRC 1987; 143:570). Apart from reactivity with parathyroid cells, the antibodies also exclusively stain renal proximal tubular cells (Juhlin et al., Proc Natl Acad Sci USA 84:2990).

Material and Methods: Immunohistochemical studies were performed on renal specimens of patients with various renal diseases, such as severe forms of glomerulonephritis and rejected transplants. A standard PAP technique was used on frozen tissues. Renal proximal tubular cells of the rat were prepared for the study of a functional effect of the antibodies on cytoplasmic ionized calcium. Single cells were prepared without collagenase and the FURA-2 microfluorometry technique was used for measuring cytoplasmic ionized calcium concentration.

Results: Stainings of renal sections were distinct and consistent. Intratubular cell fragments were easily demonstrated, and tubular atrophy was depicted as thin tubular linings. Rat renal proximal tubular cells were identified using a functionally inactive antibody. Steady-state cytoplasmic calcium was 80 nM and upon increasing extracellular calcium concentration to 4 mM, the cytoplasmic calcium transiently rose to 275 nM. Preincubation with the functionally active antibody virtually blocked the rise in cytoplasmic ionized calcium.

Conclusions: Similar structures for the sensing of Ca^{2+} have been identified in the parathyroid and in renal proximal tubule, as shown both by immunohistochemistry and by interference with the normal rise in cytoplasmic Ca^{2+} in response to increased extracellular calcium. The antibodies may be used for diagnostic purposes and are also used for the purification and characterization of the antigen, which is either a receptor or part of calcium channel, exclusively present in parathyroid and in the renal proximal tubule.

A24. Primary Hyperparathyroidism in Patients with Nephrolithiasis in Northeastern Slavonija and Baranja

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The study covers 232 cases of nephrolithiasis in northeastern Slavonija and Baranja. Primary hyperparathyroidism was diagnosed as a result of biochemical and radioimmunological analysis in 5.2% of patients.

A25. Should Idiopathic Calcium Phosphate Stones be Separated from Calcium Oxalate Stones? – Evidence from a Comparison of Sex, Age, Stone Weight, and Acidification

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We reviewed the results of analyses of 3,119 ammonium-negative calculi of renal origin, in which only calcium phosphate and/or

calcium oxalate was detected by qualitative wet chemical and infrared (IR) methods. The oxalate-to-phosphate (O:P) ratio was determined by IR (Gault et al., Clin Chim Acta 166:103, 1987); accuracy was validated by comparison with a quantitative wet chemical procedure in another laboratory (r = 0.92). Considering the 3,119 stones in three groups: "phosphate" (17%), "intermediate" (23%) and "oxalate" (60%) with O:P ratios of ≤ 1, >1 to <10 and ≥ 10, there was a strong association between sex and O:P ratio ($P < 0.001$). The male-to-female (M:F) ratio was 0.9 for phosphate stones, 1.9 for the intermediate, and 3.2 for the oxalate group. Of the phosphate stones 51% were formed by females compared with 24% of the oxalate stones. Stone frequency/1,000 population per decade peaked for the oxalate group in the 5th decade in males, but there were peaks in the 4th and 6th decades in women. The peak for the phosphate group was in the 7th decade in males and in the 3rd decade in females. Stone weight and O:P ratio also correlated strongly (inversely). The mean weight for phosphate stones was 589 mg, or 3.7 times the 159 mg for oxalate stones. The M:F ratio for stones ≤ 20 mg was 2.7; for 21–100 mg, 2.2; for 100–500 mg, 1.8; and for those >500 mg it was 1.4 (r = −0.99 for M:F ratio and log weight). Studied prospectively, 8/22 patients with recurrent phosphate stones, but without evidence of urinary tract infection, failed to lower urine pH <5.3 after 0.1 g/kg ammonium chloride; this compared with 0/24 patients with recurrent oxalate stones (X^2 = 8.2, $P < 0.01$). Mean urine H^+ was lower and pH higher ($P = < 0.05$) in the phosphate group before, and 2, 4, and 6 h after the acid load. Urine ammonium and titratable acid excretion were lower in the phosphate group at 6 h, and also in 24-h urines ($P < 0.05$). These results suggest idiopathic phosphate stones may have differences in pathogenesis from calcium oxalate stones. Consideration should be given to separating the two types of stones in treatment protocols.

A26. A Role for the Thyroid Gland in Calcium Stone Formation?

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Hypercalciuria has been identified as one of the biochemical changes brought about by hyperthyroidism. To date, however, hyperthyroidism has not been assessed as a possible cause for calcium urolithiasis. Due to the large number of hypercalciuric cases defined as idiopathic, it seemed appropriate to us to evaluate the thyroid gland as having a possible role in the processes of formation of calcium containing urinary stones. Our trial comprised thyroid patients with proven hyper-, hypo- and euthyroid status. In all, a spectrum of variables in urine and blood was set up and critically evaluated against healthy controls. These laboratory data and those from reviews of the literature shed light on the probability of a role for the thyroid gland in the pathogenesis of calcium-containing urinary stones.

A27. The Effect of Increasing Urate Concentration on the Precipitation of Calcium Oxalate from Human Urine

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The role of urate in calcium oxalate (CaOx) urolithiasis remains polemic. In 1970, Kallistratos et al. reported that addition of urate to urine salted out CaOx, a finding that has been largely ignored, despite its potential significance in CaOx stone formation. The aim of this study was to confirm these findings at physiological urate concentrations using human urine subjected to a variety of treatments and to characterise the crystalline material deposited. Urine was collected from healthy men, divided and subjected to one of the following procedures: (1) 50 μm sieving, (2) centrifugation at 10,000 g, followed by 0.22 μm Millipore filtration; (3) 10,000 Da ultrafiltration; (4) 10,000 Da ultrafiltration + 35 mg/l Tamm-Horsfall mucoprotein (THM). Three milliliters of a filtered solution of sodium urate was added to 50 ml samples of urine so as to increase the final urate concentration by approximately 3 mM. Controls were treated with NaOH to give the same final osmolality, and all samples were adjusted to the original native pH before incubation for 90 min at 37 °C. Samples that did not precipitate CaOx upon addition of the urate were subjected to a standard load of oxalate in order to induce crystallization, which was detected and quantified using a Coulter Counter. Crystals were never detected in the absence of urate. High-particle background counts in the sieved urines complicated the assessment of crystallization. Nonetheless, in those samples (n = 10) that spontaneously deposited crystals upon the addition of urate, the amount of material deposited was significantly greater in the spun and filtered and the ultrafiltered urine than in the sieved. THM did not affect crystal deposition. Where crystallization was induced by oxalate, the addition of urate significantly lowered the metastable limits of all samples (n = 10) in relation to their controls. Despite this, addition of a standard oxalate load above these limits resulted in the deposition of approximately 3 times the amount of crystalline material in the presence of added urate. In all cases IR studies revealed the exclusive precipitation of calcium oxalate. Scanning electron microscopy demonstrated that crystals precipitated by the addition of oxalate in the presence of urate were markedly smaller, more numerous and more highly aggregated than those deposited in its absence. It was concluded that the salting out of calcium oxalate by urate at physiological concentrations may explain the apparent association between urate and calcium oxalate urolithiasis.

A28. Oxalate Transport Studies in Intestinal and Renal-Brush-Border Membrane Vesicles in Pyridoxine-Deficient Rats

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Hyperoxaluria is the major risk factor for idiopathic calcium oxalate urolithiasis. Hyperabsorption of oxalate from the gut has been implicated in the increased risk of calculogenesis in pyridoxine deficiency. Investigations on the comparative aspect of oxalate transport across intestinal and renal-brush-border membrane vesicles (BBMV) [1, 2] and their alterations in pyridoxine deficiency are of interest to understand the pathophysiology of oxalate urolithiasis. Acute, subclinical and chronic levels of pyridoxine deficiency were induced in male weanling rats and oxalate transport studied in intestinal and renal BBMV. Pyridoxine deficiency failed to modify the oxalate influx in rat intestinal BBMV but elevated the oxalate reabsorption by renal tubular cells. The sodium and potassium ions did not affect oxalate transport in either intestinal or renal BBMV. Thiol-group blocking agents did not alter oxalate uptake in intestinal BBMV; however, oxalate translocation across renal tubular cells was significantly decreased. Thus, in pyridoxine deficiency, rat kidneys appear to be more specific for including oxalate lithiasis as compared to oxalate influx through the intestine.

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A29. Diurnal Changes in Urine Chemistry of Normal Muslim Subjects in Day Fasting (Roza)

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Roza, i.e. complete fasting from dawn to dusk (no food and no water) during the period of Ramzan is very common among Muslim communities. In view of the observed higher prevalence of urolithiasis in Muslims and the possibility of a sharp transient rise in the concentration of urinary solutes inducing nucleation or spontaneous precipitation culminating in urolith formation, we examined the diurnal variation in the urine chemistry of 11 Muslim subjects in Roza. The subjects had their first meal at 4 a.m. and did not take any food or water until 7 p.m. when they took their last meal. The urine passed between 4 a.m. to 10 a.m. was pooled and labelled S_1, that between 10 a.m. and 7 p.m. S_2, and that from 7 p.m. to 4 a.m. S_3. S_2 was thus collected during a period of complete deprivation of food and water. Mean volume of S_2, collected during complete fasting, was 135 ± 142 ml significantly lower than S_1 and S_3. Creatinine calcium, magnesium, phosphorus, oxalic acid, amino-nitrogen, uric acid, uromucoid, Tamm Horsfall protein and sodium was lower in S_2 than S_1 and S_3, but the concentration (mg/dl) of all were higher. The GAG concentration progressively decreased in the three samples (0.41 ± 0.34, 0.24 ± 0.19, 0.10 ± 0.08 CPC units/dl). The urinary pH was lower in S_2 than in S_1 and S_3. No significant difference was observed in the calcium/magnesium ratio, calcium/citrate ratio, calcium oxalate risk index, or ionic activity product index among the three samples. All things considered, we feel that a "dawn to dusk" fast may not materially predispose the studied population to an increased risk of urolithiasis.

A30. Vitamin D_3-Induced Glycolic Aciduria in an Experimental Stone Model

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Urinary glycolate is a precursor of oxalic acid, which is a risk factor for kidney stone formation. One alpha vitamin D_3 has been reported to induce renal stones in rats fed ethylene glycol (EG) (Urinary Stone, 2nd International Conference, Singapore, Okada, et al. pp. 378–383, 1984). In the present study we examined the effect of one alpha hydroxy vitamin D_3 on the excretion of oxalate precursors in EG-fed rats. Twenty-four male Wistar rats were randomly allotted to two groups. Group I received daily 0.5% EG. Group II received, in addition, one alpha hydroxy vitamin D_3, 0.5 g, every other day by gavage for 3 weeks. Urinary oxalate, glycolate, citrate and hydroxyproline were determined by HPLC and ion chromatographic methods. No differences were noted in urinary citrate and hydroxyproline between the groups. Urinary glycolate increased 10-fold in group I compared with 100-fold in group II (0.37–5.6 vs 0.40–51.0 mg/day $P < 0.001$, respectively). Urinary oxalate increased 4-fold in both groups. The source for the increased urinary glycolate, determined by feeding of ^{14}C EG, demonstrated that ^{14}C recovery as glycolate was 1% in group I and 21% in group II. Subsequent administration of sodium glycolate alone, together with one alpha vitamin D_3 or with the vehicle, did not show any differences in urinary glycolate between the three groups. Our results indicate that: (1) one alpha vitamin D_3 increases the urinary glycolate without a parallel increase in urinary oxalate in EG fed rats; (2) increased glycolate resulted from the synergistic effect of both drugs; (3) this effect probably takes place prior to glycolic acid in the oxalate pathway.

A31. Withdrawn

A32. Epidemiology of Stone Disease in the U. S. as Discerned From Stone Risk Profile

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The introduction of Stone Risk Profile, a method for the analysis of stone-forming risks in urine, offers a unique opportunity for an epidemiological study of nephrolithiasis. A total of 2516 patients underwent this analysis, 418 from the Northeast (NE), 659 from the Southeast (SE), 358 from the North Central (NC), 719 from the South Central (SC), and 362 from the West (W). Low urine volume (↓TV) was found in 67%–77% of patients, hypercalciuria (↑Ca) in 35%–45%, hyperoxaluria (↑Ox) in 17%–24%, hyperuricosuria (↑UA) in 15%–19%, hypocitraturia (↓Cit) in 21%–31%, and high urinary sodium (↑Na) in 23%–32%.

		↓T.V. l/d	↑Ca mg/d	↑Ox mg/d	↑UA mg/d	↓Cit mg/d	↑Na mEq/d
NE	$\bar{x}$	1.28	351	59	847	203	258
	%	77	35	23	19	31	24
SE	$\bar{x}$	1.25	349	60	831	182	251
	%	77	41	20	15	30	25
NC	$\bar{x}$	1.27	349	60	809	207	266
	%	77	45	19	18	21	32
SC	$\bar{x}$	1.27	347	57	813	204	261
	%	75	45	17	17	25	25
W	$\bar{x}$	1.30	367	62	831	195	260
	%	67	40	24	19	24	23

Percentages are calculated on the basis of total number of patients in each region.

The pattern of distribution of these abnormalities was remarkably similar among the five regions of the country. Thus, the frequency of occurrence of the above risks was not necessarily greater in the Southeast region (believed to be the stone belt). The results suggest that the majority of patients with stones present with abnormal stone-forming risks that are environmental (↓TV and ↑Na) as well as largely "metabolic" in origin (↑Ca, ↑Ox, ↑UA, ↓Cit). The presentation in the stone belt was not characterized by exaggerated risk factors.

A33. Risk of Stone Formation in Rock Phosphate Mine Workers

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Udaipur (southeastern part of India) is an area rich in minerals with a large number of phosphate, soapstone, zinc, silver and cadmium mines. Matoon Mines are open cast-rock phosphate (P_2O_5: 25%–32%) mines. In view of the apparently higher incidence of stones in this area, we investigated the urine chemistry of 113 normal miners and compared it with the normal population of Udaipur. The trace metal (iron, zinc, manganese, copper, lead, chromium and cadmium) content of water and food items in this area are within permissible limits. Interestingly, phosphate excretion was significantly lower in miners (299.5 ± 245.6 mg/g creatinine) as compared to controls (872.8 ± 927.4 mg/g creatinine). Among the stone-promoter substances, the excretion of oxalate was quite high (71.56 ± 122.58 mg/g creatinine) and among the inhibitors, that of magnesium (44.4 ± 133.8 mg/g creatinine) and citric acid

(441.4 ± 475.1 mg/g creatinine) was lower. Zinc, copper and manganese are reported to be inhibitors; their respective excretion was 704.9 ± 471.2, 108.4 ± 415 mg/g creatine in miners, and the latter two minerals were significantly higher than in the controls. Higher oxalate and lower magnesium excretion were the commonest risk factors in miners, oxalate being contributed by an endogenous pool.

A34. The Significance of Annual Rhythms for the Excretion of Lithogenic and Inhibitor Substances in the Urine

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In view of the varying climatic influences and the changing dietary habits of the population over the seasons of the year, there is reason to believe that the risk of urolithiasis is subject to a seasonal rhythm. In this study, the 24-h urine of healthy women (n = 30) and healthy men (n = 40) was collected once each month for 1 year. The following parameters were determined: pH, specific weight, Na, K, NH_4, Ca, Mg, Cl, P, SO_4, creatinine, uric acid, titrable acid, GAGs, and relative supersaturation (EQUIL). Parallel to these, a dietary record was kept in each case in order to check the calcium and oxalate uptake. A statistically significant annual course was discovered for all urolithiasis-related parameters. There were also differences between the sexes. For example, the seasonal rhythm of calcium excretion and concentration peaks from August to November and is even more marked in men. In contrast to this, peaks for oxalic acid and uric acid were recorded from January to March. These results conform to those gained from evaluation of the diet records. The citric acid and magnesium excretions were highest in the summer and autumn months and were regarded as being seasonal protective factors. Women excreted more citric acid than men. When calculating the relative supersaturation, all these results affect the CaOx figure. In autumn and winter the figures for relative supersaturation are at their highest.

Conclusions: The annual rhythm of urolithiasis-related parameters for men and women were studied in the course of a longitudinal study. Statistically significant courses were found for the majority of the urine parameters, which were linked to seasonal variations in diet.

A35. Urine Studies in Xanthinuria

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Xanthine stones are among the rarest urinary stones and are always the result of autosomal recessive hereditary deficiency in xanthine oxidase. Being faced with the case of a 4-year-old boy with xanthine urolithiasis, a urine study program was developed. Urine was collected every 12 and 24 h. Uric acid excretion per 24 h, at 12 mol/day, was greatly reduced (normal: 1.5–3.0 mmol/day). Taken in conjunction with the low uric acid level in the serum (24 mmol/l), this is a reliable sign of a xanthine oxidase deficiency. A HPLC method was developed to determine the xanthine and hypoxanthine excretion. For this, an isocratic system with UV detection was employed; 4 mM NaH_2PO_4 with 0.5 mM tetrabutyl ammonium chloride was used as a mobile phase. Separation was accomplished in a Lichrosorb RP 18 column. Xanthine excretion in the boy's 24-h urine was measured and found to be 180–220 mol/day (normal: 34–57 mol/day). Analysis of the 12-h urine revealed that nocturnal concentration of up to 800 mol/l occurred. On examining other members of the family, both the boy's father (stone diathesis already known) and the boy's 9-year-old sister were found to be suffering from xanthinuria. Quantification of the xanthine in the urine enabled us to check the effectiveness of prophylaxis against relapse.

Conclusions: Xanthine stone disease can be reliably diagnosed by quantification of the uric acid and xanthine in the urine. A new HPLC method for xanthine determination is described.

A36. Trace Element Contents of Serum, Urine and Kidney Stones of Urinary Stone Formers

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The importance of trace elements for urolithiasis has not yet been fully investigated. We therefore determined trace elements in serum, urine and kidney stones of stone-forming patients. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used as a method of analytical determination. This technique has the advantage of simultaneous measurement of all interesting elements of low sample weight (100 mg stone) and a superior accuracy over conventional methods. Investigation of 25 patients led to the following results: no significant distribution of trace elements within urinary calculi could be found according to the chemical composition of the stones evaluated by X-ray diffraction. However, there seems to be a difference between serum and urine concentrations of some elements in patients with urinary lithiasis compared to normal controls.

A37. Renal Calculi and Bacterial Adherence – An Ultrastructural Study of Adhesion to Hydroxyapatite Particles by Urinary Tract Pathogens

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Urinary tract infections associated with renal calculi are often impossible to cure with antibiotics unless the stone is removed surgically. Calculi may be "infection-induced" (struvite) or may be formed in idiopathic stone disease (calcium oxalate and/or hydroxyapatite, HAP). The adhesion of urinary tract pathogens to HAP has not been studied. The present study is aimed at defining the role of different surface fimbriae on binding of urinary tract isolates to the HAP particles.

Strains: Two *E. coli* strains (A & E) from urine cultures of stone patients with persistent urinary tract infection, one *Proteus mirabilis* strain from a struvite calculus and two *E. coli* strains [2683a, mannose-resistant haemagglutination (MRHA) and M7810, mannose-sensitive haemagglutination (MSHA)] with well-defined surface properties were included in the study.

Methods: Strains were grown for 24 h at 37 °C to express and at 18 °C to suppress fimbriae. Expression of fimbriae and cell-surface properties was measured by the salt aggregation test (SAT) and haemagglutination (HA) test. Bacterial suspensions were incubated with HAP in synthetic urine to study bacterial binding to HAP. The samples were prepared for scanning electron microscopy and observed for bacterial binding.

Results: Strains cultured at 37 °C expressed high cell-surface hydrophobicity and HA, indicating good expression of surface fimbriae, and showed a high degree of binding to HAP particles. Bacteria cultured at 18 °C showed poor surface hydrophobicity and HA properties. Binding to HAP particles of 37 °C grown cells was in the following order: *Proteus* ~M7810 ~2683a > *E. coli A* ≫ *E.*

coli E. The same strains when grown at 18 °C showed poor binding to HAP.

Conclusions: Both MRHA and MSHA strains show high binding at 37 °C, which was nearly abolished by growth at 18 °C. Different expression of bacterial surface fimbriae (MSHA/MRHA and hydrophobicity) seems to be involved in the binding of some uropathogens to HAP.

A38. A Rapid Ion Chromatographic Procedure for the Estimation of Glycolate in Plasma and Urine

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We describe a direct and rapid method for the determination of glycolic acid in urine. A 50-µl filtered sample of diluted urine (1:2 with purified water) was injected into the high-performance liquid chromatograph (HPLC). The separated glycolate peak was measured by a conductivity detector and compared with a standard sample of glycolic acid. The purity of the separated glycolate peak was confirmed by utilizing the enzyme glycolate oxidase, which completely abolished the glycolate peak. The mean normal 24-h urinary excretion of glycolic acid was 0.56 ± 0.18 mM (1 S.D.) (n = 24) for adults; the mean, for males (n = 11) was 0.65 ± 0.15 mM and for females (n = 13), 0.47 ± 0.17 mM. The mean normal plasma glycolic acid was 7.22 ± 0.95 µM/l (1 S.D.) (n = 12) for adults, as determined from a protein-free filtrate injected into the HPLC. Recovery of glycolic acid added to a series of urines ranged from 95% to 102%, mean 98.8 ± 2 S.D. Intra-assay precision was 2.5%, n = 20, for urine and 7.8%, n = 11, for plasma.

A39. Amino Acid Excretion in Urinary Calculous Disease

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In view of reports that urinary amino acids, except for hydroxyproline and cystine, act as inhibitors of the stone-forming process and that amino nitrogen excretion is lower in some stone-former populations, we studied 24-h amino nitrogen excretion in 30 healthy controls (HC) and 247 stone-formers (SF) (renal 113, ureteric 63, vesical 71). Individual amino acids were studied by paper chromatography and chemical methods in 27 HC and 49 SF (renal 21, ureteric 16, vesical 12). The amino nitrogen excretion in HC, renal SF, ureteric SF and vesical SF was 101.41 ± 70.60, 82.58 ± 46.17, 133.85 ± 96.12, 95.31 ± 46.88 mg/24 h, respectively; concentration was 10.44 ± 6.80, 7.98 ± 5.78, 10.23 ± 7.63, 9.21 ± 6.04 mg%, respectively. There was no frank case of cystinuria or moderate case to serve as hydroxyprolinuria, and there was also no gross variation in the excretion of other amino acids in HC or SF. Slightly lower excretion of taurine in SF was observed. The in vitro experiments indicated that changes in urinary amino acids within physiological limits did not influence the mineralization process to any noticeable degree. Considering all the results together, we are inclined to believe that urinary amino acid levels are not associated with the etiology of urinary calculus disease in the local population. Furthermore, we feel convinced that any variation in the excretion of amino acids within physiological limits does not influence the lithogenic process to any substantial degree.

A40. Soft Drinking Water and Urolithiasis

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It is well known that soft drinking water has an influence on urolithiasis and areas with soft drinking water have a high rate of urolithiasis. The area where our study was carried out has extremely soft drinking water with a mineral content of less than one German grade. Because of this, we did a biochemical investigation on 21 stone-formers (12 males and 9 females) and 6 male volunteers. Twenty-four hour urines were collected, and the excretion of sodium, potassium, calcium, magnesium, phosphates, and chlorides, was measured. Our investigation showed that stone-formers had an urinary excretion of: sodium, 321.7 ± 134.9 mmol; potassium, 39.4 ± 16.2 mmol; calcium, 4.8 ± 2.2 mmol; magnesium, 4.1 ± 1.7 mmol; phosphates, 22.6 ± 22.6 mmol; chlorides, 180.2 ± 69.7 mmol. The same investigation was carried out in the volunteers, all of them medical doctors, and showed a urinary excretion of: sodium, 352.0 ± 85.5 mmol; potassium, 44.2 ± 8.2 mmol; calcium, 4.2 ± 0.4 mmol; magnesium, 3.1 ± 1 mmol; phosphates 19.9 ± 7.2 mmol; chlorides, 205.7 ± 39.8 mmol. Our results are very interesting because we found hypercalciuria in only 5 (19%) of the stone-formers; however, there was a very high rate of hypernatriuria and moderate hyperchloriuria in 90% of stone-formers and 100% of volunteers. The significant high level of sodium excretion by stone-formers and volunteers in our area with extremely soft drinking water is the result of salt in their food, which is probably added to the food. Hypernatriuria is probably a risk factor associated with hypercalciuria or normocalciuria in stone-formers in our area.

A41. Seromucoids, Urinary Mucoproteins, Tamm-Horsfall Protein, Glycosaminoglycans and Carbohydrates in Stone Disease

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Seromucoids, 24-h excretion of mucoproteins (MP), Tamm-Horsfall protein (T-HP), glycosaminoglycans (GAGs) by the CPC method, and total carbohydrates (TC) were determined in 30 healthy controls (HC) and 46 stone-formers (SF). GAGs containing hexuronic acid (HUA) were also determined in 12 HC and 16 SF. The urinary mucoprotein excretion was significantly raised in vesical SF (6.43 ± 6.69 mg%, 56.82 ± 53.67 mg/24 h, 79.32 ± 84.78 mg/g creatine and 40.13 ± 35.22 mg/m^2 BSA), but not in renal SF (3.15 ± 2.59 mg%, 46.93 ± 52.02 mg/24 h, 48.58 ± 38.66 mg/Cr., 28.54 ± 30.33 mg/m^2 BSA) as compared to HC (2.64 ± 1.57 mg%, 31.74 ± 23.88 mg/24 h, 42.76 ± 34.78 mg/g Creatine and 20.64 ± 15.71 mg/m^2 BSA). The seromucoid levels were not affected in SF (32.24 ± 15.03 mg%) as compared to HC (30.51 ± 12.35 mg%). The 24-h urinary excretion of T-HP, GAGs (CPC units) and TC in HC was 3.39 ± 2.68 mg, 7.27 ± 16.21 mg, and 1,772.76 ± 1,413.98 mg, respectively. No significant differences were observed in SF. However, HUA-GAGs excretion was lower in the latter (0.48 ± 0.41 mg%, 5.53 ± 4.28 mg/24 h, 7.12 ± 4.77 mg/g Creatine and 3.68 ± 2.82 mg/m^2 BSA) than in HC (1.31 ± 1.28 mg%, 15.84 ± 13.22 mg/24 h, 22.13 ± 13.26 mg/g Creatine and 10.10 ± 8.32 mg/m^2 BSA). The CPC method determines total GAGs, including keratan sulfate. Since total GAGs (CPC method) excretion was no different in SF than in HC but HUA-GAGs was lower in the former, keratan sulfate excretion was therefore higher in SF. The overall results indicate the possible involvement of urinary mucoproteins and GAGs but not of T-HP in urinary calculus disease.

A42. Prevalence of Permanent Idiopathic Hypocitraturia in Calcium Stone Formers

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Hypocitraturia is considered a risk factor of calcium nephrolithiasis, but its frequency is variably estimated according to the criteria defining the degree and persistence of the disorder. We evaluated the prevalence of hypocitraturia in 309 consecutive calcium stone formers (205 male, 104 female) followed at Necker Hospital between January 1983 and June 1987, who underwent serial determinations of urine citrate excretion (UCitV). Among them 127, or 41.1%, exhibited at least once UCitV $\leqslant$ 320 mg/day (according to Nicar & Pak, Urology 1983) with otherwise normal values in most cases, whereas only 46, or 14.8%, had UCitV $\leqslant$ 215 mg/day (according to Rudman, JCEM 1982) on at least one occasion. Using a threshold of 1.50 mmol (290 mg)/day, the lowest value observed in our healthy controls, hypocitraturia was found at least once in 99 patients, or 32%, but was persistent, i.e., present in at least two instances in only 52, or 16.8%, most of them (46) having values < 215 mg/day. In 33, hypocitraturia was concomitant with either active urinary tract infection (15), hypocalemia (thiazide-induced, 15; from other causes, 2), or chronic diarrhea (1). In the other 19 patients, or 6.1%, it was apparently idiopathic, associated with hypercalciuria in 6 and presenting as an isolated disorder in 13 (8 male, 5 female), i.e., 4.2% of the whole series. First morning urine pH was 5.8 or more in 11/19, all of whom had normal plasma concentration, thus suggesting a subtle defect in tubular acidification. Protein intake was high, in excess of 120 g/day, in 2 patients. Crystalluria was present in 9 of 16 cases studied and preponderantly made up of calcium oxalate dihydrate crystals in 8 cases, with a high proportion of large-sized crystals and agglomerates. In conclusion, mild intermittent hypocitraturia is often found in calcium stone formers, but marked permanent, idiopathic hypocitraturia appears to be an infrequent disorder, the prevalence of which is about 6% in our experience.

A43. Circadian Variation of Plasma Oxalate in Normal Healthy Subjects

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A reliable and routine enzyme method for the measurement of plasma oxalate, using immobilised oxalate oxidase, was recently reported [1]. It became possible to study circadian variation of plasma oxalate in seven healthy adult subjects. Marked postprandial rises in plasma oxalate were found in these subjects whilst they were on unrestricted dietary regimens. The plasma oxalate levels in these subjects progressively rose during the daytime hours, varying between a mean of 1.8 μmol/l at 0930 hours to a mean of 3.2 μmol/l at 2,000 hours and presumably returned, during the night, to the lower levels found in the morning samples. The effect of changes in the diet on the circadian variation of plasma oxalate was studied in the same subjects. Both restriction of the intake of oxalate in the diet and fasting prevented fluctuations and the progressive daytime rises of plasma oxalate. The mean level of plasma oxalate during fasting or whilst subjects were maintained on diets with no oxalate was 1.6 μmol/l, considerably lower than in samples taken from subjects on unrestricted diets, and the former values never rose above 2.3 μmol/l. Circadian variation of plasma oxalate is quite large and it was shown to be diet dependent. The collection of blood samples for plasma oxalate measurements should take account of this. More importantly, the estimation of oxalate clearance using single-point plasma oxalate and 24-h urine oxalate levels in subjects on unrestricted diets should be avoided.

References: 1. Kasidas GP, Rose GA (1986) Measurement of plasma oxalate in healthy subjects and in patients with chronic renal failure using immobilised oxalate oxidase. Clin Chim Acta 154:49–58

A44. Membrane-Induced Calcium Oxalate Crystal Nucleation

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Calcium oxalate (CaOx) crystals experimentally induced in rat kidneys as well as those found in human kidneys are almost always associated with PAS and colloidal iron-positive material. Transmission electron microscopy of the crystalline deposits in rat renal tubules revealed the associated material to comprise amorphous as well as membranous cellular degradation products (MCDP). Since CaOx relative supersaturation (RS) of both stone-forming rat as well as human urine is generally under 25, much less than required for homogeneous nucleation of CaOx, it is suggested that crystal nucleation in urolithiasis is heterogeneous. Since CaOx crystal MCDP association appears universal, we decided to investigate, in vitro, the possibility of membranes playing a role in crystal nucleation. We used renal-brush-border membrane (BBM) because renal proximal tubules have been implicated in urolithiasis. Proximal tubular BBM was isolated from the rat kidney cortex. Its purity was confirmed by specific activity of alkaline phosphatase. BBM was incubated at 37 °C and 6.5 pH in a metastable CaOx solution made by mixing potassium oxalate and calcium chloride and containing ^{14}C-labelled oxalic acid. The solution had 0.4648 mM calcium and 0.4325 mM oxalate, and CaOx RS of 5.32. Incubation was carried out in 4 ml of the solution with 0.1 mg/ml of the substrate. After 24, 48, 72, and 96 h of incubation, the solutions were filtered through 0.2 μm nucleopore filters. The filters were examined by scanning electron microscopy. Filtrates were checked for final pH and analyzed for Ca^{++}, Na^{+}, and K^{+} by atomic absorption spectrophotometry and oxalate by scintillation counting. CaOx RS decreased by 27.2% after 24 h, 54.8% after 48 h, 60.4% after 72 h, and 72.4% after 96 h of incubation, and CaOx crystals were seen after 72 h. We conclude that brush-border membranes can induce CaOx crystallization from metastable solution, which would otherwise not support it.

A45. Proximal Tubular Injury and Crystallization of Calcium Oxalate in Rat Urine

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Ever since Randall proposed that damage to the renal tubular epithelial cells was essential for the permanent deposition of calcium salts, a connection between urothelial injury and urolithiasis has been suspected. The nature of this relationship is, however, still not understood. To study this we injured the renal proximal tubules of rats by daily subcutaneous injections of gentamicin sulphate (GS), 100 mg/kg, and induced hyperoxaluria by 0.25% ethylene glycol (EG) in drinking water. Rat urine was collected daily and its volume and pH measured. Urinary Ca^{++}, Na^{+}, K^{+}, Mg^{++}, ammonium, phosphate, sulphate, citrate, and oxalate were determined. With these data urinary calcium oxalate (CaOx) supersaturations (RS) were calculated, using a computer program EQUIL. Kidneys and urinary sediment were examined by light, scanning, and transmission electron microscopy. Activities of various brush-border and lysosomal enzymes were also determined. With EG alone, no morphological changes were found in the kidneys but urinary oxalate levels were elevated. GS administration resulted in progressive renal tubular damage, an increase in membrane fragments in the urine, and elevated levels of brush-border enzymes: alkaline phosphatase, gamma-glutamyl transpeptidase and lysosomal enzymes: acid phosphatase, β-galactosidase, and *N*-acetyl-β-glucosominidase. Neither EG nor GS alone induced CaOx crystals. However, simultaneous administration of the two resulted in CaOx crystalluria by day 4. These crystals were associated with membranous fragments. Urinary CaOx RS was higher in these rats than in normals but not high enough for homogeneous nucleation. It is suggested that injury

to the renal epithelium resulted in membranuria and enzymuria. Membranes provided sites for heterogeneous nucleation of crystals while enzymes may have influenced the inhibitory activity of the urine.

A46. Lipids of Calcium Oxalate Urinary Stones

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It is generally agreed that crystal nucleation during urolithiasis is heterogeneous. However, the nature of these nucleators is still unknown. Since membranes and membrane lipids have been implicated in nucleation of crystals in a number of biomineralization systems and have been isolated from calcified matrices, we decided to look for lipids in urinary stone matrix. Urinary stones of known composition, containing more than 75% calcium oxalate, were rinsed, dried, ground, and extracted with chloroform: methanol: tris, pH 7.2, at 4 °C with sonication. The organic and aqueous layers were separated from the solid residue, which was further extracted with chloroform: methanol: HCl. The organic and aqueous layers were separated from the residue. The aqueous and organic phases, as well as residue, were all lyophilized and weighed. Combined weight of all gave the weight of matrix. Organic material was the total lipids. It was resuspended in chloroform: methanol and treated with acetone to precipitate phospholipids. Acetone-soluble neutral lipids were used to determine the amount of cholesterol. Crude phospholipid fraction was then chromatographed on Sephadex LH 20. Material eluting in void volume 280 nm absorbance was collected, and various fractions were tested for the presence of protein. Protein-positive fraction was proteolipid while the others were pooled as phospholipids. These stones had 4.72% ± 0.41 matrix, 0.66% ± 0.12 total lipids, 0.048% ± 0.016 phospholipids, and 0.152% ± 0.069 cholesterol. They also contained proteolipids. Thus, urinary calcium oxalate stones do contain lipids characteristic of cellular membranes, and membranes may have played a role in their crystal nucleation.

A47. Variation of Parathyroid Hormone in Calculus Disease

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Hyperparathyroidism has been incriminated as a direct causative factor in the production of renal calculi. Hyperparathyrodism is characterized by hypercalciuria, hyperphosphatemia, hyperphosphaturia and hypercalciuria. The most reliable laboratory findings is by far elevated levels of calcium in blood, but the absence of hypercalcemia does not rule out the diagnosis of hyperparathyroidism. In previous work from this laboratory, it was found that most of the patients with stones showed no significant differences in serum and urinary calcium and phosphate values from the control subjects and that stones were mostly of mixed composition. A subclinical condition of hyperparathyroidism cannot therefore be ruled out. The present study describes the levels of parathyroid hormone in stone patients. Twenty-one control subjects and 42 urolithiasis patients were studied for serum and urine inorganic phosphate and calcium, serum alkaline phosphatase and parathyroid hormone It was found that normocalcemic, normocalciuric urolithiasis patients had a slightly higher level of serum parathyroid hormone, which was not statistically significant as compared with control subjects. A high level of serum alkaline phosphatase was observed in the patients. The possibility therefore exists that normocalciuric primary hyperparathyroidism may prevail in certain urolithiasis patients.

A48. Nutrition and Stone Disease in Pakistan

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Dietary pattern is known to play a major role in the etiology of urolithiasis. The disease is predominantly found in low socioeconomic groups, who suffer from varying degrees of undernutrition and malnutrition Bladder stone disease is common in children, who suffer from varying degrees of protein malnutrition. Vitamin deficiencies have also been assigned importance as etiological factors. In the present study, dietary assessment was made by physical examination for deficiency diseases and from the dietary history by filling in a questionnaire. A total of 224 patients with stones (183 male, 41 female; 82 bladder stones and 142 kidney stones) and 95 control subjects (67 male and 28 female) were investigated. Daily intake of total calories, proteins, fat, carbohydrate, calcium, phosphorus, and vitamin A were calculated from dietary tables and compared with the normal control subjects in different age groups of a similar socioeconomic group. In addition, urinary excretion of creatinine and hydroxyproline and serum vitamin A, beta carotene, and vitamin C were also determined. Vitamin B_6 status was estimated by tryptophan supplementation in these patients. The diets of stone-formers had less calories, proteins, fat and vitamin A as compared with normal subjects. Kidney stone patients consumed fewer calories as compared with bladder stone patients while there was no difference in other nutrients. The changes in nutrient intake of patients was not related to the type of stone. The urinary creatinine excretion in bladder stone patients was higher than control subjects. Lower levels of serum vitamin A were also found in patients. It was observed that after oral supplementation of L-tryptophan, urinary excretion of oxalate or xanthurenic acid did not show any significant increase. Vitamin B_6 deficiency was excluded.

A49. Effect of Fasting in Urinary Stone Risk

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Total fasting of varying durations (from 24 h to several days) is very common among Hindus in general and in the Jain sect in particular, as it is considered to be a holy act. Reports on changes in the urinary profile during fasting in obese subjects are available but none to our knowledge on normal subjects. This prompted us to investigate the urine chemistry of 14 women of normal weight under fasting and fed condition. During a 36-h fast, urine samples (F) were collected in the last 24 h. Non-fasting (NF) 24-h urine samples were collected from the same subjects on their normal diet. Urinary citric acid, inorganic phosphorus, uromucoid, and uric acid excretions remained unaffected. Creatinine excretion (F: 574.71 ± 233.88 mg; NF: 365.16 ± 133.14 mg) significantly increased and calcium (F: 49.24 ± 30.35 mg; NF: 87.04 ± 24.80 mg), oxalic acid (F: 13.77 ± 7.42 mg; NF: 26.90 ± 7.39 mg), and magnesium (F: 25.16 ± 10.41 mg; NF: 43.83 ± 12.42 mg) excretion significantly decreased. Urine pH mildly decreased during fasting. It is concluded that a sudden rise in stone-promotor substances in the urine and in stone-risk indices, e.g., the Ionic Activity Product Index (N: −0.58 ± 0.25; NF: −0.25 ± 0.17), Calcium Oxalate Risk Index (F: −2.25 ± 0.47; NF: −1.63 ± 0.53), Calcium Oxalate Quotient (F: 8.31 ± 6.75; NF: 25.25 ± 12.11), Ca/citrate (F: 0.16 ± 0.12; NF: 0.26 ± 0.05), Ca/Cr (F: 0.08 ± 0.04; NF: 0.26 ± 1.06), oxalate/Cr (F: 0.03 ± 0.02; NF: 0.08 ± 0.04) and citrate/Cr (F: 0.66 ± 0.03; NF: 1.03 ± 0.40) during resumption of eating after a 36-h fast enhances the chances of lithogenesis.

A50. Contribution of Different Doses of Ascorbic Acid in Urinary Oxalate and its Influence on the Mineralization Process

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The influence of daily intake of different amounts of ascorbic acid (AA) on urine chemistry and its mineralization capacity was investigated in healthy controls (HC) and in operated and nonoperated stone-formers (OSF and USF) on a controlled hospital diet. 1, 2 and 4 g AA was given to 5,6 and 5 HC and 6,7 and 4 USF, respectively. In OSF, the effect of 1 and 4 g AA was tested in 5 and 4 subjects. The 24-h urine was collected in a bottle containing 10 mmol/l EDTA, which effectively checked the in vitro conversion of AA to oxalic acid and also the interference of AA in oxalate estimation by colorimetry. In general, pH was significantly reduced and AA excretion increased. In HC, 1 and 2 g AA intake did not influence urinary oxalate while the 4 g intake caused a rise (mean ± SD) in 11.6 ± 9.2 g/24 h. In USF and OSF, intake of 1 g of AA did not affect oxalate and citrate excretion, but 2 and 4 g of AA increased oxalate and decreased citrate excretion. Inorganic phosphate, magnesium, uric acid, calcium and creatinine excretion were not influenced. The mineralization capacity of the urine at all three levels of AA intake, with respect to calcium phosphate and calcium oxalate, remained uninfluenced. Our results suggest that AA is not a risk factor in urolithiasis, as the risk of stone-formation due to slightly enhanced excretion of oxalate and lowered excretion of citrate is compensated by a reduction in pH.

A51. Citric Acid Excretion and Stone Formation – The Prevalence of Hypocitraturia in Stone Formers and its Dependence on Age and Sex

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Citrate inhibits the growth and agglomeration of calcium oxalate. This property, proved in vitro, makes citric acid the most effective inhibitor for the purposes of Ca-oxalate urolithiasis. In the present study, citrate excretion in the urine of healthy test subjects (n = 30) and Ca-oxalate stone patients (n = 277) was investigated with respect to sex under conditions of both standard and uncontrolled diet. It was also examined with respect to age. In the healthy test subjects, citrate excretion was found to be clearly sex dependent, so any consideration of this problem must take sex into account (women: 3.18, men: 2.01 mmol/24 h). Female stone patients (2.26 mmol/24 h, n = 111) on a standard diet excreted significantly less citrate in the urine than healthy women (3.18 mmol/24 h, n = 13). This reduced rate of citrate excretion in the females was also found on a standard diet. There was no difference in citrate excretion between healthy men (2.01 mmol/24 h, n = 17) and stone patients (2.29 mmol/24 h, n = 217). After setting the limit value for hypocitraturia at 2.0 mmol/day, 47% of the stone patients on an uncontrolled diet displayed reduced citrate excretion, whereas on a standard diet (a precisely balanced diet as recommended by the DEG), only 17% manifested hypocitraturia. On investigation of the age-dependency of citrate excretion in healthy people on an uncontrolled diet, reduced excretion was measured in women (n = 150) in the age groups 15–19, 40–49, and over 60. Healthy men (n = 150) displayed reduced excretion in the age group 30–39. *Conclusion:* The investigations showed that in the case of Ca-oxalate lithiasis, citrate should always be assessed on an individual basis. Generally, our investigations led to the conclusion that hypocitraturia plays a more important role in women than in men.

A52. Induction and Isolation of Oxalate Binding Protein in Rat Intestinal Brush-Border Membrane

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The role of pyridoxine in oxalate metabolism is well documented. To understand the precise role of pyridoxine in the regulation and induction of the oxalate transport carrier, the uptake of ^{14}C-oxalate in intestinal brush-border membranes vesicles (BBMV) [1, 2] was undertaken. Oxalate uptake by BBMV prepared from normal and pyridoxine deficient rats revealed an induction of a biphasic carrier-mediated transport characteristic for oxalate uptake in this deficiency. The BBMV from pyridoxine deficient intestine were solubilized by treatment with non-ionic detergent, sodium deoxycholate, which was then removed by dilution below critical micellear concentration and followed by ultracentrifugation [3]. The protein pellet was incubated with ^{14}C-oxalate. The oxalate-bound protein peak was separated and partially purified on Sephadex G-75 and Sephadex G-150 columns. It is postulated that this oxalate-binding protein may be involved in the carrier-mediated phenomenon of oxalate uptake in pyridoxine deficiency.

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A53. Mechanism of Hypercalciuria (HC) in Renal Stone (RC) Patients (P) in Northeast Thailand

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To study the mechanism of HC, calcium loading (CL) was conducted in three population groups (G): GI = 13 healthy personnel or city dwellers, GII = 12 native villagers without RC and GIII = 27 villager with RC. Daily excretion of calcium (Ca) of GII and GIII, though within normal range, tended to be higher when being standardized to .similar salt intake. Seven patients in GIII were HC (24-h urine CA > 200 mg/day). After overnight fasting with hydration, calcium lactate, 929 mg, was given orally with breakfast. Blood and urine (U) were collected before and after CL. Fasting UCa (mg/dl of glomerular filtrate) of GI, GII and GIII were 0.05 ± 0.01, 0.13 ± 0.01, 0.11 ± 0.01, respectively (normal = < 0.11). The 4-h UCa after loading: GI 38.6 ± 2; GII = 56.6 ± 5 (vs GI, $P < 0.01$); GIII = 54.5 ± 5 (vs GI, $P < 0.05$). After-CL UCa (mg/mg creatinine) of GI, GII and GIII were 0.18 ± 0.01, 0.21 ± 0.01 and 0.24 ± 0.02, respectively (normal ⩽ 0.20). There were 16 P in GIII (60%) who had an abnormal response to CL as compared to 5 P in GII (40%) and 4 P in GI (30%). About 50% of GIII (15 P) can be classified by Pak's classification (Am J Med 1980; 69:19) as absorptive HC and 4% (1 P) as renal HC. We conclude that HC should be the main contributing factor for the pathogenesis of RC in northeast Thailand. Absorptive HC is more common than renal HC. The reason for the frequent occurrence of HC among villagers who do not have RC remains a puzzle.

A54. Is Hypomagnesuria Frequent in Stone Disease?

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The frequency in stone formers (SF) of hypomagnesuria, either absolute (AHoMg) or relative to calciuria, (RHoMg), is still controversial. It was assessed 24-h urine collections in 122 male calcium stone formers with normal renal function. AHoMg, defined by a Mg excretion <1.5 mmol/day, or a Mg/Creatine (mg/Cr) < 0.1 was observed in less than 1%. RHoMg assessed by Mg/Ca < 0.5 was observed in 31 (25.4%) but Mg/Ca was correlated with Ca/Cr but not with Mg/Cr. Using a new parameter (dMg/Cr), which accounts for the relationship between Ca/Cr and Mg/Cr in normals and is independent of the level of calcium excretion, RHoMg was present in only 16 (13.1%). The urinary excretions in such RHoMg patients and of their normomagnesuric counterparts (RNMg) are listed below.

	dMg/Cr	Mg/Cr	Ca/Cr	P/Cr	UA/Cr	Na/Cr	Urea/Cr
RHoMg	−0.107	0.16	0.45	1.52	0.27	10.7	29.6
RNMg	0.046	0.31	0.46	1.91	0.25	11.3	27.6

Compared to RNMg such RHoMg patients had a lower Mg/Cr but similar Ca/Cr, and the frequency of hypercalciuria (Ca:Cr > 0.45) was similar in both groups (31.3 vs 39.6%). The Mg plsama levels, the urinary excretions, and the blood levels of other studied parameters were not significantly different in either group.

Conclusion: Absolute hypomagnesuria is rare in stone formers while relative hypomagnesuria occurs in 13.1% Mg/Ca ratio overestimates this frequency because its value mainly depends on calcium excretion. Relative hypomagnesuria does not appear to depend on sodium or protein intake and might reflect either a subclinical Mg deficiency or a specific renal abnormality in Mg handling.

A55. Comparison of Urine Composition in Male Patients Forming Calcium Stones of Different Types

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In order to evaluate the significance of small amounts of phosphate in calcium oxalate (CaOx) stones, we compared the composition of urine from male patients forming stones with different amounts of CaOx and calcium phosphate (CaP). The results were compared with urinary findings in normal men. Urine was analyzed with respect to calcium, magnesium, oxalate, creatinine, citrate, and urate. Stones were analyzed chemically with respect to calcium, magnesium, ammonium, oxalate, phosphate, uric acid, protein, and cystin (as total sulfur). The patients were subgrouped according to their stone composition: (a) Reference group (n = 102): normal men without a history of stone formation. (b) CaOxCaP group (n = 9): stones composed of CaOx and 15%–85% of CaP. (c) CaOx (CaP) group (n = 25): stones containing CaOx with less than 15% of CaP. (d) CaP group (n = 4): stones containing CaP with less than 3% of CaOx. (e) CaOx group (n = 18): pure CaOx stones without a trace of phosphate. We found that urinary excretion of calcium was higher in the CaOx (CaP) group than in the reference group. In the CaOx group, the excretion of calcium was lower than in the CaOxCaP group. The oxalate excretion was higher in the CaOx group than in any other group. No differences with respect to magnesium or urate excretion were recorded between the groups. We found that patients forming stones without phosphate had a high excretion of oxalate, which might be an important cause of stone formation in these patients. However, in patients forming stones composed of a mixture of CaOx and CaP, oxalate excretion did not differ from that in normal men although urinary calcium was increased. This indicates that stones composed of pure calcium oxalate might have another etiology than stones composed of CaOx and CaP. Further studies on this problem appear to be of importance.

A56. Increasing Dietary Calcium Intake Reduces Urinary Oxalate Excretion in Healthy Adults

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During studies of the role of serum 1,25-$(OH)_2$-D concentrations in regulating Ca metabolism, we observed that daily UoxV averaged 0.31 ± 0.08 SD mmol/day among 34 subjects fed diets providing normal amounts of Ca (22.1 ± 4.8 mmol/day) and that UoxV was significantly higher, averaging 0.45 ± 0.09 mmol/day ($P < 0.001$) among 24 subjects fed low Ca diets (4.4 ± 0.7 mmol/day). We, therefore, reviewed 106 studies in healthy adults who ate diets providing 3.4 to 34.2 mmol/day alone (n = 69) or who were also given 1,25-$(OH)_2$-D_3 (n = 37) to evaluate the relationships between daily or fasting UoxV and the following other measurements: body weight (range 53 to 88 kg), dietary Ca (22.1 ± 4.8 mmol/day), estimated dietary oxalate (0.3 to 7.1 mmol/day), estimated dietary vitamin C (100 to 300 mg/day) and estimated dietary protein (55 to 217 g/day), serum 1,25-$(OH)_2$-D (40 to 235 pM), daily and fasting urine pH (4.8 to 7.3), and net acid excretion (3 to 120 mEq/day; −38 to 154 mEq/l), as well as fecal Ca excretion (1.1 to 23.2 mmol/day), fecal Ca/kg feces (7 to 308 mmol/kg) and net intestinal absorption (−2.5 to 17.9 mmol/day). UoxV/day was inversely correlated to diet Ca ($r = -0.58$; $P < 0.001$), to fecal Ca/day ($r = -0.60$; $P < 0.001$) and to fecal Ca/kg feces ($r = -0.64$; $P < 0.001$). UoxV/day was also and unexpectedly inversely correlated to net intestinal Ca absorption ($r = -0.36$; $P < 0.005$) and positively correlated to serum 1,25-$(OH)_2$-D ($r = 0.30$; $P = 0.002$). Fasting UoxV was correlated to daily UoxV ($r = 0.34$; $P < 0.001$) but was unrelated to dietary Ca. Fasting UoxV was also directly correlated to urine pH ($r = 0.37$; $p < 0.001$), although daily UoxV was not related to pH. We conclude that among healthy adults: (a) the availability of dietary Ca and thus the presence of Ca in the feces, reduces UoxV, presumably by limiting intestinal oxalate absorption; (b) it appears unlikely that in vitro conversion of vitamin C to oxalate accounted for the dependence of fasting UoxV on pH since no such relationship was observed in 24-h urines. Speculatively, the increase in fasting UoxV with pH may reflect an alteration in renal oxalate transport/metabolism analogous to the increase in urine citrate with increasing urine pH.

A57. Analysis of an Immobilized Oxalate Oxidase Method in Urine – Problems Solved and Methods Compared

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Recent experience with urinary oxalate by the Olthius method (Lewatit ion exchange column/chromotrophic acid) suggested an unusual number of patients with oxalate levels spuriously elevated on clinical grounds. A rapid oxalate oxidase method was modified from Kasidas and Rose (Ann Clin Biochem 22:412, 1985) using a Kratos #757 HPLC spectrometer and Kipp and Zonen #BD41 recorder. The mean excretion in 99 normals was 0.237 mmol or

21 mg/24 h. Range 0.11–0.46 mmol/24 h. (9–41 mg/24 h); 0.1–0.54 mmol/1.73 m^2/24 h (mmol x 88 = mg). C.V. of method is 8.5%. Samples collected in HCl showed neither sex nor age bias. An urinary oxalate pool was stable at room temperature, refrigerated, or frozen for 14 days, but only frozen (−20 °C) thereafter (to 6 months). Mean oxalate before and after 2 gms vitamin C, in 9 normals, were 22 and 20 mg/24 h. However, after 3 months frozen samples from controls with vitamin C increased by 21% (enzymatic) and 91% (Olthius). Comparison of the oxalate oxidase (A), Olthius (B), and Dionex (C) methods were undertaken. Dionex is an HPLC with ion suppression column and conductivity detector used by Wandzilak and Williams. A double blind comparison of 53 frozen samples, thawed at 60 °C x 10 min were made with results as follows:

Method	Mean mg/24 h	Ranges mg/24 h	Compare			Intercept	Slope	*P*
			Y	X	*r*			
A	33.6	12–77	B	A	0.813	8.592	1.169	0.0002
B	47.8	17–118	B	C	0.725	9.427	1.124	0.0003
C	34.2	13–75	C	A	0.831	8.329	0.770	NS

The oxalate oxidase method compares closely with the Dionex method. Both methods had lower values than the Olthius method by 29%. Initially the oxalate oxidase method was insensitive to vitamin C; however, after 3 months spontaneous conversion of vitamin C to oxalate was noted. The larger increase of oxalate in the vitamin C containing urines, by the Olthius method, suggests conversion of vitamin C to oxalate during Olthius assay.

A58. Intestinal Absorption of Oxalate and Calcium

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In 20 healthy subjects, in 12 idiopathic calcium oxalate stone formers and in 19 patients operated with jejunoileal bypass (JIB), sodium oxalate 100 μmol and ^{14}C-oxalate 0.4 μCi was administered orally together with a standard breakfast to fasted subjects. The total amount of ^{14}C-oxalate excreted in the urine was used for the calculation of absorption related to the ingested dose. The fractional calcium absorption was calculated as the ratio between the forearm activity values after oral (10 μCi) and i.v. (2 μCi) administration of $^{47}CaCl_2$. Both idiopathic renal stone formers and patients with JIB had a greater intestinal uptake of oxalate in comparison with controls (11 ± 5.1%, $P < 0.01$; 27 ± 18%, $P < 0.001$ vs 6.2 ± 3.7%). There was a positive relationship between the fractional absorption of oxalate and the total urinary oxalate in patients with JIB (r = 0.40) but not in the idiopathic stone formers. The greatest amount of ^{14}C-oxalate was excreted during the first 6-hour period after ingestion in controls as well as in idiopathic renal stone formers but in patients with JIB after 12–24 hours. The fractional gastrointestinal calcium absorption was increased in idiopathic renal stone formers in comparison with controls (55 ± 11% vs 47 ± 9.1%, $P < 0.05$). The patients with JIB had a lower uptake of calcium (35 ± 9.7%, $P < 0.001$). There was no correlation between the fractional absorptions of calcium and the urinary calcium excretions. The patients with JIB who formed stones had, however, a lower urinary calcium excretion than those who did not form stones (1.6 ± 0.69 mmol/24 h vs 2.9 ± 1.2 mmol/24 h $P < 0.01$). There was a positive relationship (r = 0.47) between the urinary excretions of oxalate and calcium in the idiopathic stone formers. During these conditions no correlation could be demonstrated between the fractional absorptions of oxalate and calcium neither in the idiopathic renal stone formers and patients with JIB, nor in the controls.

In conclusion, patients with recurrent formation of calcium oxalate containing stones had an increased uptake of both oxalate and calcium which is considered to be of importance for their stone forming propensity. In contrary, patients with JIB had an enhanced uptake of oxalate but a reduced uptake of calcium. Altered oxalate kinetics seemed to be present in patients with JIB, which will be studied further.

A59. Role of Diet in Calcium Nephrolithiasis

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We evaluated the customary diet in a group of 88 patients (50 males and 38 females, mean age 42.9 ± 11.8 and 44.0 ± 14.7 years) affected by calcium nephrolithiasis. 64 patients had recurrent, and 45 patients had bilateral nephrolithiasis. Mean body weights for the male (73.4 ± 8.8 kg) and the female (65.0 ± 12.2 kg) groups were 13% and 18% respectively above their ideal body weight. The estimated dietary intake was the following:

	Males	Females
Calorie overload*	+ 5%	+ 12%
Calories (kcal/day)	2,915 ± 788	2,223 ± 837
Protein (gr/kg b.w.)	1.39 ± 0.43	1.32 ± 0.45
Calcium (mg/day)	1,194.9 ± 587.8	931.9 ± 423.8
Phosphate (mg/day)	1,264.4 ± 525.0	1,233.3 ± 496.3
Oxalate (mg/day)	145.1 ± 136.6	123.2 ± 118.3
Sodium (mEq/day)	130.58 ± 64.42	84.01 ± 44.25
Potassium (mEq/day)	82.09 ± 27.58	69.90 ± 27.30
Water (ml/day)	1,635.1 ± 826.6	1,310.5 ± 681.0

* compared to the ideal requirements

A multiple correlation analysis among the different dietary components showed a statistically significant correlation for: (a) total calories intake vs. sodium and protein; (b) protein vs. phosphate and potassium; (c) calcium vs. phosphate; (d) phosphate vs. calcium and protein. The significant correlation between the dietary intake of calcium and phosphate ($P < 0.001$) was mirrored by a significant correlation between urinary calcium and urinary phosphate excretion ($P < 0.001$). On the contrary no correlation was found between dietary intake and urinary excretion of other components of the diet.

A60. Urinary Citrate Excretion as a Screening Test for Distal Renal Tubular Acidosis

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The purpose of this study is to evaluate the efficiency of using urinary citrate for the screening of potential cases of distal renal tubular acidosis, in a population in the northeast of Thailand, an area known to have high prevalence of the condition. Random

urine samples from 3,007 villagers were assayed for citrate levels by citrate lyase enzymatic assay. A large population (1,002 or 33%) of villagers had an urine citrate below 0.3 mmol/l compared with less than 5% in urban Thai population. Acid loading tests were performed in 566 individuals in which 108 failed to acidify their urine (pH > = 5.5). The urinary citrates of those with acidification defect were significantly lower than those without ($P < 0.001$). When the cutting point of urinary citrate was below 0.1 mmol/l, the sensitivity and specificity in the identification of cases with acidification defect were 62.9% and 56.2% respectively. If the point was taken at 0.3 mmol/l, the sensitivity increased to 93.4% with 7.4% specificity. Depending on the purpose of surveying, urinary citrate is a useful screening tool for the detection of cases with distal renal acidosis, with sensitivity ranging from 60 to 90% and specificity upto 60% depending on the level of citrate chosen.

A61. Mineral Metabolism and Bone Mineral Content in Calcium Nephrolithiasis with and Without Hyperparathyroidism

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We undertook this study to compare different parameters of mineral metabolism and bone mineral content (BMC) in a population of pts affected by hypercalciuria (H) and recurrent calcium nephrolithiasis (RCN), in presence or absence of histologically-proven primary hyperparathyroidism (PHPT). We evaluated 20 pts (15 M and 5 F, 24–68 yrs) with idiopathic H (380.6 ± 123.0 mg/day) and RCN. All but 2 pts had normal iPTH values (223.5 ± 113.0 pg/ml), while the T_mPO_4/GFR was reduced in 8 pts. $1,25(OH)_2D$ (48.1 ± 19.4 pg/ml) was at the high normal level in 11 pts. Urinary Ca excretion was directly related to urinary PO_4 excretion ($P < 0.005$) and iPTH ($P < 0.01$). No correlation was found between H and ionized Ca, serum PO_4, alkaline phosphatase, $1,25(OH)_2D$, blood pH, FEC_a, T_mPO_4/GFR. BMC, measured by dual photon absorptiometry of the lumbar spine, was 0.881 ± 0.150 gr/cm^2, which represents 88% of our control values. We compared these pts with a group of 13 pts affected by PHPT and RCN (10 M and 3 F, 20–70 yrs), characterized by iPTH and $1,25(OH)_2D$ values of 613.4 ± 276.3 pg/ml and 26.2 ± 9.6 pg/ml respectively, and a slightly higher degree of H (436.2 ± 146.0 mg/day). BMC (0.789 ± 0.180 gr/cm^2) was 84% of the control value. Urinary Ca excretion was increased in men compared to women as far as the H group was concerned (413.8 ± 124.7 vs 280.8 ± 23.3 mg/day, M vs F, $P < 0.05$), while no difference was found in the PHPT group (461.0 ± 149.9 vs 429.1 ± 156.0 mg/day, M vs F, NS). Lumbar BMC's were 85.1% (M) and 97.2% (F) in the H group, and 77.3% (M) and 86.5% (F) in the PHPT group. We conclude that pts with IH and RCN, as a whole, have a similar degree of bone demineralization, irrespective of presence or absence of PHPT, and the osteopenic process does not appear to be correlated with either iPTH or $1,25(OH)_2D$ values. However, though similar, the degree of demineralization in IH and PHPT is unexpectedly more severe in the male than the female population.

A62. Oxalate in Urine and Plasma Revisited – Evidence in Favour of Mild Hyperoxaluria in 24 h Urine of Male and Female Patients with Recurrent Idiopathic Calcium Urolithiasis (RCU)

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The magnitude of urinary oxalate excretion in RCU is unsettled for a number of reasons; e.g., imprecision of analytical methods; comparison of controls and patients using groups comprising both males and females of greatly differing age and body weight, all biasing the hyperoxaluria results in RCU subgroups with either normocalciuria (NC) or hypercalciuria (I-HC). In plasma, the instability of oxalate led to erroneously high values (>2 μM/l) and, thereby, prevented investigation of renal oxalate handling. We adopted the principle of anion chromatography (DIONEX i 2000) to the measurement of oxalate in both urine and plasma [1, 2]: in controls, NC, I-HC subjects (classification according to ref. [3]) of comparable age (♂: 21–65 years; ♀: 20–65), and body weight (♂: 53–110 kg; ♀: 51–87).

Results (see Table at the bottom of the page): In all groups the mean value for plasma oxalate is <2 μM/l, and the range of values is comparable to the one obtained with the isotope dilution technique [4]; in RCU plasma oxalate is low, with the smallest value in I-HC. Oxalate in urine after a 12–15 h nocturnal fast is unchanged in RCU; however, the fractional oxalate clearance resulting from underlying urinary and plasma oxalate exceeds creatinine clearance (= 100%) in five of the six groups, and in RCU generally tends toward higher values. Oxalate in 24-h urine is elevated in females with RCU but in males only in the NC subgroup.

Conclusions: (1) Ion chromatography allows assessment of plasma oxalate under selected conditions of sample preparation [2]; (2) in RCU there may be altered renal handling of oxalate; (3) the source(s) of mild hyperoxaluria in RCU remain to be determined.

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	n	Males			Females		
		Controls	NC	I-HC	Controls	NC	I-HC
Plasma-OX; μM/l	7–12[3]	1.98 (1.4–25)[1]	1.78 (0.8–4.0)	1.58 (1.2–2.2)[c]	1.78 (0.7–2.9)	1.69 (0.8–3.6)	1.21 (0.8–2.1)[a]
Urinary-OX; μM/2 b[2]	7–12[3]	21 (14–30)	17 (6–27)	22 (5–31)	25 (14–45)	21 (12–30)	21 (11–32)
Fractional-OX; %[2]	7–12[3]	84 (56–109)	123 (31–357)	143 (63–297)[b]	112 (34–292)	131 (39–202)	124 (30–287)
Urinary-OX; mg/24 h	25–36[3]	24 (10–57)	28 (11–52)[a]	27 (10–50)	17 (2–33)	23 (8–37)[a]	23 (5–47)[b]

[1] Data are mean values (and range); [2] in fasting urine; [3] number of participants per group; [a,b,c]: $P < 0.05$, <0.01, <0.001 versus controls

A63. Mechanisms of Hypocitraturia in Idiopathic Calcium Stone Disease

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The patterns of citrate excretion (uCit) in our large series of idiopathic calcium stone-formers (SF) demonstrated that no differences could be found with normal controls (NC) if data were matched as to sex, age, body weight, and GFR. However, even after patients with diseases known to lead to low uCit were excluded, 68 SF could still be defined as having hypocitraturia (Hcit). We report herein the results of further investigations on the possible mechanisms of this metabolic disorder. Hcit was confirmed in samples from a mean of three separate outpatient urine collections. Besides routine biochemistry tests, serum Cit (sCit) and fasting uCit were also determined. Arterial blood was drawn for acid-base values. Citrate clearance (CitCl), fractional excretion (FEcit) as well as net acid (NAE) and total nitrogen (TNE) excretions were calculated by standard methods. Patterns of uCit were also studied in family members from 20 Hcit-SF. Our results showed that neither sCit nor ultrafiltered load of Cit differed between NC and Hcit-SF, and Hcit could be accounted for by sharp decreases in both CitCl ($P < 0.001$) and FEcit ($P = 0.012$). The decrease in FEcit was closely related to FEK ($r = 0.512$, $P < 0.001$), and inversely to NAE ($r = -0.403$, $P < 0.001$), but not to FE Na. These data confirmed our previous observations of a lower uK and higher NAE and TNE in Hcit-SF as compared to patients with normal uCit. Fasting uCit was significantly lower in Hcit-SF than in NC ($P = 0.03$). Family studies have shown that when Hcit occurs, it is most likely shared by one (or more) first-degree relatives. We conclude that Hcit of idiopathic stone-disease is the result of a decrease in fractional renal clearance of Cit. Strong evidence suggests that this could mainly depend on potassium and acid-base balance-related disorders. The observed significant familial trend still did not allow the relative role of genetic and environmental factors to be better defined regarding its pathogenesis.

A64. Sulfur Aminoacids, Thiol Drugs and Related Mixed Disulfides from Urines of Cystine Stone Patients

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To identify abnormal peaks other than CIS, ORN, LIS, ARG found in urine specimens of cystinuric patients, column chromatography of 2-mercapto-propionylglycine (MPG) or D-penicillamine (PEN) alone and after incubation with cysteine, cystine, omocysteine and omocystine was performed. The analytical results allowed us to identify on the chromatograms the peaks of both MPG and PEN and of related mixed disulfides; in particular, MPG was eluted just before TAU and MPG-CYS disulfide just before ASP; PEN was eluted after cystine, PEN-CYS disulfide after cysteine, and cysteine-omocysteine mixed disulfide after LEU. Urine specimens from 18 healthy subjects and 20 cysteine stone patients with normal GFR were then analyzed. Ten of the patients were taking MPG, 3 PEN, and 7 were not taking drugs.

Results: Cysteine, omocysteine, MPG, and PEN peaks were undetectable in the urine from both patients and normal subjects; cystathionine and methionine excretions did not differ from that of normal subjects; TAU was lower than normal in patients on drug therapy and normal in the others. The peaks corresponding to cysteine-omocysteine mixed disulfide was present in all the patients, but its quantity varied markedly. Patients on thiol therapy showed peaks corresponding to the mixed disulfides MPG-CYS and PEN-CYS on their chromatograms. This finding represents evidence of patient compliance to therapy.

A65. Mitochondrial and Cellular Metabolism in Experimental Hyperoxaluric Nephrolithiasis

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The purpose of this study was to see if hyperoxaluria could induce cellular dysfunction in an experimental model of nephrolithiasis. Nephrolithiasis was induced by feeding rats regular chow supplemented with 4.6% ammonium oxalate for 10 days. Calcium-oxalate renal calculi, and not just crystals, were seen in 75% of the rats. Histological examination of the kidneys showed no evidence of cellular or tubular damage and pronounced intratubular crystallization. Nonetheless, clearance of endogenous creatinine was significantly lower. The rats exhibited significant hyperoxaluria, hypocitraturia, and acidosis. Kidney cortical mitochondria isolated from AmOxal-treated animals did not exhibit any marked alterations in their properties. Respiration rates studied under a variety of conditions, such as at rest and when stimulated by ADP, CCCP or A23187 plus calcium, were comparable to those in control mitochondria. The mitochondrial calcium content and the rates of calcium flux were also not significantly different. We then examined the magnitude of calcium uptake and calcium-triggered mitochondrial swelling in these animals. There was an inverse relationship between mitochondrial permeability and urinary oxalate excretion ($r = -0.836$), whereas no such correlation was found between mitochondrial calcium content and urinary oxalate ($r = -0.090$). Because mitochondrial functions may be affected by procedures used for isolation, we next determined cellular and mitochondrial functions in kidney cortical slices. This technique allowed the measurement of mitochondrial and cellular functions in a system that maintains cellular integrity and orientation. Gluconeogenesis from glutamine or lactate increased significantly in rats treated with either ammonium oxalate or ammonium chloride. The addition of citrate blunted this response. The increased gluconeogenesis seen in rats fed ammonium oxalate was therefore not the result of oxalate per se, but of the acidosis induced by ammonium oxalate. In summary, hyperoxaluria induced changes in membrane permeability but no significant alteration in mitochondrial or cellular metabolism in this model of hyperoxaluric nephrolithiasis.

A66. Effect of Oxalate on Kidney Mitochondrial Function

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We have shown previously (J Biol Chem 26:12197, 1986) that oxalate is transported into the mitochondrial matrix by the dicarboxylate carrier. The current studies were designed to evaluate the effect of oxalate on kidney mitochondrial function. In medium containing glutamate plus malate or glutamate plus pyruvate, oxalate did not inhibit respiration of kidney mitochondria at a concentration of 3 mM, that is 5 to 10 times higher than that detected in renal cortex. The ability of mitochondria to phosphorylate ADP, measured by ADP/0 ratio and by the acceptor control index, was likewise unchanged by 3 mM oxalate. Oxalate impaired, however, the uncoupling process of the renal mitochondrial membrane. In the absence of ADP, mitochondrial respiration stimulated by CCCP was progressively inhibited by oxalate, as was respiration stimulated by A23187 plus calcium. Furthermore, oxalate inhibited competitively the uptake and oxidation of extramitochondrial malate as well as succinate. Oxalate did not induce these biochemical changes in liver mitochondria. At concentrations up to 5 mM, oxalate did not alter the rate of uniporter-mediated calcium uptake by energized mitochondria at extra mitochondrial 17 μM $CaCl_2$. Moreover, the subsequently determined ruthenium-red-insensitive efflux of calcium was also unchanged. When 110

ngatom of calcium/mg of protein was added to kidney mitochondria in a medium containing glutamate/malate and phosphate, they quickly accumulated the calcium and started to swell after a 30-s lag phase. Oxalate did not alter the accumulation of calcium, but inhibited markedly the rate and amplitude of mitochondrial swelling. In sharp contrast, oxalate inhibited neither calcium uptake nor mitochondrial swelling in liver mitochondria. In conclusion, oxalate at concentrations five- to tenfold higher than those detected in rat kidney cortex and medulla: (1) did not impair the supply of energy from mitochondria; (2) had no effect on the rate of calcium redistribution by mitochondria; (3) interacted specifically with kidney mitochondrial membranes or with the processes controlling their integrity.

A67. Histological and Biochemical Characterization of a Model of Nephrolithiasis Incorporating Proximal Tubular Dysfunction

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Considerable evidence suggests that nephrolithiasis is associated with underlying defects in renal tubular function. This study was designed to develop a model of renal stone formation in the background of proximal tubular dysfunction. Proximal tubular dysfunction was induced by administering 40 mg/kg per day of gentamicin SC to rats. Animals were fed Purina rat chow containing no added oxalate, whereas experimental animals were fed diets containing 4.6% ammonium oxalate, starting at day 4 of gentamicin treatment. Animals were killed at 8 and 14 days and morphological and biochemical studies were performed. The kidneys looked normal except for minimal discoloration in rats treated with gentamicin alone. The addition of oxalate caused swelling and extensive discoloration of the kidneys by 8 days (or 4 days of oxalate treatment). Calcium-oxalate renal calculi were seen in the papillary tips of animals treated with oxalate at 4 days. After 7 days of gentamicin, histological changes in the kidney were minimal. In contrast, the tubular cells were grossly enlarged and contained intracellular bifringent deposits within 4 days of oxalate feeding. Many cells were destroyed and the nuclei were extruded into the lumen of the tubule. Crystals could be seen within the proximal tubular cells within 4 days. Administration of gentamicin to rats for 7 days had no effect on respiratory capacity of kidney cortical mitochondria. Oxalate feeding had little effect on ADP-stimulated respiration but decreased uncoupled respiration. The total calcium content was markedly elevated in kidney mitochondria isolated from gentamicin-treated rats and returned to control levels after discontinuation of gentamicin. The respiration rate during calcium accumulation was also enhanced. Oxalate caused a further increase in calcium content and respiration stimulated by calcium addition. Prolonged administration of oxalate led to a decline of oxalate uptake by energized mitochondria, resulting in comparable rates of uptake in nonenergized and energized mitochondria. In summary, oxalate feeding to rats with preexisting tubular dysfunction induced mitochondrial dysfunction, intracellular calcium oxalate crystallization, and stone formation.

A68. The Pattern of Urinary-Tract Stone Disease in Croatia

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In this study, 10,000 consecutive urinary calculi that were passed or operatively removed from patients originating from the continental part of Croatia were analyzed, using the semiquantitative infrared spectrometric method: 45% of stone formers had a single stone, 16.0% multiple stones, 5.5% passed "sand," and for 34.5% there were no data. The ratio of frequency between males and females was 51%:49%. The stones weighed 0.01–123.9 g. On the cut surface in 17.5% of stones, the cores were discernible. In stones with cores, the cores and the shells were analyzed separately. In the remainder, the stones were analyzed as a whole, and information on the principal components, admixtures, and traces was obtained. A compound present in a concentration of 25% or more was considered a principal component. In stones having a core, the core was most frequently composed of phosphates (53.2%) and the shells of oxalates (56.5%). In the stones analyzed as a whole, the principal components were as follows: oxalates in 49.3%, phosphates in 37.8% (among them struvite in 10.1%), uric acid compounds in 9.7% (among them ammonium hydrogen urate in 0.06%), cystine in 0.3%, and other constituents in 2.9%. Only 10.4% of stones were found to be pure, consisting of only one component. At present, a multicentric study of urolithiasis is taking place in Croatia. Relevant clinical data are being stored in the computer center and are available for the investigations and a more detailed characterization of urolithiasis. The data presented in this paper suggest that the pattern of urinary stone composition highly resembles the pattern found in the majority of developed European countries, whereas there is a marked difference if compared with other regions of Yugoslavia, for example, Dalmatia and SAP Kosovo.

A69. A Method for the Determination of Urinary Ammonium

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In the course of an investigation concerning the formation of infection stones, we tried to find a method for rapid and reliable determination of ammonium in human and animal urine samples. For this purpose we tested the suitability of an ion-selective electrode (ISE). Ammonium was determined as ammonia using the ORION ammonia-electrode type 95-12 (Colora Meßtechnik GmbH, D-7073 Lorch) with a commercial pH/mV meter. For each of the series a logarithmic calibration curve was calculated from aqueous standards (NH_4Cl: 5–100 mmol/l). For the preparation of the specimens, 100 ml of the urine samples or standards was adjusted to pH 11. For this, the addition of 1 ml 10 N NaOH has been found to be generally appropriate. The measurements were taken by immersing the electrode into the urinary solution, which was stirred using a magnetic stirrer. The mV values were read after 2 min. Both within-run imprecision and total imprecision were estimated for this method. In a method-comparison experiment with 80 urine samples, the results obtained with the ISE were compared to those acquired by Schmidt's method, which utilizes Folin's principle. Handling of the ISE turned out to be generally easy. During our experiments, it was noted that the mV value to be read off only slowly became stabilized. Yet the value taken after 2 min was close to the final value. For the ISE, a within-run imprecision of 1.35% (CV) and a total imprecision of 11.9% (VK) were calculated. Compared to the titrimetric method (relative accuracy), it could be shown that, statistically speaking, both methods lead to identical results. Therefore, we conclude that the ion-selective electrode tested here is appropriate for the determination of urinary ammonium.

A70. Serum and Urinary Citrate in Normal Persons and Patients with Calcium Urolithiasis

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This study was carried out in order to investigate citrate metabolism (i.e., serum levels, filtered load, tubular reabsorption, total and

fasting urinary excretion) in a large group of normal subjects (n = 39) and kidney-stone patients (n = 45). Metabolic tests included a 24-h urine collection to determine citrate, Ca, Mg, P, and creatinine (Cr), followed by a 2-h collection for the determination of the citrate/cr ratio, calcium excretion, the hydroxyproline/Cr ratio and TmP/GFR. A blood sample was also taken for the measurement of citrate, Ca, Mg, P, Cr, Na, K, and iPTH. Serum and 24-h urinary uric acid levels were also assessed in kidney-stone patients. Citrate in serum and urine was measured by an enzymatic method using a Perkin Elmer Lambda 7 UV/VIS spectrophotometer. We found that mean serum citrate levels and filtered loads were similar in the two groups studied. In contrast, both the 24-h urinary citrate (487.8 ± 283.7 mg vs 673.2 ± 200.7; $P < 0.001$) and the fasting citrate/Cr ratio (0.29 ± 0.20 vs 0.46 ± 0.19; $P < 0.001$) were significantly reduced whereas the mean tubular reabsorption of citrate (86.2 ± 7.7% vs 78.4 ± 7.1; $P < 0.001$) appeared to be significantly increased in kidney-stone patients with respect to control subjects. A good correlation was found between fasting and 24-h urinary citrate excretion both in normal subjects (r = 0.72; $P < 0.001$) and in kidney-stone patients (r = 0.77; $P < 0.001$). Finally, no correlation was noted in the two groups studied between the serum-citrate levels and the direct and indirect indexes of parathyroid function, with the exception of a correlation observed in normal subjects between calcium excretion and the fasting citrate/Cr ratio ($P < 0.05$). Our data indicate that the kidney appears to be involved in the pathogenesis of hypocitraturia in kidney-stone patients and that a substantial proportion of them show a reduced urinary excretion of citrate. Finally, evaluation of the fasting citrate/Cr ratio may replace measurement of the substance on the basis of a 24-h urine collection.

A71. Relapsing Calcium Stones – The Therapeutic Implications in Daily Calciuria

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The aim of the present study was to evaluate the importance of the "real" daily calciuria in normal subjects and in stone-formers. At the same time, we analyzed whether hypercalciuria could be significantly correlated to relapsing calcium stones. Using a colorimetric method (0-cresolphtalein at 575 nm), performed on 3 consecutive days on 1,870 hospitalized patients during the last 2 years, we monitored the daily urinary calcium; the results were compared with: body weight, sex, presence or not of urinary diseases, GFR, hypertension, isolated calcium stones, relapsing calcium stones (with or without contemporaneous therapy) and diet. In normal subjects the daily calciuria was lower than the bibliographic data: on average 1.46 ± 1.52 mg/kg per day. No correlation between calciuria and clinical parameters was observed except in normal subjects (104 ± 107 mg/day) vs the patients with relapsing calcium stones (218 ± 93 mg/day), $P < 0.01$. Of these stone-formers with daily calciuria (on average) double than normal subjects, 23 cases presented significant hypercalciuria (256 ± 95 mg/day), and 19 cases were in the normal range (171 ± 58 mg/day). Oral thiazides rapidly lowered urinary calcium excretion in all 23 patients up to 95 ± 36 mg/day, but the incidence of relapsing stones decreased (from 0.92 to 0.33 stone/per year) as fast as in the 19 normocalciuric patients (from 0.89 to 0.35 stone/patient/year). In conclusion, we can consider the normal urinary calcium excretion range to be up to 3 mg/kg per day; the use of drugs in hypercalciuric patients does not seem of real therapeutic interest.

A72. Transport of Oxalate in Intact Red Blood Cells Can Identify Potential Stone-Formers

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Red blood cells (RBCs) have often been used as an index parameter for a number of metabolic disturbances in the body. Urolithiasis is associated with an apparent disturbance in calcium and oxalate metabolism. To investigate this prospect, oxalate self-exchange transport was studied in intact RBCs of 33 idiopathic stone-formers and 21 normal individuals [1]. The flux rate in the RBCs was represented by the flux constant (k) in each case, calculated on the basis of regression analysis as

$ln(A_t - A_\alpha)/ln(A_0 - A_\alpha)$ versus "time"

where A_0, A_t and A_α are the concentrations of the ^{14}C-oxalate left in the medium at time zero, t, and infinity, respectively. Stone-formers showed a very high range of flux constant with a mean value of k = 0.81 ± 0.57, as compared to the normals who showed the mean flux constant of k = 0.10 ± 0.11. This remarkable difference in flux constant of oxalate in RBCs of stone-formers and normal subjects may provide an unique method of identifying the persons who may be potential stone-formers.

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A73. Prevalence of Distal Renal Tubular Acidosis in Five Khon Kaen Villages

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Our previous study suggested a high prevalence of symptomatic primary distal renal tubular acidosis (dRTA) in Khon Kaen and Ubol. The purpose of this study was to investigate the prevalance of this condition, also including asymptomatic cases, in five villages in Khon Kaen. A total of 3,007 villagers from five villages in Khon Kaen were screened for their urinary citrate concentration. An acid loading test was done in 566 villagers whose urinary citrates were below 0.3 mmol/l; 108 were found to be unable to acidify their urine below 5.5 and 16 had complete renal tubular acidosis. The prevalence of acidification defect ranged from a minimum of 1.1% to a maximum of 5.0% in different villages. Plain KUB was performed in 369 and 64 villagers with low and normal urinary citrate, respectively. About 20% of cases in both groups showed roentgenographic abnormalities, with renal stone and/or nephrocalcinosis. However, among cases with low urinary citrate, plain KUB abnormalities is significantly higher ($P < 0.001$) in groups with acidification defect that those without. This study confirmed the high prevalence of dRTA in the Khon Kaen areas and suggested an association between urinary acidification defect and the occurrence of renal stones. Our preliminary data indicated a higher prevalence of dRTA in villages with a poorer socioeconomic status.

A74. What is Renal Stone Matrix?

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The organic material "matrix" in renal stones is generally considered to be mucoproteins. However, in the fragment material

obtained from stones subjected to extracorporal shock-wave lithotrypsy (ESWL), fibers were observed. Similar fibers were also present in intact stones. Obviously, fibers were really a component of the stones and not an artifact resulting from the ESWL procedure. We attempted to characterize the fibers chemically and examined the fibers both under ordinary and ultraviolet light, as well as by scanning electron microscopy. Morphologically, we distinguished the following groups: (a) coarse fibers: no color, no fluorescence; (b) white fibers: no color, blue-white fluorescence, forming a meshwork through the material; (c) red fibers: red color, red or no fluorescence; (d) black, green, blue, and violet fibers: colored and nonfluorescent; (3) membranes: nonfluorescent and transparent. After an attempt to hydrolyze the material in 6 mol/l HCl at 110 °C for 5 days, most of the material remained unaffected. Amino acids were present in the solution, but also components that reacted with ninhydrin without being identified with any known reference amino acid. Boiling with 10 mol/l NaOH did not alter the material. Sodium hypochloride destroyed coarse fibers, but the others were unaffected. A lot of organic solvents were tried, none of which dissolved the material. Concentrated nitric acid and bichromate sulfuric acid were the only agents found to dissolve the material. Scanning electron microscopy, combined with X-ray energy analysis, revealed the absence of atom numbers greater than 8. Infrared spectroscopy and pyrolysis gas-chromatography mass spectrometry gave no key information on the nature of the material. The fibers could not be characterized as any known class of organic compounds. They may be of protein origin, but modified (cross linkage?) to give the described shape and chemical stability. They may be an important factor in the stone-formation process.

Conclusion: Renal stone "matrix" is evidently of a far more complicated structure than earlier suggested and contains materials of an undefined class of organic substances.

A75. Increased Frequency of Hypercalciuria in Stone Formers with High Protein Intake

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The frequency of a high protein intake (above 100 g protein/day) and of associated urinary abnormalities was assessed in 120 male calcium stone formers with normal renal function. The patients were divided into three groups according to their 24-h urea excretion (Ue): group I, $n = 42$, Ue < 400 mmol/day (312 + 76); group II, $n = 38$, 400 < Ue < 500 mmol/day (444 + 31); group III, $n = 39$, Ue > 500 mmol/day (606 + 46). The urinary excretions (mmol) expressed as ratios to creatinine (Cr) showed a tendency to increase with high Ue (see below).

	Urea	Ca	Na	Mg	Uric acid	Phosphate
G I	21.7	0.42	10.5	0.28	0.24	1.82
+/− SD	6.8	0.18	4.0	0.11	0.08	0.80
G II	26.3	0.44	11	0.30	0.26	1.80
+/− SD	5.3	0.20	4.8	0.10	0.12	0.39
G III	33.4	0.53	11.7	0.30	0.26	1.95
+/− SD	7.0	0.23	4.2	0.12	0.09	0.74

Urea and calcium in GIII vs GI and GII: $P < 0.01$

Hypercalciuria (Ca/Cr > 0.45) occurred more frequently ($P > 0.01$) in GIII (61.5%) than in GI (34.5%) and GII (34.2%), while in normocalciuric subjects, Ca/Cr was similar in all groups.

Conclusion: High protein intake is frequent (32.5%) in male stone formers and is associated with increased excretion of most lithogenic substances. Hypercalciuria is particularly frequent in those subjects, a finding which might be related to acidosis or dietary habits and should lead to primary dietary management of stone disease in such patients.

A76. Clinical Application of a New HPLC Determination of Urinary Glycolate

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Glycolic (GLY) and glyoxylic (GLX) acids are the main known precursors of oxalate in man. An increase in these carboxylic acids is a well-known finding in cases of type-I primary hyperoxaluria. The availability of an accurate method for determination of the above acids is also suggested as a useful tool in the metabolic study of the mild hyperoxaluria syndromes. In this work we propose a new HPLC technique for the determination of urinary GLY, based on α-ketoacids precolumn derivatization by means of phenylhydrazine coupled with enzymic oxidation of GLY to GLX. The resulting phenylhydrazone strongly absorbs at 324 nm and may be separated and detected by LC. The sensitivity of the method is 10 μmol/l for GLY and 0.5 μmol/l for GLX. Contrary to GLY, GLX has poor stability in biological fluids; therefore, for clinical purposes, only GLY was considered. Urinary GLY was determined in 24-h samples from 20 normal subjects and 45 idiopathic calcium stone-formers (ICaSF). Urine GLY was not significantly different in controls as compared to ICaSF ($P = 0.506$). GLY excretion was independent on sex, age, and body weight. A mild correlation was found with GFR ($P < 0.01$). There was a close correlation between GLY and oxalate excretions ($P < 0.001$). Both parameters were independent of both total nitrogen and main cation excretion. Our results confirm that the accuracy and reliability of the method proposed here make it suitable for clinical metabolic studies. The data obtained support the view that GLY excretion can be regarded as a faithful index of the rate of oxalate biosynthesis and excretion.

A77. Differentiation of Unclassified Hypercalciuria Utilizing an SCP Trial

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Many patients with hypercalciuric nephrolithiasis present with unclassified hypercalciuria (fasting hypercalciuria without parathyroid stimulation), which may mask the diagnosis of absorptive or renal hypercalciuria. Proper classification of patients with undifferentiated hypercalciuria has important diagnostic as well as therapeutic implications. A total of 42 patients having undergone our standard outpatients evaluation were identified as having fasting hypercalciuria (>0.10 mg/100 ml GF) with normal parathyroid hormone (PTH) levels (<400 pg/ml). Sodium cellulose phosphate (SCP) was given orally with meals for 3 days in order to remove the effects of absorbed calcium (Ca). On the 2nd day, a 24-h urine sample was collected for Ca and on the morning following SCP administration, a 2-h fasting urine sample was obtained for Ca, and a blood sample for PTH. The SCP trial results suggest that of the patients studied 25 may actually have absorptive hypercalciuria (AH), since the SCP restored normal fasting urinary calcium without causing parathyroid stimulation. In 6 patients. SCP unmasked parathyroid stimulation, thus suggesting true renal hypercalciuria (RH). However, 11 patients

	n	Initial evaluation			SCP trial		
		Ca (mg/day)	Fast (Ca/Cr)	PTH (pg/ml)	Ca (mg/day)	Fast (Ca/Cr)	PTH (pg/ml)
AH	25	299	0.15	225	156^{+}	0.06^{+}	257*
RH	6	300	0.17	202	119**	0.10**	438^{+}
FH	11	310	0.19	217	119**	9.16	242

* $P < 0.05$, ** $P < 0.01$, $^{+}P < 0.001$

showed persistent fasting hypercalciuria (FH) without parathyroid stimulation. It is believed that the persistent FH patients may represent a primary overproduction of 1,25-$(OH)_2$ vitamin D or resoprtion of bone.

A78. Urolithiasis and Atherosclerosis – Is There an Association?

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Idiopathic urolithiasis and coronary artery disease have been described as diseases of the affluent communities. Both conditions are strongly influenced by environmental factors such as diet. During the course of an investigation of the effects of various nutrients on risk factors for stone in normal subjects and idiopathic stone formers, an opportunity also arose to assess the risk factors for atherosclerotic disease. Eleven normal subjects and 9 male stone formers were studied. The tests were performed on 4 separate days on each individual. On day 1, after overnight fasting, venous blood samples were obtained just before 0900 hours when 400 ml of distilled water was given by mouth. Blood samples were obtained at 2-h intervals over the ensuing 8 h. On days 2, 3 and 4, the test was repeated in a similar fashion, except for a test meal at 0900 hours of isocaloric quantities of glucose, animal protein and fat, respectively. The blood samples were analyzed for cholesterol, triglycerides and apolipoproteins A1 and B. Stone formers were found to have higher plasma cholesterol and triglyceride levels when compared to normal subjects. The levels of cholesterol, triglycerides and apolipoproteins A1 and B showed an increase in both groups after the ingestion of animal protein and fat. The results suggest that the dietary risk factors for urolithiasis and degenerative arterial disease are similar, and stone formers may be at an increased risk of atherosclerosis and related conditions. This indicates a need for epidemiological studies to investigate the relationship between the two disorders.

A79. The Role of Insulin and $1,25(OH)_2D_3$ in Idiopathic Urolithiasis

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The mechanism for the calciuric effect of refined carbohydrates is not clear, but it may be mediated through insulin. $1,25(OH)_2D_3$ has also been implicated in certain forms of hypercalciuria. There is some evidence to suggest that insulin may stimulate the renal synthesis of $1,25(OH)_2D_3$. Nine recurrent idiopathic renal stone formers and 11 normal subjects were investigated on 4 days. On each day, after overnight fasting, a test meal of distilled water, 100 g of glucose, steamed haddock (400 g) and cream (50 ml) was given at 0900 hours, respectively. Blood samples were obtained before and at frequent intervals for 8 h after ingestion of the test meal and analyzed for insulin, PTH, $25(OH)D_3$, $1,25(OH)_2D_3$, calcium, phosphate, glucose, and creatinine. Urinary calcium and creatinine were monitored in the fasting state and after the test meals. The fasting urinary calcium was significantly higher in stone formers ($P < 0.001$). Ingestion of glucose and animal protein increased the calcium excretion in both groups, but the response was exaggerated in stone formers. The plasma insulin response to glucose was significantly higher in stone formers. There was a positive correlation between plasma insulin levels and urinary calcium (patients $r = 0.9$, $P < 0.01$; controls $r = 0.8$, $P < 0.02$). There was no difference at any time between the two groups in respect of $25(OH)D_3$, $1,25(OH)_2D_3$, PTH, serum calcium and phosphate, although the levels in stone formers exhibited wide fluctuations. The results suggest that the hypercalciuria induced by glucose is mediated through insulin. It appears that $1,25(OH)_2D_3$ may not be the primary cause for hypercalciuria and the abnormal values reported in stone formers by others merely reflect the adaptive changes due to the labile nature of calcium metabolism in stone formers.

A80. Role of Vitamin A Deficiency in Experimental Urolithiasis

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Experimental vitamin A deficiency in rats is reported to result in a high incidence of urolithiasis. To confirm these findings under more physiological conditions, and to understand the underlying mechanism, studies were undertaken in rats. Weanling male rats were grouped into three groups: (1) ad libitum control (2) pair-fed control, and (3) vitamin A deficient, with 18 rats in each group. All the rats were fed a potential calculogenic diet high in calcium content. The diet of the experimental group was devoid of any source of vitamin A. After 20 weeks, important lithogenic properties of urine were studied. Size of urinary crystals, urinary creatinine, oxalic acid, uric acid, calcium, magnesium, phosphorus, citrate and glycosaminoglycans were estimated. Incidence and size of urinary calculi were recorded. Vitamin A deficiency was characterized by massive aggregates of calcium oxalate crystals in urine. Most of calcium -oxalate crystals (68%) were in the range of 20–70 μm in vitamin A deficient rats. In contrast, in urine of control rats, the proportion of oxalate crystals of these sizes was only 25%. The incidence of bladder calculi was 70% in vitamin A deficient group. This incidence was only 20% in control groups. Calcium oxalate was the predominant component of these stones. The vitamin A deficient state led to increased excretion of oxalate and uric acid. A significant fall in the concentration of glycosaminoglycans of urine was also observed in vitamin A deficient rats. Urine of these deficient rats exhibited significantly less inhibitory activity towards calcium oxalate crystal growth compared to the control groups. A significant positive correlation between inhibitory activity and glycosaminoglycans of urine, was observed in all the groups ($r = 0.82$; $P < 0.05$). This study clearly demonstrated that vitamin A deficiency, even under more physiological conditions, increases the risk of urolithiasis. This effect appears to be due to increased excretion of urinary risk factors – oxalic acid, uric acid, and decreased excretion of important inhibitor compounds, namely, glycosaminoglycans.

A81. Binding of Calcium Oxalate and Apatite Crystals to Renal Papillary Collecting Tubule Cells in Primary Culture

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Attachment of microcrystallites within the renal papilla may be an important component of the pathophysiology of urolithiasis. These studies use rat renal papillary collecting tubule (RPCT) cells in primary culture to characterize crystal-cell binding as an in vitro model of nephrolithiasis. Primary cultures of medullary tubule cells pooled from the kidneys of 150–250 g male Sprague-Dawley rats were grown on 12 mm diameter circular glass cover slips. Cultures became confluent in 3–5 days. To study the concentration-dependent binding of microcrystallites, the cells on the cover slips were incubated with ^{14}C-calcium-oxalate monohydrate (CaOx) and ^{45}Ca-apatite (AP) crystals for 30 min at 37 °C in an artifical urine buffer at pH 6.0. The cells were washed and quantified by microscopy and adherent radioactivity. CaOx crystals were found to be bound preferentially to clumps of cells distributed throughout the monolayer. CaOx and AP showed concentration-dependent saturation with a $1/\alpha$ value (maximum μg of crystallites that would adhere to one cm^2 of binding area) for CaOx of 287 μg/cm^2, and a $1/\alpha$ value for AP of 113 μg/cm^2. Simultaneous incubation of ^{14}C-CaOx crystals with cold AP crystals demonstrated concentration-dependent inhibition of CaOx binding. To show that this inhibition was occurring at the cell-binding regions, RPCT cells were incubated with cold AP crystals, washed, and reincubated with ^{14}C-CaOx crystals. The inhibition of CaOx binding was similar in magnitude to the previous inhibition experiment. By using low concentrations of crystals, the β-values (fraction of cross-sectional cell area which bound the crystallites) for AP and CaOx were compared. The β-values for both crystals varied from 0.08 to 0.28, depending on the cell preparation and intensity of wash. However, AP consistently demonstrated a slightly higher β-value for all cell preparations and washes. In conclusion, CaOx and AP adherence to RPCT cells demonstrated selectivity for the cellular clumps and concentration-dependent saturation. Inhibition of CaOx adherence by either simultaneous or previous incubation with AP crystals implies the existence of common binding areas for CaOx and AP microcrystallites. Also, the higher β-value for AP suggests that AP may bind to more sites than CaOx. Finally, all of these data, when taken together, suggest specificity in binding of CaOx and AP microcrystallites to RPCT cells in primary culture.

A82. The Calculation of Stone Risk in the Urine of Middle Eastern Men and Western Expatriates Living in Saudi Arabia

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Twenty-four-hour urine specimens were collected from groups of normal Middle Eastern men (NME), Middle Eastern idiopathic stone-formers and normal Western expatriate men (NWE) living and working in Saudi Arabia. The stone-formers were divided into those who had predominantly calcium-containing stones (CaSF) and those with predominantly uric acid or urate-containing stones (UASF). The urines were analyzed and various parameters of the risk of stone-formation were calculated. The latter included the supersaturation of urine with respect to calcium oxalate (CaOx), calcium phosphate (CaP) and uric acid (UA), and the overall biochemical risk of forming Ca-containing stones (PSF_{Ca}) and UA-containing stones (PSF_{UA}). The data were compared with corresponding figures from normal men living in the UK (NUK). The results showed that the mean values for PSF_{Ca} were in the order CaSF, NME, NWE, NUK. The corresponding values for PSF_{UA} were in the order UASF, NME, NWE, NUK. The data on the supersaturation of urine showed a similar pattern of risk of stones in the various populations. We conclude that the risk of both CaOx and UA stones is increased in normal Western men when they move from the West to live in Saudi Arabia. However, the risk of both types of stone is markedly higher in Middle Eastern men than in either NWE or NUK.

A83. Insulin Stimulates Intestinal Ca Absorption in Man and the Rat

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Abnormally high postprandial plasma insulin levels have been observed in idiopathic hypercalciuric (IHC) recurrent Ca-urolithiasis patients. On the other hand, the mechanisms and mediators of the accompanying elevated intestinal Ca absorption (CaA) in these patients are largely unknown. Starting which the hypothesis that postprandial hyperinsulinemia and hyperabsorption of Ca might be interrelated, we have performed studies on the effects of the postprandial-like plasma levels of insulin on CaA in man and the rat [1]. In 36 male SPD rats (mean BW 250 g), the duodenal bidirectional fluxes (lumen-to-plasma, LP; plasma-to-lumen, PL) and net absorption of Ca were evaluated using an in situ loop technique under hyperinsulinemic euglycemic clamp conditions with three different steady-state plasma insulin levels (66 ± SEM 16, 187 ± 13, 263 ± 24 μU Eq/ml) obtained by IV infusion of 20, 40 or 60 mU/h of insulin (Actrapid, Novo, Denmark), or during vehicle infusions (steady-state plasma insulin 22 ± 2 μU Eq/ml). In 9 healthy males, 3-h cumulative CaA was studied using in ^{85}Sr/^{47}Ca double isotope technique, during either hyperinsulinemic euglycemic clamp and IV infusion of 40 mU/m^2 min insulin (H-Insulin, Hoechst, FRG) or vehicle infusions. The subjects served as their own controls. Mean plasma insulin was 6 ± 1 and 172 ± 10 μU/ml during vehicle infusion and glucose clamp, respectively. CaA was calculated by deconvolution. LP and net CaA of the rats increased significantly and were dose-dependently under all conditions, while PL was not changed by any of the three insulin infusion doses. Serum parathyroid hormone (PTH) was not influenced by insulin. In the healthy men, 3-h cumulative CaA rose by 14% during glucose clamp ($P < 0.01$ vs vehicle), while the accompanying urinary ^{40}Ca excretion rose and the excretion of the orally administered tracer ^{47}Ca dropped significantly. PTH as well as 1,25(OH)$_2$ vitamin D were unchanged throughout the clamp procedure in comparison to the vehicle trial. We conclude that the physiological degree of hyperinsulinemia enhances CaA above normal in man and the rat. This action appears to be independent of PTH and 1,25(OH)$_2$ vitamin D. Hyperinsulinemia might possibly contribute directly to the hyperabsorption of Ca in IHC patients.

References: 1. Rümenapf G, Issa S, Schwille PO (1987) Metabolism 36:60–65

A84. Biochemistry of Crystalluric Versus Noncrystalluric Stone Patients

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Crystalluria is a definite finding in a significant number of urinary stone patients, but it is not yet established whether or not positive evidence of crystalluria is related to any specific biochemical ab-

normality. In this paper, the biochemistry results of 100 stone patients with crystalluria are compared with a similar number without crystalluria. Twenty-four-hour urine samples were collected and, together with the serum samples, calcium, phosphorus, uric acid and creatinine were estimated. The mean values of the two groups were compared and the statistical differences worked out. The study findings regarding the statistical significance of the difference between values in the two groups are shown below:

Parameter	Crystalluric (mean and SE)	Noncrystalluric (mean and SE)	*P* value of difference
Serum calcium (mg%)	10.03 (0.12)	10.32 (0.06)	NS
Serum phosphorus (mg%)	3.56 (0.14)	3.68 (0.08)	NS
Serum uric acid (mg%)	4.86 (0.16)	4.80 (0.09)	NS
Serum creatinine (mg%)	1.15 (0.05)	1.25 (0.08)	NS
Urine calcium (mg/day)	213 (4.58)	208 (4.49)	NS
Urine phosphorus (mg/day)	568 (37.15)	593 (14.6)	$P < 0.001$
Urine uric acid (mg/day)	533 (26.85)	528 (13.08)	NS
Urine creatinine (g/day)	1.32 (0.1)	1.31 (0.3)	NS

NS, Not significant; SE, Standard error

The findings indicate that the biochemical values of the two groups did not vary significantly, except for the urine uric acid values. The study exemplifies the fact that crystalluria is independent of the biochemistry of the stone patient. Multiple factors play a role in the causation of urolithiasis.

A85. Contrasting Effects of Various Potassium Salts on Acid-Base Status, Urinary Citrate Excretion, and Renal Citrate Clearance

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Recently we have demonstrated that hypocitraturia is a common finding in patients with calcium (Ca) and uric acid (UA) nephrolithiasis. Oral potassium citrate (K-Cit) treatment has been effective in enhancing renal citrate excretion in such patients (Am J Med 79: 284–288, 1985). The present study is designed to determine if the citraturic action is: (a) a result of the alkali load provided by K-Cit and the effect of alkali on renal citrate excretion; (b) in addition to the role played by changes in systemic pH, bicarbonate and potassium ions might directly affect renal citrate metabolism; and/or (c) a result of absorbed citrate (via increased filtered load), which might contribute to urinary citrate excretion and citrate clearance. Based upon the above considerations, we compared the effects of oral K-Cit therapy with those of $KHCO_3$ and KCl. To test this hypothesis, eight patients with Ca and UA nephrolithiasis participated in four phases of study, control (no drug), and three randomly timed treatment phases, each of 2 weeks duration, with treatment consisting of 80 mEq/day of each potassium salt, while maintained on a neutral ash diet. At the end of each 2-week period, patients were admitted to the General Clinical Research Center, and fed a constant neutral ash metabolic diet (consisting of 400 mg Ca, 800 mg P, 100 mEq Na) for 4 days. Both K-Cit and $KHCO_3$ therapies increased urinary citrate significantly from 474 ± 298 (SD) to 983 ± 319 and 860 ± 296 mg/day ($P < 0.05$) and renal citrate clearance from 8.0 ± 13.1 to 27.4 ± 9.1 and 25.8 ± 6.3 ml/min ($P < 0.05$), respectively. Both K-Cit and $KHCO_3$ significantly decreased urinary ammonium and titratable acid and significantly increased urinary bicarbonate and total CO_2 excretion. Due to the aforementioned changes, urinary net acid excretion fell significantly during both K-Cit and $KHCO_3$ therapies to −7.6 ± 3.8 mEq/day and 7.3 ± 16.7 mEq/day from 32.7 ± 16.2 mEq/day ($P < 0.05$), respectively, and both were significantly correlated with renal citrate clearance ($r = 0.85$) ($P < 0.05$). Moreover, the effect of K-Cit treatment on acid-base, citrate excretion, renal citrate clearance, and plasma citrate was more prominent than with an equivalent amount of $KHCO_3$. KCl treatment did not alter urinary citrate or renal citrate clearance. Therefore, we conclude: (1) both K-Cit and $KHCO_3$ therapies are effective in raising urinary citrate and renal citrate clearance principally due to alkali effect and alteration in acid-base and (2) absorbed citrate (via increased filtered load) contributed only slightly to the rise in urinary citrate.

A86. External Quality Assessment of Urine Oxalate Analysis – 3 Years Experience

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A quality assessment scheme has been organized to examine the reliability of urinary oxalate results produced from laboratories. Sixty departments covering a range of workloads have participated in the scheme, the majority of these being from the UK. They have been circulated at regular intervals with lyophilised urines and standard solutions embracing a range of oxalate (and ascorbate) concentrations. The Sigma Kit oxalate-oxidase procedure has been the most popular method, but precipitation, immobilised enzyme, and HPLC techniques have all been represented. Significant points to emerge from the study so far include: (a) the wide scatter of results between laboratories with C.V. values on a given urine sample usually exceeding 20%; (b) the poor precision and accuracy exhibited by the precipitation techniques, particularly at lower concentrations; (c) the positive interference caused by ascorbate, at least in the Sigma Kit procedure; (d) the adverse effect on precision caused by ferric-chloride-based ascorbate-removal techniques, with sodium nitrite emerging as the preferred agent for this purpose; (e) the encouraging performance of the automated immobilised oxalate oxidase technique in the two departments adopting this approach. The scheme has shown that in many departments the standard of oxalate analysis may not be meeting the clinical need, particularly with respect to the detection of marginal hyperoxaluria. In laboratories with low workloads, the Sigma Kit procedure continues to be popular but must be coupled with a suitable ascorbate-removal stage. Centralisation of oxalate analysis within those departments able to utilise more sophisticated approaches (e.g. immobilised enzymes, HPLC) may represent an alternative approach to reducing the unacceptable variation of results between laboratories. It is hoped that as more date are generated by the scheme, ideally with a larger number of participants, some much-needed recommendations will be possible regarding the approach to this assay.

A87. Statistical Characterization of a Selected Group of Patients with Recurrent Calcium Nephrolithiasis

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We studied 253 subjects, aged 17 to 70 years, out of a total population of 540 patients affected by recurrent calcium nephrolithiasis,

who were subsequently referred to our clinic. The sample population was stratified into five different classes according to age. Patients affected by diabetes, obesity, hyperuricemia, hypertension, suspected hyperparathyroidism, obstructive nephropathies, "staghorn" nephrolithiasis, persistent urinary infection, and renal insufficiency were excluded from the study. The statistical analysis of the main biochemical parameters emphasized significant correlations related to the "surgical" activity of the disease (115 patients), which is associated with a longer duration of the disease ($P < 0.001$), the "clinical" activity ($P < 0.005$), and the occurrence of bilateral nephrolithiasis ($P < 0.001$). The "clinical" activity of the disease (49 patients with more than two newly formed calculi in the last 2 years) correlated with some metabolic parameters such as urinary calcium excretion, both in the basal state (280.9 ± 133.4 vs 246.1 ± 98.0 mg/day, high vs low clinical activity, $P < 0.05$), and after a low-calcium diet (241.6 ± 133.3 vs 202.8 ± 83.8 mg/day, $P < 0.01$), and uric acid excretion ($P < 0.001$). Using the same parameters, we also found a significant correlation between the "surgical" and the "metabolic" activity of the disease ($P < 0.001$). Moreover, an increased incidence in previous urinary infections, both in the female group and in patients with bilateral nephrolithiasis, was also suggested. On the other hand, in the presence of an increased urinary pH, there was a prevalence of mixed calcium-oxalate and calcium-phosphate stones. Finally, a "cluster analysis" of the data showed no dependency between age and "metabolic" activity of the disease.

A88. Long-Term Observation of the Metabolism and Adapted Therapy in Cystine-Stone Patients

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After a 10-day basic checkup as inpatients on standard diets, 20 of our cystine-stone patients were subjected to further examination both as in- and outpatients over the course of the following 2–12 years. In addition to measuring cystine, calcium, uric acid, and oxalate, calcium loading and purine loading were also checked and ammonium chloride tests and stone analyses performed. Thirteen patients began therapy with ascorbic acid, 5 with alpha-mercaptopropionyl glycine, 1 with alpha-mercaptopropionyl glycine plus Allopurinol, and 1 was merely placed on a diet combined with a liberal fluid intake. Nineteen out of 20 of patients exhibited further metabolic disorders (dietic or absorptive hypercalciuria, hyperuricosuria, hyperoxaluria). In view of these results and the clinical course of the study, the therapy was left unchanged for 11 of the patients. Four of the patients discontinued the therapy themselves; 2 took their medicine (ascorbic acid) regularly for 8 years, but did not appear for any checkups during the period. Of the 14 patients on a regular diet and under constant monitoring, 3 suffered relapses (21.4%); of the 2 on a regular diet but where monitoring was inadequate, 1 suffered a relapse. Of the 4 patients who broke off the therapy of their own accord, 3 suffered relapses. The metabolism of cystine-stone patients requires regular monitoring in order to check the extent to which they are complying with the rules, check the effects of the therapy, and to extend the treatment to include any additional metabolic disorders that may be discovered. Of the patients who were examined and whose treatment was adapted to any changes in their situation, 21.4% suffered relapses; among those who were not examined or where the treatment was not adapted with time, the relapse rate was 75%.

A89. Bearing of Drinking Water Quality on the Prevalence of Urolithiasis

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A census survey of localities comprising a population of 30,954 persons was carried out to ascertain the prevalence of urinary stone disease in the Udaipur region. The average incidence of disease was observed to be 293 ± 54.7/100,000 population. An analysis of drinking water consumed by this population was also done for hardness, Ca, Mg, Cl, F, SiO_2, SO_4, Na, K, Mn, Zn, and Cu. As per the recommendations of the Indian Council of Medical Research, the calcium present was beyond the maximum permissible limits in 3.3% samples and fluoride in 23.3% samples. Levels of hardness, calcium and fluoride were in the highest desirable range in 33.3%, 6.7%, and 53.3% of samples, respectively, and between this and maximum permissible limits in 66.6%, 93.3%, and 46.7% of samples, respectively. Chi-square test for the association of attributes was employed to test the association between the prevalence of urolithiasis and various parameters analyzed in the water samples. This test was applied using two criteria. In the first case, the frequencies below and above the mean levels of the parameters studied in the water samples were considered as per the levels recommended by the Indian Council of Medical Research. The statistical evaluation indicated no association in any respect. Hence, it is concluded that the prevalence of urolithiasis is independent of the quality of drinking water in this region.

A90. Hypercalciuria and Hyperoxaluria in Stone Formers

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Hypercalciuria and hyperoxaluria are considered to be the two major risk factors in urolithiasis. We investigated hypercalciuria (>200 mg/24 h) and hyperoxaluria (≥40 mg/24 h) in 276 stone formers on a restricted calcium (≤400 mg/day) and oxalate (<200 mg/day) diet. In general, hypercalciuria and hyperoxaluria were present in 21.0% and 45.3% patients, respectively; 9% of the patients suffered from both hypercalciuria and hyperoxaluria. All three groups of stone formers had almost the same prevalence for both these risk factors. The mean 24-h excretion of calcium in hypercalciuric renal, ureteric, and vesical stone formers was 281.1 ± 76.8, 288.4 ± 83.2, and 285.2 ± 42.4 mg, respectively. The mean 24-h excretion of oxalate in hyperoxaluric renal, ureteric and vesical stone formers was 66.0 ± 29.6, 61.7 ± 21.5, and 58.7 ± 16.5 mg, respectively. The calcium oxalate risk index and ionic activity product were higher in all groups compared to the controls. The hypercalciuria appears to be mainly of the absorptive type and hyperoxaluria of endogenous origin. The latter appears to be the most important determination of lithogenic process in the local population.

A91. Prevalence of Upper Urinary Tract Stone (UTC) in Northeast Thailand

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Thailand, a developing country in Southeast Asia, is believed to have a high prevalence of both lower and upper urinary tract stones, especially in the northeastern region (population = 20 millions). Yet no epidemiological study of the true incidence of UTC has been reported. We conducted an epidemiological survey in an adminis-

trative subdivision (Amphur Ban Fang) of the Khonkaen Province. It consists of 8,292 households in 53 villages (area = 153.5 sq. mile; population = 48,420). Case contact and direct interview were made possible via "Village Health Volunteers (VHVs)", the grass-root paramedical personnel of the whole-nation, integrated primary health-care system, native residents of individual villages. Positive cases were defined as those who had a surgical scar from a stone operation (pyelo/nephrolithotomy) or had previously been seen by physicians with X-ray confirmation for UTC. Ninety percent (144 cases) of those eligible were available for direct interview and were included in this study. The prevalence of positive cases was 3.1/1,000. By extrapolation, UTC patients of the whole region were estimated to be 55,000. The male/female ratio was 2: 1. Age averaged 37.6 ± 13.6 (SD) and the range 3–71 years. Common associated symptoms were pain in the flank (64%), abdominal pain (40%), red urine (21%), and urination difficulties (12%). Although this is a rough estimation, it strongly suggests that northeast Thailand has a high prevalence of UTC. Further study is urgently needed to solve this noncommunicable national health problem.

A92. Blood (B) and Urinary (UC) Aggregators and Inhibitor Compositions of Renal Stone (RS) Patients in Northeast Thailand

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Evaluation of B and UC is important for studying the pathogenesis of renal calculi. We compared B and UC in 15 healthy hospital personnel (G I), 12 local villagers without RS (G II) and 25 villagers with RS (G III). In each subject, both B and two consecutive 24-h urine specimens (24 hU) were collected and analyzed for creatinine, Na, K, Cl, HCO_3, Ca, Mg, P, and uric acid. In addition, 24 hU was also analyzed for sulfate and F. Urinary pH was measured from a separate morning-urine specimen.

A significant difference between groups was observed for the following data (mean ± SEM)

	G I	G II	G III
Serum K (mEq/l)	4.1 ± 0.1	3.5 ± 0.1****	3.7 ± 0.1****
24 hU Na (mEq)	154 ± 13	87 ± 11****	80 ± 9
24 hU K (mEq)	29 ± 3	16 ± 2****	18 ± 2****
24 hU P (mg)	652 ± 54	501 ± 52	466 ± 38***
24 hU uric acid (mg)	554 ± 40	415 ± 57*	390 ± 41**
24 hU citrate (mg)	193 ± 26	190 ± 116	55.7 ± 15****
24 hU Ca (100/24 hU Na)	2.4 ± 0.2	5.9 ± 0.7****	6.7 ± 1.0***
Hypercalciuria (>200 mg/d)	6%	25%	32%

* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$

Our data suggest that G II and G III consumed less salt and protein compared to G I. However, when standardized to similar salt intakes, G II and G III tended to excrete more Ca than G III. Hypocitraturia is common in G III. Hypercalciuria is also more common in G II and G III. The mechanisms involved in hyper- and hypocitraturia are unknown.

A93. The Tübingen Urolithiasis Documentation System

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Starting with the patient documentation system, UROPAD, which was developed in our department to record the number of patients suffering from bladder cancer, we drew up a record for patients suffering from urolithiasis as well. As UROPAD is a data bank with maximal information, only a few stone-specific forms had to be supplied. To a large extent codification is done using the systemized nomenclature of medicine (SNOMED). There is a special form at hand to answer statistical problems; correlations between different parameters can be examined in this way. A total of 128 stone patients were selected from the year 1984 and their data recorded. One-third had infection-induced stones; the findings were evaluated in detail. In contrast to oxalate stones, infection-induced stones were located more often in the kidneys and had to be removed with instruments or surgically. Urinary obstruction was found much more frequently in struvite-apatite stones; as far as the bacteria spectrum was concerned, we found mainly urease-forming bacteria. The recurrence rate was 39%, and calcium excretion was higher in infection-stone patients than in others. The Tübingen urolithiasis recording system enables us to record and evaluate large amounts of information; thus, it is suitable for scientific evaluation as well as for follow-up in a stone clinic.

A94. Pharmacokinetic and Autoradiographic Studies on Oxalate in Rats

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The pharmacokinetics of oxalate were studied in normal and nephrectomized rats, using radioisotope-labeled oxalate. Plasma disappearance of ^{14}C-oxalate was analyzed with a two-compartment open model in order to obtain the half-life of elimination, each compartment volume, and total clearance. These values were compared with those for inulin. In normal rats, the half-life of elimination for oxalate was 20 min and that for inulin was 16 min. The total distribution volume of oxalate was 1.7 times larger than that for inulin, and the total clearance for oxalate was 1.2 times higher than that for inulin with statistical significance. In nephrectomized rats, on the other hand, the half-life of elimination extended to 1.85 h for oxalate and 4.26 h for inulin. The total clearance for oxalate resulted in a much lower level than that in normal rats. However, the total clearance for oxalate was much larger than that for inulin in nephrectomized rats because oxalate was excreted from the bile while inulin was rarely excreted. In support of these data, an autoradiographic study showed that ^{14}C-inulin activity was mainly localized in the kidney and extracellular space. Moreover, ^{14}C-oxalate was distributed in the bone, and the radioactivity in the liver, spleen, and muscle was much higher than that for inulin. These results suggest that oxalate is more diffusible than inulin, and that oxalate is mainly excreted from the kidneys, although a part of the circulating oxalate is excreted from the liver.

A95. Chemical Composition of Drinking Water and the Incidence of Stone

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Calcium concentration in the drinking water and its contribution to the daily dietary intake of calcium could be an important factor in the etiology of urinary tract stones. In India, 80% of the population lives in villages where ground water is the main source of drinking water. This study was therefore performed on the rural population with the objective of discovering such a relationship. An epidemiological screening survey was performed for stone disease using a standard questionnaire in a rural population of 30,000 individuals residing in 10 villages during the period 1972–1987. Five hundred drinking-water samples were analyzed for hardness, calcium, and other relevant constituents. The dietary intake of calcium and magnesium per day and the intake from water alone were separately correlated with the prevalence rate of stone disease. Similar studies were performed on 200 cases of stone patients attending the clinic. Subjects with confirmed stone disease were evaluated for dietary intake of calcium and magnesium through the repeat 24-h recall method. The duration of stay and consumption of drinking water were noted in each case. The incidence of urinary tract stone was high in areas with hard water and high calcium content. Calcium intake through water contributed significantly to the daily intake of calcium. The Mean contribution of water calcium to daily intake ranged from 200–400 mg. The reasons for this lack of correlation between the prevalence rate of urinary tract stones, and water hardness and its calcium content with that reported in the Western literature will be discussed.

A96. Lack of Prevalence of Stone Disease in Areas Endemic for Fluorosis

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Urinary tract stone disease and fluorosis are widely prevalent in several states of India and affect rural farming communities. An environmental, epidemiological, clinical, nutritional, biochemical, and radiological study was therefore designed to discover the prevalence of stone disease in areas endemic for fluorosis and vice versa: 3,000 patients of endemic skeletal fluorosis were investigated for stone disease, and an equal number of patients with endemic stone disease were examined for the simultaneous occurrence of fluorosis during the period 1965–1987. Our results suggest that fluorosis and renal stone disease, although both endemic, are unrelated regarding their epidemiological prevalence. In the areas endemic for fluorosis, the incidence of stone was low. The water in these areas was soft, alkaline with a low calcium and high fluoride content (1.1–25 ppm), while in the areas endemic for stone disease, the water was hard, acidic with a high calcium and low fluoride content (<1.0 ppm). The plasma and urinary excretion of fluoride was within the normal range. Chemical analysis of the stones showed that calcium oxalate was the major constituent. The fluoride content of the stones was not directly proportional to the calcium content. No significant difference was detected in the fluoride content of the nucleus and peripheral parts of these stones. A low incidence in urinary tract stone in the areas endemic for fluorosis strongly suggests that fluoride is not involved in the mechanisms of stone formation.

A97. Chronological Variation in Chemical Composition of Urinary Calculi Between 1965–1968 and 1982–1986 in Northwestern India

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To investigate the effect of an improvement in health status and nutrition over two decades with regard to the occurrence of various types of urinary calculi, the chemical composition of 210 urinary calculi, either spontaneously passed or surgically removed during 1965–1968, were compared with 1,338 stones obtained during 1982–1986. The proportion of vesical calculi had significantly decreased from 30.48% during 1965–1968 to 8.37% during 1982–1986 ($P < 0.001$). During 1982–1986, the number of vesical calculi containing magnesium or ammonium decreased to zero, while the percentage of vesical calculi containing urate increased from 33.4% during 1965–1968 to 76.8% during 1982–1986. Similarly, during 1965–1968, 35.6% of renal calculi contained magnesium, 20% contained ammonium, 13.6% contained carbonate while urate was present in 32.2% of renal calculi. In contrast, during 1982–1986 magnesium was detected in only 0.24% of renal calculi, ammonium in 0.16%, carbonate in 3.9%, but 57.4% of renal calculi contained urate. The proportion of urinary calculi containing calcium-oxalate-urate, calcium-oxalate-phosphate and calcium-oxalate-phosphate-urate showed a significant increase in 1982–1986. The data on the disappearance of vesical calculi and preponderance of oxalate- and uric-acid-containing stones confirm the earlier observations in other developed countries of the world.

A98. Urine Composition in Patients with Calcium Stone Disease Before and During Follow-Up in an Outpatient Stone Clinic

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We analyzed the urine composition in 802 patients with calcium stone disease before and during follow-up at our outpatient stone clinic. Urine was analyzed with respect to calcium, oxalate, citrate, magnesium, and creatinine. Supersaturation with CaOx was expressed both as an AP(CaOx) index calculated for the measured 24-h urine volume and as AP(CaOx) index (S) for a standardized 24-h urine volume of 1,500 ml. Of the patients, only 392 were given advice concerning dietary and drinking habits, whereas 410 in addition received some form of medical treatment such as thiazide, allopurinol, magnesium oxide, or alkaline citrate. Urinary oxalate remained unaffected during follow-up in both groups. In male patients, the AP(CaOx) index was reduced in those who did not form new stones during follow-up. However, the AP(CaOx) index (S) was reduced only in the recurrence-free patients who were given medical treatment. An increase in urine volume was the main reason for reduction of the AP(CaOx) index in patients without medical prophylactic treatment. In the group that continued to form stones, neither the AP(CaOx) index nor the AP(CaOx) index (S) were significantly affected by therapeutic management. A similar pattern was also observed for women, but here a reduced AP(CaOx) index was observed even in women who formed new stones during medical treatment. To some extent, this could be explained by the more abnormal urine composition in the latter group before treatment. The results obtained show that both giving advice on drinking habits and medical treatment favorably affect urine composition. The response to treatment in terms of recurrent stone formation was at least to some extent reflected in the effects on the AP(CaOx) index and AP(CaOx) index (S).

A99. Renal Stone Disease and Nutrient Intake in Italy

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The aim of this study was to investigate the relationship between diet and renal stone disease. A comprehensive analysis of the relationship of certain nutrients to the 24-h urinary concentrations of lithogenic and inhibitory substances was performed. The subjects studied were 165 adults (aged 13–73 years) (78 M, 87 F), who were chosen randomly from family practitioner files. The study participants provided complete 24-h diet recall and timed 24-h urine samples for the same 24-h period. The nutrients and calories were calculated with the use of food-composition tables. Sodium, potassium, calcium, phosphorus, magnesium, zinc, oxalate, citrate, uric acid, glycosaminoglycans, creatinine levels, and pH were measured in the urine samples. The participants were divided into three groups: 16 renal stone formers; 25 controls with a family history of renal stones; 124 controls without a family history of renal stones. A renal stone prevalence rate of 9.6% was observed and a positive family history of stones was more common in renal stone formers (37%) than in controls (17%). Daily intakes of energy, protein and calcium were higher (2,169 ± 710 kcal, 100 ± 27 g, 917 ± 406 mg) in renal stone formers than in controls with positive (2,102 ± 580 kcal, 98 ± 29 g, 879 ± 479 mg) and negative family histories (2,027 ± 551 kcal, 91 ± 21 g, 881 ± 504 mg). Daily excretions of calcium and uric acid were higher (212 ± 95 and 562 ± 113 mg) in renal stone formers than in controls with positive (188 ± 99 and 494 ± 196 mg) and negative family histories (176 ± 83 and 513 ± 189 mg). Although there were no statistically significant differences using the two-way analysis of variance these findings indicate that there are potentially important relationships between nutrients and renal stone disease. In particular, the relatives of stone formers could be predisposed to stones because they have dietary patterns that are similar to those of the patients; in fact, their caloric and protein intakes are higher than in subjects without a family history of renal stones.

A100. An Analysis of 265 Samples of Calcium-Oxalate Concrements in the Uropoetic Tract in Patients of the Osijek Region

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This article presents the results of an analysis of ingredients in urolithiasis with special attention to calcium-oxalate stones. This work was done in a complex research program in the field of urolithiasis in Croatia. The results were compared with the results in other countries. Infrared spectrometry was used analyzing the concrements. From 406 analyzed stones, 265 (or 65.24%) mainly consisted of calcium-oxalate. Pure calcium-oxalate was found in 178 analyzed calculi or in 43.84%. In 78 patients (or 19.21%), it was combined with phosphate as the result of uroinfection. The male patients had an average age of 46.17 years and the women had an average age of 42.84 years. Almost all stones were located in the upper part of the urinary tract and most of them were solitary. When comparing the results of this calcium-oxalate research study with the results of other authors, we conclude that the results achieved are similar to those in the industrialized developed countries of Europe.

A101. Obesity and Calcium Stone Disease

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Calcium stone disease prevails in developed countries and has continued to increase during the present century with concomitant industrialization and affluence. Therefore, in 120 patients (pts) with idiopathic calcium urolithiasis (ICU) and in 75 age- and sex-matched normal subjects, the obesity incidence was evaluated and its relationship with certain urinary parameters was investigated in patients. The amount of overweight was determined by the weight index (WI), defined as the ratio of the actual to the ideal mean body weight. Daily urinary excretion of calcium (uCa), phosphorous (uPi), uric acid (uUA), oxalate (uOx), and magnesium (uMg) was measured on individual diets with no drug administration. The correlation between WI and urinary parameters was assessed by the linear correlation coefficient. WI was significantly ($P < 0.001$) higher in the pts (1.13 ± 0.14) than in normal subjects (1.05 ± 0.13), as was the incidence of obesity, defined by a WI $\geqslant$ 1.2 (30.8% vs 12%, $P < 0.01$). The mean urinary excretions in the pts with ICU were the following (mg/day): uCa 230 ± 114, uPi 877 ± 277, uUA 714 ± 200, uOx 24.5 ± 13.6, uMg 98 ± 39. WI correlated closely with uUA and uPi ($P < 0.001$, in both cases), and weakly with uCa ($P < 0.05$); but not with uOx and uMg. This study shows that pts with ICU tend to be overweight and obese in comparison to normals. The high caloric intake is also due to excessive animal protein because of the close correlation between WI and uUA found in the pts studied. In fact, high animal protein intake is associated with an increase in uUA, which is an important factor favoring calcium stone formation. Therefore, the overweight pts with ICU should normalize their body weight and also reduce animal protein intake in order to prevent the risk of urinary stone relapse.

A102. Medullary Sponge Kidney and Calcium Nephrolithiasis

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A wide variation in the incidence of medullary sponge kidney (MSK) among calcium stone formers (CSF) has been reported, and the existence of peculiar metabolic abnormalities with respect to idiopathic calcium nephrolithiasis (ICN) has been debated. Thus, the incidence of MSK in 138 consecutive CSF was evaluated, and possible clinical and biochemical differences were investigated between MSK patients (pts) and a group of 82 pts with ICN. MSK was diagnosed in 16 pts (11.6%) on the basis of typical radiological features. A comparison of the clinical patterns between MSK and ICN pts showed: a significant prevalence of females in MSK (50% vs 18%, $P < 0.01$); a more frequent, but not significantly different family history of nephrolithiasis in MSK (56% vs 32%); more stones at X-ray in MSK ($P < 0.001$); a significantly lower weight index (ratio of the actual to the ideal body weight) in MSK ($P < 0.005$); no difference in the age of clinical onset and in the number of stone episodes/patient x year. No significant difference in biochemical parameters between the two groups except for urinary magnesium excretion (uMg), which was higher in the MSK than in ICN pts (1.83 ± 0.81 vs 1.23 ± 0.47 mg/kg/day, $P < 0.005$). The incidence of MSK in this study is close to the highest so far reported. The presence of the above clinical and biochemical features in CSF might suggest the coexistence of MSK whenever a satisfactory urogram is not available. The relative hypermagnesiuria found in pts with MSK might be attributed to reduced Mg reabsorption in the dilated collecting ducts with a resulting Mg leak, Mg also being reabsorbed to a small extent along the distal nephron. The postulated protective effect of elevated uMg on calcium-oxalate cristallization could be overwhelmed by the known urinary acidification defect and by prolonged urinary transit in the ectatic ducts.

A103. Morbidity of Urolithiasis Beetween 1979 and 1984 in the Federal Republic of Germany

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To estimate the incidence and prevalence of urolithiasis in the Federal Republic of Germany (FRG) in 1979 and 1984, nationwide surveys were undertaken, together with the Institute for Applied Social Sciences (INFAS). In 1980, a total of 10,130 and in 1985 a total of 10,238 individuals over 18 years of age were interviewed. The approach guaranted that the distribution of sex, age, occupation, and place of residence correspondend well with the total population. Children under 18 were not included because it is difficult to get a representative sample of them in the survey and because we already know that stone formation in this group does not exceed 2% of all stone formers. The incidence amounted to 0.5% in 1979 and 0.4% in 1984. In both years 2/5 first stone manifestations and 3/5 recurrences were registered. There was no difference between sexes. The prevalence for both years was stated as 4% without any difference between sexes. In 1979 as well as in 1984, an increase with rising age was found. Mortality was 427 cases in 1979 and 297 cases in 1984 (data obtained by Statistisches Bundesamt).

Conclusions: Incidence and prevalence of urolithiasis in the FRG showed no statistically significant differences between 1979 and 1984. The data indicate that in the FRG around 200,000 inhabitants annually suffer from urinary stone episodes. No significant differences between sexes were found concerning incidence (in contrast to former examinations) and prevalence, although women under 50 years showed significantly higher stone formation than men under 50 years. Women above 65 have significantly less stone formation than men above 65 years. In 1979 as well as in 1984, a similar increase with rising age was found. The mortality for 1979 and 1984 confirmed that there has been a steadily decreasing tendency since 1974.

A104. 2.8-Dihydroxyadenine Stone Formation

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Are there reliable methods for the detection, evaluation, and monitoring of adenine phosphoribosyl transferase (APRT) activity, 2.8 DHA stone formation, and appropriate therapy for the disease? Enzyme deficiency can be characterized by radiochemically measuring the APRT activity in the erythrocyte hemolysate, thus allowing us to study hereditary factors. Using chemical methods of urinary stone analysis, 2.8 DHA stones will constantly be classified as uric acid calculi. IR spectroscopy enables us to identify these stones clearly and reliably. For quantitative measurement of 2.8 DHA we developed a specific HPLC technique in conjunction with a separating system for purine. Dependent upon the degree of APRT deficiency, 2.8 DHA crystals with a concentric, spherolitic arrangement could be demonstrated in both of our patients. Enzyme deficiencies could also be detected in the patients' families. 2.8 DHA was not excreted by normal individuals, but in our patients it varied from 245–414 μmol/l, correlating with the urinary volume. Excretion and concentration exhibited a characteristic circadian rhythm, with strongly increased levels during the night and in the early morning. Treatment with allopurinol produced a dramatic drop in the excretion rates down to 40 μmol/l. Under this therapy, excretion was on a constantly low level, eliminating circadian peaks, although not below the critical supersaturation level of 20 μmol/l. Consequently, for urinary stone analysis, modern methods, preferably IR spectroscopy, must be used. The enzyme deficiency in 2.8 DHA stone formation can be characterized by determining APRT activity. With the HPLC method we have developed a quick and reliable method to measure 2.8 DHA excretion quantitatively for the diagnosis and monitoring of therapy. Constant urinary dilution (2–2.5l), together with allopurinol therapy, proved able to prevent crystallization in these patients.

A105. Is Urinary Tract Infection Concomitant with Urinary Stone Disease?

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Various reports are available that detail the incidence of urinary infection in patients with urinary stone disease. However, whether the urinary infection is the cause of stone formation or vice versa is still being debated. In this study, urinary infection in 400 cases of urinary stones was assessed. The occurrence of urinary infection in the different groups of stones (upper tract, lower tract, stone passing, renal, ureteric, bladder, urethral and staghorn calculi) was established. It could be seen that urinary infection was present in 24% of all stone patients. It played a role in 27% of the upper-tract stone patients compared to 18% of the lower-tract stone patients. The incidence of infection in the renal, ureteric, bladder, and urethral stone patients showed no significant differences. Staghorn calculi patients showed only a 9% infection rate. There was a 67% correlation between the culture reports of urine and the stones retrieved during surgery. Positive evidence of infection was not related to any particular type of biochemical abnormality. Neither was it significantly different in the group of idiopathic stone formers. The patients with and without crystalluria also did not show any differences in the incidence of urinary infection. The study findings indicate that urinary tract infection is not a very significant entity in urinary stone patients in this part of the world. In staghorn calculi, infection is not as commonly encountered as is a uric acid problem. In lower-tract stones the incidence of urinary infection was also lower than in upper-tract stones. The problems involved in the eradication of urinary tract infection in a small group of recurrent stone formers will be presented.

A106. Response to Exogenous $1,25(OH)_2$ Vitamin D_3 (1,25D) Under a Low-Calcium Diet in Normal Controls and Idiopathic Renal Stone Formers

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The pathogenesis of hypercalciuria in renal stone formers is not clear, but may be due to a primary increase in intestinal calcium (Ca) absorption, an increase in sensitivity to or the production of 1,25D or a renal Ca leak. In order to examine the pathophysiology of hypercalciuria, we studied 72 stone formers and 21 controls (C) under a prospective protocol. After two consecutive 24-h urine and blood samples were collected, under a normal Ca diet (1 g/day), the subjects were instructed to ingest a low-Ca (2 mg/kg). After 7 days on the diet, new 24-h urine and blood samples were collected and a Ca-load test was performed. The subjects were then started on 1 μg per day of 1,25D for another 6 days, and 24-h urine and blood samples and a calcium load test were repeated. The stone patients were subdivided according to Ca excretion under the normal Ca diet, in hypercalciuric (H) (n = 24) and normocalciuric (N) (n = 48). After 1 week on a low Ca diet, urinary Ca excretion (UCa) decreased significantly in C, whereas no significant change

was observed in N or H. Administration of 1,25D resulted in a significant increase in UCa in both C and N. In contrast, in the H group UCa remained unchanged. Intestinal Ca absorption, mediated by exogenous 1,25D, as assessed from the change in fractional excretion of Ca, post-Ca load minus fasting, was significantly increased in C and N [C = 0.11 ± 0.02 vs 0.23 ± 0.03 mg/dl GF ($P < 0.001$) and N = 0.14 ± 0.01 vs 0.20 ± 0.02 mg/dl GF ($P < 0.001$)]. In the H group, there was no further increase in Ca absorption after exogenous 1,25D (0.19 ± 0.03 vs 0.22 ± 0.03, NS). Serum PTH, urinary cAMP or citrate excretion were unchanged after 1,25D. These results suggest that hypercalciuric stone formers are not hypersensitive to exogenous 1,25D and that other factors besides 1,25D could be involved in the pathogenesis of hypercalciuria.

A107. Oxalate-Synthesizing Enzymes in Rat Liver

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The enzymes responsible for the synthesis of oxalate from glycolate in rat liver were studied in vitro. Upon ammonium sulfate fractionation of the 16,500 x g supernatant, glycolate dehydrogenase (GDH) activity, the activity for the direct formation of oxalate from glycolate, and glycolate oxidase (GOD), xanthine oxidase (XOD), and lactate dehydrogenase (LDH) activities were recovered, mostly in a fraction precipitated at 35%–60% saturation (AS_{35-60}). When the dialyzed AS_{35-60} was subjected to DEAE-cellulose column chromatography, in which LDH, XOD and GOD were well separated from each other, the GDH activity was no longer detectable in the eluate. The GDH activity detected in the AS_{35-60} in the absence and presence of NAD was thus entirely accounted for by the combination of GOD and XOD, and by GOD and LDH, respectively, indicating that in rat liver, GDH does not contribute significantly to the synthesis of oxalate from glycolate, and the oxalate synthesis takes place *via* glyoxylate. The kinetic properties of XOD and GOD examined in vitro are not preferable for the oxidation of glyoxylate to oxalate under physiological conditions, suggesting the main contribution of LDH. In man, however, urinary oxalate excretion is not profoundly decreased in patients with LDH-M subunit deficiency or with LDH-H subunit deficiency. The search for other LDH species contributing to the oxalate synthesis is underway.

A108. Increased Urinary Glycolate in Idiopathic Calcium-Oxalate Nephrolithiasis

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The objective of this study was to assess endogenous oxalate (OX) production in patients with idiopathic calcium OX nephrolithiasis. We measured urinary OX by the method of Hallson and Rose and urinary glycolate (GLYC) by the method of Kasidas and Rose in 79 untreated male patients eating self-selected diets at home. This patient group comprised all of the untreated male patients with "idiopathic" calcium OX stones with mild hyperoxaluria (>450 μmol/day) who have been investigated in our clinic since 1980 ($n = 32$) plus a randomly selected group with normal urine OX ($n = 47$); in 14 of the latter group, urine OX was in the high normal range (400–450 μmol/day). Urinary GLYC was elevated (>450 μmol/day) in 17 of the 46 patients (37%) in whom urine OX exceeded 400 μmol/day but in only 5 of the 33 patients (15%) with urine OX < 400 μmol/day. In the entire group of 79 patients, there were highly significant positive correlations ($P < 0.001$) between urinary GLYC and urinary uric acid ($r = 0.603$) and urinary creatinine ($r = 0.471$). Urinary GLYC was also positively correlated with urine calcium and phosphate and body weight ($P < 0.01$). Analysis by multiple linear regression indicated that urinary uric acid was the only significant predictor of urine GLYC. In three patients, long-term administration of pyridoxine (PYR) in doses of 25 to 200 mg/day did not restore urine GLYC to normal. Preliminary dietary studies, however, suggest that the abnormality is responsive to dietary protein restriction. Our data suggest that our patients with elevated urine GLYC have a reversible diet-related abnormality of OX metabolism. Type I primary hyperoxaluria (PH) is unlikely because of the onset in adult life, the intermittent nature of the disorder, the response to protein restriction, the failure to respond to PYR, and also because urinary GLYC usually exceeds urinary OX; the reverse is true in type I PH. In addition to the 22 untreated patients, we also identified 49 patients with elevated urine GLYC on one or more occasions during long-term treatment with thiazides. Most of these patients continue to have mild intermittent hyperoxaluria, whereas in patients with normal urine GLYC, urine OX tends to fall after the first 1–2 years of thiazide treatment.

A109. The Prevalence of Urolithiasis in the Western Region of Saudi Arabia – A Population Survey

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This study, a stratified population survey to establish the prevalence of urolithiasis in the three main cities of the western region (Jeddah, Mecca, and Taif) is the first in Saudi Arabia. It included 7,000 completed questionnaires: 5,000 from Jeddah, 1,000 from Mecca, and 1,000 from Taif, yielding information on a total of 16,777 individuals. The questionnaire, designed specifically for this purpose, was distributed through school children to their parents. The results shown in Table 1 demonstrate a high prevalence of stone disease with prominent regional variations. Also, in different regions the relationship of the prevalence rate to various age and sex groups and socioeconomic conditions was studied and compared with reports from Sweden, Germany, Scotland, and California. In addition, in Jeddah, Mecca, and Taif, the possible role of climatic factors and chemical composition of the drinking water in relation to the shown regional variations in the prevalence rate of stone disease was investigated in light of current references.

Table 1. Sample size and prevalence rate of stones

City	Sample size			Stone prevalence rates (%)			
	Total	Males	Females	In males	In females	Overall	Ages (25 years +)
Jeddah	11,080	7,409	3,671	9.0	4.0	7.4	10.0
Mecca	2,984	2,352	623	6.2	4.1	5.8	10.2
Taif	2,713	1,834	879	5.9	3.9	5.0	7.4

A110. Changing Trends in Pediatric Urolithiasis in Kerala

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In Western countries, an increase in the incidence of upper urinary stones was followed by a decrease in the incidence of lower tract stones. Pediatric urolithiasis, now uncommon in the Western world, is confined to certain stone belts. This paper presents the changing pattern of pediatric urolithiasis in Kerala, the southern state of India. Forty-four patients below 12 years of age were studied over 20 years. They formed 2.5% of the total stone patients. The mean incidence of pediatric stones has risen from two per year in the 1960s to six in the 1980s. The maximum incidence was in the 8th year. Males predominated by 8:1. Bladder stones formed 54%, renal 26%, ureteric 16%, and urethral 12%. There was a positive family history in 6%. Forty-four percent had crystalluria, primarily of the oxalate variety; 27% had urinary infection, and 47% had biochemical anomalies. There was hyperuricemia in 29%, hyperuricosuria in 24%, hypercalciuria in 24%, and hypercalcemia in 6%. Congenital anomalies were associated in 24%, namely, posterior urethral valves, anorectal anomalies, hypospadias, and congenital megaureter. Of the stones 87% were mixed and 13% were pure phosphate. Stone prophylaxis and follow-up resulted in a very significant reduction in the stone-episode rate. The increased incidence in recent times appears to be an increase in bladder stones. Uric acid abnormality appears to be on the increase in stone disease. The increased incidence of bladder stones in the absence of famine or significant malnutrition will be discussed.

A111. Prostatic Hyperplasia Complicated with Vesical Calculus

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From 1980 to 1984, we treated 181 cases of prostatic hypertrophy. Vesical calculus was diagnosed in 12 of 103 patients who were examined by X-ray, cystoscopy, or at operation. It accounts for 6.0% of urinary stones (199) and 57.1% of vesical stones (21) at the same time. The age of the patients was between 56 and 86 years (average 67.2). The main symptoms were disuria (9) and hematuria (3). In only one case was there a complicated interruption of the urinary stream. In all others, vesical stones were diagnosed by other examinations or during an operation after a diagnosis of prostatic hyperplasia. *E. coli* was determined in only two of ten cases. All stones were removed by operation except in one case. A single stone was found in six cases and multiple stones in five. Seven calculi were analyzed: CaOx, 2; uric acid, 3; phosphate, 1; CaOx and uric acid, 1. Three were of a crystalline structure and 4 were of a ollitic structure. In the Gansu Province patients over 60 years accounted for 27% of the lower urinary stones. Prostatic hyperplasia complicated with vesical calculus accounted for 57.1% of vesical stones. This fact suggests that prostatic hyperplasia has become an important factor in vesical stone formation in recent years. Because the bladder has a big cavity, the calculi often have an ollitic structure. In our patients, infection is not a main factor of stone formation, and uric acid stone accounts for a considerable proportion; it may be related to anormal uric acid metabolism in elderly men.

A112. Descriptive Epidemiology of Urolithiasis in Japan

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Nationwide surveys on urolithiasis in Japan were carried out in 1979 and 1988 in an effort to clarify the incidence and prevalence of urinary stones in Japan. The range of the investigations covered 1965 to 1987. The life expectancy of patients with urinary calculous disease was also estimated among the Japanese people, who are racially and ethnically homogenous, all having similar customs and living habits. It was estimated that the annual prevalence of urolithiasis in Japan was 69.4 per 100,000 population, and about 4% of the general population was expected to form a stone at some time in their lives in 1975. The data from 1985 are now under investigation. Statistical data showed that upper urinary tract stones increased from 50% in 1945 to 95% in 1975, and the male preponderance decreased from 5.6 in 1945 to 2.4 in 1975. Analysis of the composition of stones summarized in 1979 disclosed that calcium-oxalate and/or calcium-phosphate stones were 76.2%, struvite-containing stone 12.7%, uric acid 4.2%, and cystine 1.5%. "Idiopathic" calcium-containing upper urinary tract stones have been increasing since 1955, as the daily style of living in the average Japanese home has become Westernized. The geographic as well as historic aspects of urolithiasis in Japan are reviewed and compared with those of other countries and races.

A113. Chemical Composition of Urinary Stones in Jeddah – A Study of 231 Stones and a Proposed New Classification

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The chemical composition of 231 urinary stones from the Western region of Saudi Arabia was studied using various quantitative and qualitative analytical techniques. In agreement with Abdel-Halim et al. (1985), the use of elemental microanalysis for the determination of carbon, hydrogen and nitrogen enables us to identify urate, oxalate and phosphate stones. Abdel-Halim's classification for urate stones (1985) [1] encompassed satisfactorily all the identified urates, which constituted 17.4% of the samples in the present study. Furthermore, in addition to the precise identification of an oxalate stone as a stone containing 40% or more oxalates and a phosphate stone as that containing oxalate <40%, urate <20% and phosphate 10% or more, a proposed further classification is suggested. According to the proposed classification, the oxalate stones (63.7% of the samples) are classified into three groups according to whether the stone is mixed with either urate (group I) or phosphate (group II) or both (group III). On the other hand, on the basis of magnesium content, the phosphate stones (18.9% of the samples) are classed into two categories; category I, noninfection phosphate stones with a magnesium content ≤3% and category II, infection phosphate stones with a magnesium content >3%. Then, on the basis of oxalate content, category I is further divided into two distinct groups. However, in category II phosphate stones are further classed according to the calcium content into two types (A and B); on the basis of the oxalate content, type A includes two groups. This classification adequately encompassed all 328 samples, whether a whole-stone sample (171) or a lamellar sample (157), and correlated well with the clinical and biochemical picture of the patients.

References: 1. Abdel-Halim RE, Baghlaf AO, Farag AFB (1985) Clinical chemical study of urinary stones in Saudi Arabia. I. Uric acid stones. In: Schwille PO et al. (eds) Urolithiasis and related clinical research. Plenum Press, New York London

A114. Piridoxilate-Induced Calcium Oxalate Nephrolithiasis – A Nationwide Epidemiologic Evaluation of Incidence

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Piridoxilate (P) is a drug made of an equimolar combination of glyoxylate and pyridoxine. During the past 5 years, calcium oxalate (CaOx) stones from 36 patients (27 male, 9 female) receiving long-term P therapy for coronary disease have been referred to us from various French regional hospitals. Their mean age was 63 ± 10 years and 12% had a past history of urolithiasis. The mean daily dose of glyoxylate was 154 mg and the mean duration of treatment 55 months (range 4 to 120). Calculi were often recurrent, with an average of 9.7 per patient (1 to 70) and had to be removed in 30 (83%) by open (18) or percutaneous (5) surgery, or shock-wave lithotripsy (7). Urine oxalate was 727 ± 246 μmol/day while on P and fell to 382 ± 201 μmol/day after P withdrawal. Whewellite was the major component of calculi and of freshly voided urine, but several stones and most urine specimens also contained CaOx trihydrate crystals, never previously observed in urine. In view of the apparently frequent occurrence of such drug-induced lithiasis, we looked for nationwide epidemiologic data at two levels: (1) we were informed of 27 additional cases recorded in other nephrology units, leading to a total number of 63 cases among about 25,000 patients with P, a 5-year incidence of 0.25%; (2) a questionnaire was sent to the 3,200 physicians who prescribed the drug, of whom 796 (corresponding to about 6,250 treated patients) replied. They identified 22 additional patients with CaOx calculi clearly attributable to P, whose mean age (63 ± 10 years), sex (77% male), past history of calculi (18%) and mean daily consumption of P (160 mg) did not differ from our 36 patients. The corresponding 5-year incidence was 22/6,250, or 0.35%, whereas the annual incidence of idiopathic calcium stones in the middle-age population is about 0.5%. In conclusion, CaOx nephrolithiasis in patients on long-term therapy with P appears to be an infrequent adverse event, despite the increased oxalate excretion induced by the drug. This and the undue presence of CaOx trihydrate suggest the role of some still unidentified risk factors, such as lack of CaOx trihydrate crystal inhibitors in affected subjects.

A115. Effect of a Short Photoperiod on Glycolate Oxidase in Rats

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The photoperiod (length of day) has an influence on stone formation, with a higher incidence during the light seasons. In animals, the photoperiod regulates vitamin D, calcium, and androgen metabolism; however, its role in the regulation of oxalate biosynthesis is as yet unknown. Thus, the levels of glycolate oxidase (GAO), glycolate dehydrogenase (GAD), and lactate dehydrogenase (LDH) were assayed (Biochem Int, 1981, 3:507) in male Wistar rats (100–120 g BW) kept on: (1) a light-dark (L:D) cycle of 12 h:12 h; (2) a L:D cycle of 2 h:22 h for a period of 15 days. The level of GAO, a major enzyme of oxalate biosynthesis, was reduced by 70% following exposure to a short photoperiod. However, the levels of GAD and LDH remained unaltered.

Enzyme (U/mg protein)	L:D cycle 12 h:12 h	L:D cycle 2 h:22 h
Liver		
1. GAO	10.39 ± 1.25	2.90 ± 0.25*
2. GAD	0.22 ± 0.04	0.14 ± 0.01
3. LDH	2.79 ± 0.15	2.86 ± 0.11
Kidney		
3. LDH	3.46 ± 0.20	3.90 + 0.23

All values are mean ± SE of eight animals
* $P < 0.001$ as compared to the other group.

One unit of GAO is defined as the enzyme required for production of 1 nmol of glyoxylate/min at 37 °C. One unit of GAD is defined as the enzyme required for production of 1 nmol of oxalate/min at 37 °C. One unit of LDH is defined as the enzyme required to produce a change of 0.01 O.D. at 340 nmol at 25 °C. The results indicate that a short photoperiod markedly reduces GAO levels which, in turn, may lead to lower production of oxalate and lower risk of stone formation.

A116. Cimetidine Inhibition of Hepatic Aldehyde Dehydrogenase and Oxalate Production

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Since almost 90% of urinary oxalate is derived from endogenous metabolic processes, we have evaluated the effect of a number of drugs and inhibitors on oxalate production in isolated rat hepatocytes. Several clinically significant, cimetidine-drug interactions, leading to decreased hepatic clearance, accumulation and even toxicity of the non-cimetidine drug, have now been identified and attributed to either a cimetidine-induced reduction in hepatic blood flow or an interaction of cimetidine with microsomal liver enzymes, specifically cytochrome P-450. We have found, however, that cimetidine (1–10 mM) has more generalized metabolic effects in isolated rat hepatocytes prepared from fasted rats. Glycogenolysis and the lactate/pyruvate ratio, but not the D-3-hydrobutyrate/acetoacetate ratio, are increased. Gluconeogenesis from lactate, fructose and xylitol, and ketogenesis from palmitate, are all inhibited, These findings suggest that cimetidine inhibits mitochondrial as well as microsomal function. We have also found that cimetidine inhibits oxalate production from xylitol, glycollate and glyoxylate, but that the most profound inhibition occurs when ethylene glycol is the substrate. Aldehyde dehydrogenase, which is involved in alcohol, ethylene glycol and oxalate metabolism, was purified from human liver. With either acetaldehyde or glycolaldehyde as substrate, the enzyme is inhibited by cimetidine, ranitidine and disulfiram but not diethanolamine, histidine or histamine. These findings suggest that drugs that reduce the metabolic oxidation of reduced pyridine nucleotides may inhibit endogenous oxalate production in stone-formers.

A117. Endogenous Oxalate Production, Clinical Disasters and Oxalate Urolithiasis

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Urinary oxalate is derived largely from endogenous metabolic processes. Using isolated rat hepatocytes, human liver homogenates and purified human enzyme preparations, we have studied the metabolic production of glycolaldehyde, a precursor of glycollate, and the metabolism of hydroxypyruvate, a precursor of glycolaldehyde. Our studies have led us to propose the existence of relatively minor metabolic pathways that link oxalate production to the metabolism of various carbohydrates, including glucose, fructose, sucrose, galactose, sorbitol, xylitol and glycerol. In clinical practice there arise, on occasion, disasters in patient management that were not initially predicted from the previous biochemical

knowledge of the nutrient or drug used. Such a toxicity syndrome of liver, renal and cerebral disturbances, metabolic acidosis and tissue calcium oxalate deposition has now been reported in association with ethylene glycol poisoning, the parenteral use of xylitol and glycerol, the use of the anaesthetic methoxyflurane and the intravenous use of vitamin E preparations (E-Ferol) in premature infants. We have shown that the tissue oxalosis can be understood in terms of metabolic pathways (e.g., fructokinase, aldolase, cytochrome P-450 system) that produce two-carbon metabolites with an oxygen and/or halogen on both carbons. Further metabolism can only produce oxalate precursors and not acetate or acetyl-CoA. It can be predicted, for example, that dichloroacetate, fluorouracil and 1,2-dibromo-ethane may be metabolised to oxalate. The seemingly trivial conversion (less than 1%) of these substances to oxalate in these minor metabolic pathways is biologically significant when considered in an one-compartment model for the renal clearance of oxalate. Given the recognition of these pathways for oxalate production, the role of diet, hormones and drugs in oxalate urolithiasis should now be reassessed.

A118. Nutrient Energy Intake, Fasting Serum Insulin and Urinary Oxalate Excretion

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Some investigators consider oxalate excretion to be the most critical factor in urine for determining the risk of formation of calcium oxalate stones. Since urinary oxalate comes largely from endogenous sources, it has been suggested that animal proteins or refined carbohydrates are dietary sources of oxalate precursors in metabolism. However, dietary studies reported from a number of laboratories, including ours, have provided contradictory results. We have re-examined human data, published by Nordenvall et al. (1981) and obtained by us in studies of dietary variation in healthy volunteers and parenteral nutrition in cancer patients, and found that oxalate excretion (nmol/day · kJ intake) is a function of nutrient energy intake (kJ/day · kg body wt) with $y = 488.1x^{-0.523}$ ($n = 152$; $r = 0.791$; $P < 0.001$): that is, dietary energy intake explains 60% of the variance in oxalate excretion. Urinary oxalate excretion (mmol/day) did not correlate with protein or carbohydrate intake but did correlate with urinary urea excretion (mmol/day), with $y = 0.184 + 0.00032x$ ($r = 0.472$), and with fasting serum insulin (μU/ml), with $y = 0.164 + 0.015x$ ($r = 0.587$). It also correlated with fasting blood glucose and negatively with cortisol, but insulin did not correlate with glucose. These correlations can be best explained by a model of dietary-induced, insulin-counter-regulatory hormone (i.e. glucagon) coupling. Insulin might be expected to stimulate the overload of minor metabolic pathways leading to oxalate production and glucagon to cause urea production. Such a model also provides a logical synthesis of the effects of sex, dietary carbohydrate and protein, indomethacin and thiazides, and intestinal bypass operations on oxalate excretion.

A119. Dietary Purine and Allopurinol Affect Urinary Uric Acid, but not Urinary Oxalate Levels

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There has been considerable debate regarding the effect of dietary purine or allopurinol on urinary oxalate levels, as well as the beneficial effects of allopurinol therapy in oxalate stone formers. In an earlier study we demonstrated that dietary purine increased both urinary uric acid and oxalate levels. Both these effects were reduced by concomitant allopurinol therapy. Although the subjects were on a basal low or high purine diet, the composition was not specific and the oxalate levels were not controlled. Careful attention was paid to all these details in a repeat study were healthy subjects were on a constant low-purine low-oxalate diet throughout. A specific purine guanosine was used as purine additive, because beer – often consumed in quantity by stone formers – is rich in this purine (up to 0.3 mmol/l). A variety of methods for oxalate estimation were compared. Under these controlled conditions, no effect of either dietary purine or allopurinol on urinary oxalate could be demonstrated using methods specific for the estimation of oxalate. These results confirm the importance of adequate attention to diet, sample collection and storage, as well as the use of specific methods for oxalate estimation. The study supported our earlier contention (based on animal studies using radiolabelled purines) that one of the beneficial effects of allopurinol was to reduce the absorption of dietary purine from the gut, thereby having a dual effect in lowering urinary uric acid levels. The latter results would provide a logical explanation for the seemingly paradoxical effect of allopurinol in reducing stone formation in subjects addicted to purine-rich foods and beverages.

A120. Urinary Excretion of Glycosaminoglycans in Calcium Lithiasis – The Role of Protein Intake

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It has long been recognized that protein intake is an important modulator of calcium, uric acid, and oxalate urinary excretion both in stone formers (SF) and in healthy subjects (HS). In contrast, there are very few reports about the protein intake influence on urinary inhibitors' excretion and particularly on glycosaminoglycans (GAGs) excretion. The aim of this work was to evaluate GAGs excretion in SF (184) and in HS (52), as well as to study the influence of several factors, mainly a different kind of protein intake, on GAGs urinary excretion. All subjects, on a normal free diet, were examined in an ambulatory setting. At the same time, another study was carried out on 14 volunteers (50% of them presented a GAGs excretion smaller than or close to the lower limit of the normal range) who were submitted to a different kind of animal protein intake (normal and then high protein intake for a week). Our results showed a significant decrease in GAGs excretion in SF when compared to HS (SF = 30.9 ± 10.6 μM/24 h; HS = 35.3 ± 14.6 μM/24 h; $P < 0.02$). The GAGs concentration, too, was significantly lower in SF than in HS (SF = 18.4 ± 9.9 μM/l; HS = 31.3 ± 18 μM/l; $P < 0.0001$). GAGs excretion was greater in males than in females in the control group ($P < 0.01$), whereas it was similar in SF of both sexes. GAGs excretion (and even more concentration) was significantly correlated with sulfate, uric acid, phosphate, magnesium and calcium excretion – both in SF and in HS. Some of these correlations could be linked with animal protein intake. Moreover, subjects submitted to high-protein intake displayed an increase in urinary excretion of uric acid ($P < 0.001$), sulfate ($P < 0.01$) and GAGs ($P < 0.025$). Furthermore, the positive relationship between these factors remained significant, and normalization of GAGs excretion was also observed in subjects with low basal GAGs excretion. In conclusion, our results suggest that GAGs excretion, too, can be modulated by protein intake.

A121. Vitamin B_6 – Status and Oxalate Excretion in Patients with Calcium Lithiasis

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Several research studies have shown that reduced intake of vitamin B_6 can induce an increase in oxalate urinary excretion. The treatment with pyridoxine in patients with urinary stones (SF) led to contrasting results, as the excretion of oxalate was either decreased, uninfluenced or increased. To evaluate the reason for these different data, vitamin B_6 status was studied both in SF and in normal subjects (NS). The total vitamin activity in plasma and urine (microbiological method) and urinary pyridoxic acid, the main metabolic end product of vitamin B_6 (high performance liquid chromatography) were determined. In the same subjects oxalate excretion was also assayed (enzymatic method), together with one of the other main lithogenetic factors. There was no statistically significant difference between the plasma vitamin B_6 mean value of NS (15.06 ± 3.39 ng/ml) and SF (14.28 ± 8.94 ng/ml), but the latter presented a larger dispersion of data. Vitamin B_6, pyridoxic acid, and oxalate excretion tend to be higher in SF than in NS, even if not statistically significant. The data showed a linear positive relationship between plasma vitamin B_6 and urinary oxalate in SF ($r = 0.751$; $P < 0.001$) and in NS ($r = 0.66$; $P < 0.05$). A linear positive relationship between urinary vitamin B_6 and oxalate excretion ($r = 0.676$; $P < 0.0025$), as well as between pyridoxic acid and oxalate excretion ($r = 0.573$; $P < 0.01$) was found only in SF. From the present results it appears that the vitamin B_6 mean status in SF does not differ substantially from NS. The positive correlations between the vitamin content of plasma and urine and oxalate excretion do not support the hypothesis of a deficient intake of vitamin B_6 in SF.

A122. Renal Stone Disease in a Medical Outpatient Clinic

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Four thousand patients were studied over a period of 2 years from February 1985 to February 1987 in the Medical Outpatient Clinic in Dhaka to determine the prevalence and clinical presentation of renal stone disease. Renal stones were found in 139 of them (3.4%). The mean age of these patients was 37 ± 12 years (M107, F32). The clinical presentations were renal of ureteric colic (59), macroscopic hematuria (32), hypertension (22), stone passing (17), urinary tract infection (5), acute renal failure (2), and chronic renal failure (2). Microscopic hematuria was detected in 104 patients and 20 had significant proteinuria. Forty-five patients had pyuria but only five showed significant growth on urine culture. Blood urea and creatinine were elevated in 12 patients. Serum calcium was elevated in 14, phosphate in 15 and uric acid in 13. A total of 123 patients had unilateral stones, 9 had bilateral stones, and 7 had stones in the urinary bladder. A single stone was present in 122 patients and 17 had multiple stones. In 111 patients, conservative treatment was given, and in 28 patients surgical intervention was needed. Twelve patients had stone recurrence during the mean follow-up period of 2 years. It is concluded that renal stone disease is common in Bangladesh and that its clinical presentation does not differ from other countries.

A123. Interdependence of Certain Selected Parameters on Calcium Excretion in Kidney-Stone Patients

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In view of the fact that Ca-lithiasis is widely prevalent in both developing and industrialized countries and that Ca, oxalic acid (OA), phosphate (Pi), uric acid (UA), Mg, and citric acid (CA) are reported to be its promotor or inhibitor, we carried out a "Path" analysis to ascertain the direct and indirect influence of these parameters on Ca-excretion in 132 renal stone formers. The results of 24-h excretion revealed that Pi and OA had a direct positive influence on Ca-excretion. Notably, UA had a substantially direct influence but did not show a direct significant correlation with Ca due to the negative influence of other parameters. The results on concentration provided a clearer picture. Pi and OA revealed a similar influence. The direct effect of Mg and UA on their respective significant correlation with Ca was meagre, showing that the observed correlations were due to the indirect effect of other parameters. CA had a strong direct inhibitory effect on Ca-excretion. It also inhibited the value of an observed correlation of Ca with Pi, OA, and UA. CA thus appeared to be one of the strong urinary inhibitors. Paradoxically, this CA effect was reduced due to its own inverse relationship with Ca excretion and a similar effect on Mg. In conclusion, we find that OA and Pi act as double culprits, i.e., as promotor substances and also enhancing Ca excretion. CA acted as an inhibitor in its limited capacity.

A124. Bladder Stone Disease in Udaipur (Southern Part of Rajasthan, India)

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A hospital-based prevalence study on bladder stone disease in the Udaipur region over a 15-year period (1971–1985) showed a fairly high prevalence (approximately 33 per 10,000 hospital admissions). However, a census survey of 38,538 persons of the same population from two primary health center blocks in 1986–1987 showed only 3 cases with a present or past history of this disease (7.8/100,000 population). The etiological aspects of 59 bladder stone patients from our hospital were also extensively investigated. Urinary excretion in mmol/24 h (and in mmol/l) of calcium, oxalic acid, inorganic phosphate, magnesium and citric acid were: 3.63 ± 0.27 (2.77 ± 0.17), 0.37 ± 0.03 (0.31 ± 0.03), 17.94 ± 1.20 (14.56 ± 1.30), 1.52 ± 0.14 (1.18 ± 0.09), and 1.32 ± 0.09 (1.23 ± 0.14), respectively. In the present study the association of oxalic acid, inorganic phosphate, magnesium and citric acid with calcium was ascertained using the path analysis (PA), partial correlation coefficient (PCC) and the multiple correlation coefficient (MCC). These statistical analyses led us to conclude that: (a) magnesium has a definite bearing on calcium excretion; (b) citric acid has no bearing; (c) the role of oxalic acid and phosphate does not show a uniform trend and hence requires further study in a larger series.

B. Physiology

B1. Premature Infants Treated with Furosemide Have Increased Urinary Calcium and Unchanged Urinary Citrate Excretion Compared to Controls

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Furosemide (F) use for bronchopulmonary dysplasia (BPD) in premature infants has been associated with hypercalciuria, nephrocalcinosis (NC), and secondary hyperparathyroid bone disease. Potassium wasting with hypokalemia is another common complica-

tion of F use. Hypokalemia is associated with decreased urinary citrate excretion (UcitV) due to enhanced proximal tubular citrate reabsorption, further increasing the risk of NC, as urinary citrate may complex up to 70% of urinary calcium. We have observed that 50% of prematures treated with F have NC by ultrasound. We have studied 16 premature infants weekly from day 1 postnatal age until 40 weeks' gestational age or discharge (110 24-hour urines) to determine changes in urine composition among controls (C, n = 3), F babies (n = 8), those treated with aminophylline or theophylline alone (A/T, n = 3), and those also receiving thiazide diuretics (TZ, n = 2) in addition to F and A/T. Urinary calcium excretion (UCaV) was increased in F babies: 181 ± 188 (SD) mmol/kg BW/day, compared to C: 63 ± 41 mmol/kg BW/day, $P < 0.05$; and compared to A/T babies, 73 ± 60 mmol/kg BW/day, $P < 0.05$. No change in UCaV with postnatal or postconceptual age was observed. UcitV was not different between drug treatment groups, averaging 104 ± 89 mmol/kg BW/day in C and 108 ± 120 mmol/kg BW/day in F. On the other hand, UcitV was significantly increased with postconceptual age for all collections: $y = 2x - 361.5$, $R = 0.46$, $P < 0.0001$, as well as in C alone. Thus, an absolute decrease in UcitV in F babies is not an additional risk factor for NC. Further evaluation of F babies, and the time course of age-related increased citrate excretion and F-induced calciuria, may demonstrate times when citrate excretion fails to compensate the hypercalciuria.

B2. Combined Primary Hyperparathyroidism and Absorptive Hypercalciuria – Clinical Implications

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Primary hyperparathyroidism and absorptive hypercalciuria are common causes of nephrolithiasis and their occasional co-occurrence would not be unexpected. However, when these disorders occur together, there are important implications both before and after parathyroidectomy (PTX). Five patients with nephrolithiasis and severe hypercalciuria (570 ± 90 mg daily on random diet) were studied on a Ca-restricted metabolic diet before and at 6–12 months following removal of their parathyroid adenomas, which weighed from 0.3 to 3.1 g.

	S_{Ca} (mg/dl)	PTH (pg/ml)	$1,25\text{-}(OH)_2D$ (pg/ml)
Before PTX	10.6 ± 0.3	385 ± 100	79 ± 20
After PTX	9.2 ± 0.5*	150 ± 33*	57 ± 17
	^{47}Ca Abs. Frac.	Fast/Load (mg Ca/dl GF)	U_{Ca} (mg/d)
Before PTX	0.82 ± 0.06	0.21 ± 0.05 0.51 ± 0.17	441 ± 84
After PTX	0.75 ± 0.13	0.08 ± 0.03* 0.22 ± 0.04	239 ± 26*

Values are presented as the mean ± SD. Values in control subjects are serum Ca < 10.5, PTH <400, $1,25\text{-}(OH)_2D$ <50, intestinal ^{47}Ca absorption <0.60, fasting urine Ca <0.11, post-1 g Ca load urine Ca <0.20, 24-h urine Ca <200; *$P < 0.01$ after PTX. Before PTX, the patients were characterized by long-standing nephrolithiasis and a family history of stones. Biochemically, they had mild hypercalcemia, but marked hypercalciuria. They had the "absorptive" type of hyperparathyroidism in that serum $1,25\text{-}(OH)_2D$, intestinal Ca-absorption and response to oral Ca-load were all increased. The diagnosis of primary hyperparathyroidism was delayed because serum PTH was usually in the normal range, bone X-ray scans were normal and bone density above average. Following PTX, serum-Ca, PTH and fasting urine-Ca decreased significantly and were normal. Although there were reductions in serum $1,25\text{-}(OH)_2D$, intestinal Ca-absorption, urinary Ca-load response and 24-h urine-Ca, these values remained well above normal levels and met criteria for absorptive hypercalciuria. Four patients formed new kidney stones following PTX. We conclude that the co-occurrence of hyperparathyroidism with absorptive hypercalciuria may impede diagnosis of hyperparathyroidism because high serum $1,25\text{-}(OH)_2D$ levels and Ca-absorption may reduce PTH secretion. Moreover, hyperparathyroid patients with kidney stones should have 24-h urine Ca checked following PTX to exclude co-existing abnormalities such as absorptive hypercalciuria.

B3. The Effects of Indomethacin on Hypercalciuria in Streptozotocin-Diabetic Rats

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Paradoxically, both glycosuria and insulin are calciuretic, and this effect is believed to be due to a direct action on renal tubular calcium reabsorption. However, other mechanisms may be involved, i.e. hyperglycemia and insulin can stimulate prostaglandin synthesis via β-adrenergic stimulation. To investigate the role of prostaglandins in these metabolic interactions, experiments were performed in the streptozotocin-diabetic rat model.

Methods: All experiments were performed in streptozotocin-treated male Sprague-Dawley rats. Urine calcium excretion was measured in animals rendered diabetic *with* and *without* glycosuria. Both groups of animals were treated with i.v. indomethacin (10 mg/kg) and urine calcium excretion measured before and after indomethacin. Urine calcium was also measured in a group of streptozotocin-diabetic rats treated with insulin (10 μm/kg per min).

Results: Urine calcium excretion was markedly elevated in the glycosuric animals ($\bar{x}$ 334.6 nmol/min) by comparison with non-glycosuric rats ($\bar{x}$ 219.75 nmol/min). Indomethacin reduced urinary calcium excretion in both glycosuric diabetic ($\bar{x}$ 282 nmol/min) and in the non-glycosuric rats ($\bar{x}$ 100.9 nmol/min). The fall in urine calcium excretion was much greater in the non-glycosuric animals. Insulin abolished glycosuria and hyperglycemia in the diabetic rats, and there was a fall in urinary calcium with insulin (pre-insulin urine Ca $\bar{x}$ 393.35 nmol/min; post-insulin urine Ca $\bar{x}$ 286.7 nmol/min). These results indicate: (1) that PG inhibition reduces hypercalciuria associated with glycosuria; (2) insulin was found to reduce urinary calcium excretion in this experimental diabetic model. The mechanisms of these actions will be discussed.

B4. Erythrocyte Abnormality in Oxalate Self-Exchange and Urinary Acidification in Idiopathic Calcium-Oxalate Stone Formers

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We recently demonstrated an abnormality in oxalate self-exchange in red blood cells (RBC) of idiopathic stone formers (ISF). Since transmembrane oxalate flow can be blocked by anion carrier inhibitors such as stilbenes, the defect most probably resides in band 3 protein. Indeed, we were able to establish an anomalous phosphorylation state of this protein. However, the crucial problem not yet resolved is the demonstration of a pathogenetic link

between the RBC abnormality and stone formation. Since a protein cross-reacting with antibodies raised against band 3 has been demonstrated in renal tubule, and considering that the anion carrier plays a major role in urine acidification, we attempted an indirect answer evaluating urinary acidification in 28 ISF, of whom 15 had an abnormal RBC oxalate transport and 13 did not. Urinary acidification was studied by the NH_4Cl long test and by evaluating the pCO_2 gradient between urine and plasma (U-B pCO_2) after bicarbonate infusion. The study disclosed that 33% of patients with an abnormally high RBC oxalate self-exchange had a defective distal acidification, which was present in none of the ISF with a normal transport ($X^2 = 5.28$; $P < 0.025$). Moreover, as a whole, the group with the RBC abnormality showed a lower U-BpCO_2 (52.29 ± 17.30 SD mm Hg vs 68.24 ± 20.43; $t = 2.24$; $P < 0.05$), and a negative correlation between net acid excretion and RBC oxalate flux rate ($r = -0.69$; $P < 0.01$) was found. Taken together, these data give indirect support to the hypothesis that, when present in RBC, a defect in anion transport is not absent in other cell lines, namely, renal tubular cells.

B5. Stone Formation in the Human Kidney

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The aim of the study was to reveal sites and types of submicroscopic calcifications and to study their correlation with tissue and urine concentrations.

Materials and methods: Nephrectomy specimens were divided into wedge sections containing cortex, medulla, and papilla. One part was used for scanning electron microscopy and the other part for chemical analysis of alkali metals, as well as zinc, copper, cadmium, and lead. In a 12-h urine sample collected before surgery, urine supersaturation was calculated by Finlayson's EQUIL 1 program.

Results: Submicroscopic crystals were found in kidneys of stone-forming as well as in kidneys of none-stone-forming patients. In most samples calcifications were intratubular in the collecting ducts near the papillary tip. Most crystals were inbedded in an organic substance covering the tubular epithelium. Both groups showed higher cadmium values in the cortex or medulla when crystals were found in the corresponding papillary samples. Urine supersaturation was higher in the stone-forming group, caused by higher calcium and oxalate, but magnesium excretion was lower.

B6. Response to Acute Acid Load in Patients with Medullary Sponge Kidneys and Calcium Nephrolithiasis

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A defect in distal tubular acidification concomitant with low-citrate and high-calcium urine concentration is considered a risk factor of calcium stone formation in patients with medullary sponge kidneys (MSK). We assessed the response to an acute acid load in 9 patients (6 male, 3 female) with bilateral, pluricaliceal MSK and recurrent calcium nephrolithiasis, by comparison to 9 healthy adult controls. All patients had normal glomerular filtration rate (GFR ≥ 80 ml/mn/1.73 m^2), had neither an urinary tract infection nor a recent obstructive episode, and had received no drug therapy for at least 3 weeks. Urinary pH (UpH), titratable acid (TA), NH4 (NH4), and net acid excretion (NAE) were determined before and following an oral NH4Cl load (2 mmol/kg BW, or 1 g per 10 kg BW). Maximal response (μmol/min) in patients and controls (mean ± SD) are shown in the following table.

	UpH	TA	NH4	NAE
Patients	5.13 ± 0.24	40.9 ± 15.9	59.8 ± 18.2	95.5 ± 31.4
Controls	4.82 ± 0.24	25.4 ± 20.9	72.2 ± 29.7	97.7 ± 40.8

In only 2 MSK patients was postload UpH greater than 5.3 (>2 SD from controls). In all patients, maximal response for TA, NH4 and NAE did not significantly differ from controls. Hypocitraturia (UCitV ≤ 1.7 mmol/day) was found in 1/9, but hypercalciuria (UCaV ≥ 0.1 mmol/kg/day), 0.125 ± 0.031 as a mean, was present in 8/9 patients (89%). In conclusion, no evidence of impaired distal acidification capacity in response to an acute acid load was shown in MSK patients with recurrent calcium stones. Hypocitraturia was an uncommon finding, and hypercalciuria was the most frequently identified risk factor for calcium stone formation.

B7. The Role of the Organic Matrix in the Architecture of Urinary Stones

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Urinary stones are formed in four steps: crystal nucleation, crystal growth, crystal aggregation, and concretion. The following studies were performed to understand the yet unclear mechanism of concretion. More than 100 small stones of whewellite, weddellite, uric acid, and apatite were obtained from 14 patients and used for the studies. To identify the core area, the stones were bisected and observed by scanning electron microscopy. To observe the crystal-matrix interrelations, some of the stones were dissolved in a fixative mixture containing EDTA. The remaining materials were thin sectioned and observed by light and transmission electron microscopy. Pure apatite stones were cut with a diamond knife and observed by transmission electron microscopy without staining. One or several core areas were identified in most of the stones. The core area is composed of randomly aggregated crystals and has a free space among the crystals. The core area is surrounded by mantle layers, in which crystals are packed compactly, displaying concentric laminations. Whewellite and uric acid crystals are radially oriented in the mantle layers. The organic matrix is always laminated in the mantle layers and fills the intercrystalline space except in uric acid stones. The stone surface is often covered with a thick layer of the organic matrix. The mantle layers of apatite stones are scattered with needlelike crystals about 0.2 μm in length, and the crystal density differs from layer to layer. In conclusion, the core area and mantle layers are formed by different mechanisms. The mantle layers are formed by crystal growth in the gel-state matrix, which continuously encrusts on the stone surface. This stone-growth mechanism allows no free space among the crystals, resulting in a firm architecture. This crystal-matrix interrelationship is probably the mechanism of concretion.

B8. Red Blood Cell Oxalate Flux in Patients with Calcium Urolithiasis

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Baggio et al. initially found that red blood cell transmembrane oxalate flux is greater in patients with calcium urolithiasis than in individuals without stone disease (NEJM 314:599–604, 1986). The present study examined this transport system in a North

American population. Transmembrane oxalate flux was measured in red blood cells from 14 healthy subjects, 13 patients with calcium stone disease, and 1 patient with primary hyperoxaluria. Uptake of radiolabeled oxalate by washed red cells was measured as a function of time. The flux rate, K, was calculated from the formula: $\ln(A_t - A_e)/(A_o - A_e) = -Kt$, where t denotes times, and A the quantity of oxalate at time o, t, and at equilibrium (24-h). The mean flux rate was -0.07 ± 0.07 (SD) in the control subjects, -0.21 ± 0.27 (SD) in the patients, and 1.21 in the patient with primary hyperoxaluria. The difference between the controls and patients was significant ($P < 0.05$). There was no correlation between oxalate flux and age, sex, severity of disease, or stone burden. If the upper limit of normal oxalate flux is defined as two standard deviations above the mean control flux, 36% of the patients (5 of 14) and 7% of the controls (1 of 14) had abnormally elevated flux rates. In vitro hydrochlorothiazide had no effect on red cell membrane oxalate transport in preliminary experiments. A subset of patients with calcium urolithiasis appears to have more facile transport of oxalate across the red blood cell membrane. This may be indicative of a more generalized membrane transport defect in the kidney or gastrointestinal tract.

B9. Characteristics of Oxalate Uptake Along the Villus-Crypt Axis in Rat Intestine

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Dietary oxalate is an important contributor to the body oxalate pool. Oxalate absorption from the gut has been well studied, using everted sacs or brush border membrane vesicles (BBMV). Oxalate uptake has been shown to be due to simple passive diffusion. Enterocytes were isolated sequentially along the villus-crypt axis based on the distribution of sucrase activity [1, 2]. An increase in activities of alkaline phosphatase and leucine amino peptidase from crypt base to villus tip was observed. Oxalate uptake was studied as a function of varying concentrations, time, and pH. Optimal uptake was obtained at pH 7.0 and 20 °C. The capacities of oxalate uptake varied in the different enterocyte populations. At oxalate concentrations of 0.1 mM and 0.5 mM, maximum uptake was seen in enterocytes near the villus tip, while at higher concentration (1.0 mM), the maximum uptake was seen in enterocytes from crypt base. No correlation could be seen between distribution of enzyme activities and oxalate uptake. The study suggests that specific channels are utilized for oxalate uptake, which have varying responses in different cell populations.

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B10. Calcitonin and Parathyroid Hormone Provocative Tests in Fasting Hypercalciuria

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Recent papers have given evidence of a primary increase of bone turnover (BT) in fasting hypercalciuria (FH) in stone-forming patients (SF). In order to evaluate the role of calcitonin (CT) and PTH, we studied 55 SF, 30 with and 25 without FH, divided into the following study protocols: *A*. Fifteen FH and 15 non-FH (NFH) patients were subjected to an oral Ca-load (1 g): CT, ucAMP, fast urinary hydroxyproline (uOHP), urinary and seric Ca were measured before and 2 h after the Ca-load. *B*. Ten FH and 5 NFH patients were treated for 1 month with hydrochlorothiazide (HTZ, 50 mg/day): before and after the treatment, fractional intestinal Ca absorption (I. Ca A.), Ca pool (kinetic methodology), uCa, uOHP, ucAMP and PTH were measured. *C*. Five FH and 5 NFH patients were subjected to an i.v. phosphate infusion (3 mg/kg per min over 120 min): at the beginning and at 40-min intervals ionized Ca, sPi, and PTH-NH2 were measured (results = M ± SD). The basal values of fast uOHP were significantly higher in FH than in NFH (28 ± 13.2 vs 19.8 ± 8.6 mg/g Cr, $P < 0.01$). *A*. The basal CT levels did not differ in the two groups (FH 28.9 ± 17.5, NFH 28.3 ± 21 pg/ml. The Ca-load resulted in a decrease of uOHP significantly higher in FH (FH -40 ± 5 vs NFH $-13 \pm 6\%$, $P < 0.01$) without any difference in CT response (FH $+20 \pm 15$, NFH $+21 \pm 11\%$) and ucAMP (FH $+0.18 \pm 0.78$, NFH -0.2 ± 0.36 nmol/dl GFR). *B*. HTZ significantly reduced fast uCa (0.97 ± 0.039 vs 0.141 ± 0.04 mg/g Cr, $P < 0.01$) uOHP (20 ± 9 vs 28.6 ± 8 mg/g Cr, $P < 0.001$) and Ca pool (35.4 ± 8.4 vs 42.1 ± 12.9 1, $P < 0.05$) in FH, without any significant change in I. Ca A., PTH, or ucAMP. The changes in fast uCa/Cr were strictly correlated with both changes in uOHP ($r = 0.929$, $P < 0.001$) and Ca pool ($r = 0.674$, $P < 0.05$). *C*. The Pi infusion resulted in an identical reduction of iCa in both groups (FH -0.61 ± 0.04, NFH -0.54 ± 0.03 mg/dl), but the % increase in PTH was strikingly less in FH (FH $+5 \pm 26$ vs NFH $+200 \pm 60\%$, $P < 0.01$). We conclude that in FH there is an increased BT; both Ca-load and HTZ reduce the BT without substantial changes in Ct and PTH secretion; a reduced response of PTH to hypocalcemia is evident in FH patients, a finding very similar to that recently reported in osteoporotic patients.

B11. Bacterial Ecology in Struvite Calculogenesis

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The bacteria associated with struvite stones provide for a more etiologically important part of infection stone calculogenesis than just providing the alkaline environment required for crystallization. To arrive at this hypothesis, we studied infected stone genesis in in vitro and animal models of infection – induced stone formation as well as clinical specimens. Our study examined in detail the sequential events in the evolving microbial ecology of progressive struvite calculogenesis employing conventional microbiological techniques, direct ultrastructural observation, newly developed ultrastructural cytochemical localization techniques, and immunological procedures for stabilization of the biofilm glycocalyx and stone matrix in our various models. We conclude that the renal uroepithelium is initially colonized by ureolytic pathogens. These adherent bacteria secrete a glycocalyx or exopolysaccharide, which facilitates bacterial adhesion in microcolonies, provides a degree of relative antibiotic protection, and is in part responsible for the metal binding of struvite and apatite crystals that result from the local bacterial urease activity. The crystals trapped within the gel matrix of the enlarging bacterial aggregate form multiple stone nidi coalescing on the uroepithelial surface, upon which succeeding bacterial biofilms develop and, with the incorporation of other urine components such as urinary mucoproteins, establish a matrix skeleton that becomes mineralized, thus allowing for the growth of the stone in concentric layers.

B12. Calcium Transport by Red Cells of Hypercalciuric Stone Formers

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Intestinal hyperabsorption, renal leak and/or bone hyperabsorption have been implied as mechanisms of hypercalciuria. Digestive

calcium hyperabsorption is explained by an increase in 1,25 dihydroxycholecalciferol plasma levels. However, the calcium hyperabsorption appears to be greater in jejunum than in ileum. This finding contradicts the experimental data after 1,25-dihydroxycholecalciferol administration. Furthermore, kinetic differences in the intestinal calcium binding protein between stone formers and controls have been described. We studied the calcium transport by red cells of 14 hypercalciuric stone formers (11 males and 3 females) and 16 normocalciuric controls (7 males and 9 females). The stone formers were selected in a previous study comprising blood determinations and 24-h urine samples of calcium, magnesium, phosphate urate, and creatinine. Oxalate and cystine were specifically determined in urine samples. Additionally, in all individuals PTH, calcitonine and 1,25-dihydroxycholecalciferol in plasma were determined. Definition of the type of hypercalciuria was carried out by means of a calcium oral load containing ^{45}Ca. The patients were kept on a 400 mg/24 h calcium diet for 7 days. In this test the ^{45}Ca absorption, radio calcium clearance, and calcium/creatinine ratio were established and used for the classification. The calcium transport by red cells (in patients and controls) was determined by incubating 3×10^6 red cells with 1,300 μmol calcium chloride and 5 μCi of ^{47}Ca at pH 7.4 and 0.9% sodium chloride. Aliquots at 1, 6, 10, and 15 min were taken. After centrifugation, the radioactivity in the pellets was counted. This study shows an increase in the calcium transportation rate of hypercalciuric stone formers as compared to the controls. No differences in 1,25-dihydroxycholecalciferol plasma levels were detected between groups. These findings suggest that the hypercalciuria is a generalized process and that intestinal hyperabsorption and/or renal leak are different phases of the same disease.

B13. Effect of Hypovitaminosis A and Supplementation of Vitamin D3 on Calcium and Oxalate Absorption by Rat Intestinal Brush Border Membrane

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Intestinal absorption of various lithogenic substances is known to be altered in calcium-oxalate lithiasis. Vitamin A deficiency plays an important role in the etiopathogenesis of this disease. Therefore, biochemical changes produced by vitamin A deficiency on absorption of calcium and oxalate by rat intestinal brush border membrane vesicles (BBMV) were studied [1, 2]. Calcium influx by intestinal BBMV demonstrated saturable kinetics, followed by 40% decrease in calcium uptake in vitamin A deficient rats. In contrast, hypovitaminosis A led to significant ($P < 0.01$) hyperabsorption of oxalate through the gut. Since vitamin A is known to stimulate the activity of 25-hydroxyvitamin D_3-1-hydroxylase in kidneys, the possible effects of $1,25(OH)_2$ vitamin D_3 supplementation on intestinal absorption of calcium and oxalate were also studied. The calcium and oxalate accumulation by intestinal BBMV was markedly stimulated ($P < 0.001$) immediately after 4 h vitamin D_3 administration. The results suggest that the disturbance in intestinal ionic transport by vitamin A deficiency may be vitamin D_3 dependent, accountable by stimulation of vitamin A dependent $1,25(OH)_2$ vitamin D_3 synthesis.

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B14. Study of Circadian Rhythmicity of Urinary Excretion of Glycosaminoglycans in Normals and Stone-Formers

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Glycosaminoglycans (GAGs) have been reported to exercise an inhibitory effect on the crystallization of urinary calcium salts. Fluctuations in GAGs excretion can occur depending on the time of day [1]. To investigate the significance of GAGs in the etiopathogenesis of urinary calculi, circadian rhythmicity in GAGs excretion was studied in 26 idiopathic calcium oxalate stone-formers and 32 healthy subjects. Urinary GAGs were determined in terms of glucuronic $acid_2$ in 3-h urine samples collected for 24 h, starting at 0 h. The urinary concentration of GAGs was significantly decreased in patients of renal stones as compared to healthy subjects (9.599 μmol/l and 18.347 μmol/l, respectively, $P < 0.001$). The healthy subjects exhibited a circadian rhythmicity in the urinary excretion of GAGs ($F_{2,31} = 18.422$; $P < 0.001$), similar to that of renal stone patients ($F_{2,25} = 23.084$; $P < 0.001$). However, a significant difference was found in the amplitude-acrophase between the two populations. In healthy controls, the amplitude was 7.028 μmol/l in contrast to 2.354 μmol/l in renal calculi patients ($T^2 = 16.9$; $F_{2,57} = 8.3046$; $P < 0.001$). Thus in renal calculi patients, the urinary excretion of GAGs was significantly less compared to healthy adults and although both groups exhibited a statistically validated circadian rhythmicity in urinary excretion of GAGs, the amplitude-acrophase was different in patients of renal calculi.

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B15. Lack of a Relationship Between Urinary Calcium Excretion and Sodium Excretion in Stone-Formers with High-Fasting Calcium Creatinine Ratios

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A relationship between urinary sodium and calcium excretion has been found in animals and man. We examined this relationship in 158 individuals with a history of stone formation (SF) and 8 individuals with no history of stone formation (NSF) on ad lib. diets. For SF, two 24-h specimens were analyzed for UCaV and UNaV and the results averaged. NSF had a single 24-h specimen analyzed for UCaV and UNaV. The SF were divided into two groups: normocalciuria (NH) if UCaV $\leq$250 mg/day in males or $\leq$200 mg/day in females and hypercalciuria if these values were exceeded. Individuals with hypercalciuria were placed on a diet containing 400 mg calcium, 800 mg phosphorus, and 100 mEq sodium for 5 days. Thereafter, urine was collected and analyzed for calcium/creatinine in a fasting state (FCa/Cr) and after a 1 g oral calcium load (LCa/Cr). Individuals with a FCa/Cr <0.12 and LCa/Cr >0.20 were considered to have absorptive hypercalciuria (AH). Individuals with FCa/Cr $\geq$0.12 were considered as high FCa/Cr (H-FCa/Cr). Of the 158 SF, 93 had normocalciuria (NH), 29 had absorptive hypercalciuria (AH), and 36 had high FCa/Cr (H-FCa/Cr). Linear regression analysis was used to test for a relationship between UNaV and UCaV in each group ($\bar{x} \pm$ SD). A significant positive relationship between UCaV and UNaV was found among NSF, NH, and AH. There was no difference in the slope of the regression line in NSF, NH or AH, but the constant

	UCaV	UNaV	Regression	r	P
NSF	93 ± 47	160 ± 68	Y = 0 + 0.58x	0.83	<0.05
NH	149 ± 83	166 ± 68	Y = 26 + 0.74x	0.60	<0.05
AH	214 ± 62	163 ± 56	Y = 125 + 0.55x	0.49	<0.05
H-FCa/Cr	330 ± 111	183 ± 72		0.13	NS

was statistically higher in the AH compared to NSF and NH. No relationship between UCaV and UNaV could be found in those with H-FCa/Cr. This is compatible with an altered renal handling of calcium and/or sodium in subjects with H-FCa/Cr.

B16. Clearance Studies in Normal and Nephrocalcinotic Rats on Nifedipine

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Our previous studies demonstrated that Nifedipine attenuates experimental nephrocalcinosis in rats, although it causes simultaneous hypercalciuria. Moreover, increased urinary citrate and magnesium were found. The underlying mechanisms, however, were not clear. Thus we performed urine and clearance studies in 40 male rats divided into five groups: (1) normal diet; (2) normal diet plus Nifedipine; (3) atherogenic diet; (4) atherogenic diet + Nifedipine. After 4 weeks the rats were given a low-calcium diet and clearance studies were performed. Urine was collected in week 1, 4, and 5. Atherogenic diet decreased the clearances of inulin, sodium, and magnesium. Nifedipine increased the clearances of inulin, calcium and sodium. Urinary excretion of calcium, magnesium, and citrate was raised by Nifedipine. We conclude that Nifedipine improves the glomerular filtration rate. The effects of sodium and calcium excretion seem to be of renal origin.

B17. A Model for the Study of the Effects of Stones and Drugs on the Pig Ureter

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The management of ureteric colic is unsatisfactory, and there is need for a model to provide knowledge of the pathophysiology of stone obstruction and a scientific basis for drug therapy. In female pigs anesthetised with halothane, the abdomen is opened and the right kidney partially mobilised to permit a needle nephrostomy. A 0.038 guide wire is passed down the ureter to the bladder, which is also opened. The nephrostomy is dilated and a 16 F (5.3 mm) cannula inserted into the pelvis. A fine nylon thread tied to a 4-mm calculus is pulled down the ureter by the guide wire to the bladder and the stone positioned in the renal pelvis. The tips of an 8 F recording and a 6 F perfusing catheter are positioned in the pelvis through the cannula, and the extrarenal end of the cannula is sealed. The ureter is perfused with green dye to delineate the lumen, and ureteric movements and diameter are recorded on tape with a video camera for later computer analysis. Pressures are recorded from the renal pelvis and distal ureter and from the femoral artery. Urine flow is measured. Following baseline recording, the stone is gently pulled from the pelvis until it impacts, generally in the mid-ureter. With one exception, stone impaction did not produce a rise in pressure and hyperperistalsis. Glucagon was administered intravenously in doses of 1 mg, 2 mg, 4 mg, and 8 mg at 20-min intervals to six pigs weighing 32–35 kg. In one animal a fall in wave amplitude and baseline upper tract pressure was repeatedly produced in spite of an increase in urine flow, but in none of the others was there a consistent change in the parameters, and the stone position was unchanged. Nifedipine was administered intravenously in a dose of 1 mg, followed 30 min later by 2 mg to ten animals weighing 31–44 kg. In all instances blood pressure and ureteric wave amplitude fell promptly, as did urine flow, but distal stone movement was not facilitated. These studies suggest this model may be useful in identifying drugs that will promote stone expulsion.

B18. Hereditary Hypophosphatemic Hypercalciuria

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We studied three kindreds with hereditary hypercalciuria. Kindred I: parents' first cousins, father, two sons and a daughter (out of seven siblings) suffered from recurrent nephrolithiasis, rickets, and/or osteomalacia. Kindred II: in 59 closely related members of one kindred, 30 had hypercalciuria; 9 of them suffered also from rickets and/or osteomalacia. Kindred III: parents' first cousins, two siblings out of seven, had primary Fanconi syndrome, hypercalciuria, rickets, and/or osteomalacia with normal acid-base balance. The common denominators in these three kindreds, in addition to hypercalciuria that fell to the normal range after 18 h fasting, were: decreased tubular reabsorption of phosphate (TmP/GRF), hypophosphatemia, normocalcemia, elevated 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) serum concentrations, tendency towards low serum levels of immunoassayable PTH (iPTH), and urinary cyclic-AMP excretion, increased gastrointestinal absorption of calcium and phosphorus, and elevated serum concentrations of alkaline phosphatase of bone origin in patients with bone disease. We believe that the primary hereditary defect in these kindreds is decreased tubular reabsorption of phosphate (Pi), leading to the following sequence of events: hypophosphatemia, stimulation of renal 1α-hydroxylase 25-hydroxyvitamin D, elevated serum $1,25(OH)_2D$, enhanced intestinal calcium (Ca) and Pi-absorption, increased renal filtered load of Ca and hypercalciuria. Hyperabsorption of Ca may suppress PTH release and renal tubular Ca reabsorption, adding to the hypercalciuria. Treatment with oral Pi (2–2.8 g/day) resulted in normocalciuria, reduction of serum $1,25(OH)_2D$ levels, cure of rickets and/or osteomalacia in patients with bone disease, with no change in the renal Pi leak. In conclusion: (1) a hereditary renal Pi-leak may lead to absorptive hypercalciuria via increased concentrations of $1,25(OH)_2F$; (2) depending on the degree of hypophosphatemia, defective mineralization of bone matrix may accompany the disorder; (3) the mode of inheritance seems to be autosomal; (4) these kindreds differ from classic X-linked hypophosphatemic rickets in the normal response of the renal tubular 1α-hydroxylase 25-hydroxyvitamin D to low Pi and may point to a different localization of the primary tubular defect along the nephron.

B19. Alterations in Renal Brush-Border Membrane Enzymes of Vitamin A, B_1, and B_6 Deficient Rats

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Hyperoxaluria is a common feature in patients with urolithiasis [1]. To investigate the effect of vitamin deficiencies on oxalate metabolism, experimental hyperoxaluria was induced in male

weanling rats by implementing vitamin A, B_1 and B_6 deficiencies, and the activities of renal brush-border enzymes such as alkaline phosphatase (AP), leucine aminopeptidase (LAP), and gamma-glutamyl transpeptidase (GGT) were assayed. A significant enhancement in urinary excretion of oxalate was observed in vitamin-deficient ($P < 0.05$ in vitamin A; $P < 0.01$ in vitamin B_1; and $P < 0.001$ in vitamin B_6) rats as compared to their pair-fed controls. Vitamin B_1 and B_6 deficient rats showed significant decrease in the activities of LAP ($P < 0.01$ and $P < 0.001$, respectively) and AP ($P < 0.05$ and $P < 0.001$, respectively). However, in vitamin A deficiency, only LAP was decreased ($P < 0.001$). The K_m and V_{max} of these enzymes reveal that the decreased activities of both AP and LAP in vitamin B_1 deficiency and LAP in vitamin A deficiency are due to a reduction in the respective active enzyme molecular numbers, while in pyridoxine deficiency the decrease in AP and LAP activities is due to decreased affinity for their respective substrates. Significant increase in GGT ($P < 0.001$) was observed only in thiamine-deficient rats, which is probably due to an enhanced turnover of enzyme rather than an increased affinity for the substrate.

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B20. Circadian Rhythmicity in Urinary Citrate Excretion in Healthy Men and Male Calcium-Oxalate Stone-Formers

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Urinary citrate is a potent inhibitor of calcium-oxalate and calcium-phosphate crystal formation and growth. Chronobiological studies on urinary excretion of calcium, oxalate, uric acid, and inorganic phosphate showed absence of circadian rhythmicity in lithogenic substances in stone-formers as compared to healthy subjects [1]. This study has been extended and circadian variation of urinary citrate excretion was estimated in 3-h urine samples collected for 24 h from 11 healthy men (group I) and 17 male calcium-oxalate stone-formers (group II). The 24-h urinary citrate excretion was 530.27 ± 87.77 mg in group I and 505.34 ± 40.17 mg in group II ($t = 0.2896$; $P > 0.05$). Cosinor rhythmometry [2] revealed a significant circadian rhythmicity in urinary citrate excretion in healthy men ($F_{2,5} = 10.73$; $P < 0.05$) but not in stone-formers ($F_{2,5} = 0.455$; $P > 0.05$). The amplitude was 14.09 mg in group I and 3.66 mg in group II. The acrophase was located at 1435 hours in group I and at 2330 hours in group II. Although there was no significant difference in the total 24-h urinary citrate excretion between the two groups, chronobiological study revealed that urinary citrate excretion showed a circadian rhythmicity in healthy men, which was absent in patients with calcium-oxalate nephrolithiasis.

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B21. Circadian Rhythmicity in the Urinary Excretion of Calcium, Oxalate, Uric Acid and Inorganic Phosphate in Stone-Formers and Their Cohorts in Northwestern India

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Environmental factors such as diet, climate, geological factors, and fluid intake play important roles in the etiology of urolithiasis. Cohorts of stone-formers who belong to same ethnic group, share the same food habits, and are exposed to similar climatic conditions may be equally susceptible to stone disease, which may possibly be present in the form of subtle biochemical changes. In this study circadian rhythmicity of urinary volume and excretion of creatinine, calcium, oxalate, uric acid, and phosphate were studied in 15 idiopathic stone-formers and in 17 cohorts of stone-formers who had no clinical evidence of stone disease. Three-hourly urine samples were collected, starting from 0 h for 24 h and analyzed for various lithogenic substances. The time series of data were analyzed by cosinor rhythmometry. Although a significant circadian rhythmicity has been detected in urinary volume, and excretion of creatinine, calcium, oxalate, uric acid and phosphate in healthy adults, circadian rhythmicity was found only in urinary volume ($P < 0.01$) and creatinine excretion ($P < 0.05$) in cohorts of stone-formers. No rhythmicity was detected in any of the parameters in stone-formers. Thus, cohorts of stone-formers fall between healthy adults who exhibit circadian rhythmicity in urinary volume and excretion of creatinine, calcium, oxalate, uric acid, and inorganic phosphorus and the stone-formers in whom rhythmicity was not manifested in any of these parameters.

B22. Oxalate Transport System Across Brush-Border Membrane in the Rat Kidney

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To examine the oxalate transport system on the brush-border membrane of rat kidney, brush-border membrane vesicles (BBMV) were isolated from the rat kidney cortex by an EGTA/magnesium precipitation method. An inside alkaline (pH 8.5 inside, pH 6.5 outside) pH gradient stimulated the uptake of [14-C] oxalate in BBMV (about five-fold). In the absence of a pH gradient (pH 6.5 outside and inside), no stimulation was observed. Evidence that this pH-stimulated oxalate (Ox) uptake represents Ox:OH exchange included: (1) no overshoot of oxalate uptake with inside positive diffusion potential by using inwardly directed potassium gradient and valinomycin (electroneutral transport process); (2) inhibition of oxalate uptake by 4,4′-disothiocyanostilbene-2,2′-disulfonate (disodium salt: DIDS), which is an anion exchange inhibitor; (3) enhancement of oxalate uptake by imposition of outwardly oxalate gradient (transstimulation); (4) saturation of oxalate uptake by BBMV according to the increase of extravesical oxalate concentration. In conclusion, an Ox:OH exchange exists on the brush-border membrane of rat kidney, and then oxalate transport across the brush-border membrane consists not only of passive diffusion but also of an Ox: OH exchange pathway.

B23. Hyperuricosuria – A Risk Factor for Uric Urolithiasis in Saudi Arabia

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One-third of all patients referred to urologists in Saudi Arabia present with a history of urolithiasis. Apart from CaOx and CaOx/CaP, uric acid or mixed urate stones are the most frequent stone types found here. In order to determine what factors might contribute to this high incidence, we have completed metabolic investigations and dietary questionnaires in 260 middle eastern subjects living in Saudi Arabia. The metabolic investigations included a 2-h urine collection following a 13-h overnight fast and two (where possible) 24-h non-fasting urine collections in each indiv-

idual. Results from these procedures were compared with data from Vancouver, Canada, where an identical protocol was followed.

Urate/Creatinine in (mg/mg)	Saudi Arabian vs stone-formers (145)	Vancouver stone-formers (45)
Fasting	0.55 ± 0.02	0.35 ± 0.02**
Non-fasting	0.49 ± 0.03	0.37 ± 0.03*

Values shown are mean ± SEM; (*n*) in parentheses;
* $P < 0.01$; ** $P < 0.001$

The 60% greater fasting and the 32% higher non-fasting urate excretion in stone-formers in Saudi Arabia cannot be accounted for by differences in analytical procedures. It would appear that this Middle Eastern population has a higher urate load to excrete than those in the West since serum urates averaged 1 mg/dl higher as well. Dietary questionnaires revealed a purine intake double that found in the West. This, compounded with a low urine volume and a more acid urine secondary in part to the hot and arid climate, renders these individuals at greater risk for uric acid stone production.

B24. Urinary Calcium and Oxalate Excretion in Stone-Formers and Normal Subjects in Saudi Arabia

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Urolithiasis is a major health care concern in Saudi Arabia. In an investigation of this problem, we have carried out metabolic studies in male idiopathic stone-formers (SF) and normal subjects (NS) in Saudi Arabia. Since Ca and Ox comprise the major constituents of stones that are found here, we have examined the excretion of these ions initially following 13-h overnight fast in normal subjects and in fasting normocalciuric (FNC) and fasting hypercalciuric (FHC) stone-formers, none of whom demonstrated hyperabsorption of dietary Ca. We then examined them in 24-h urine collections when subjects were consuming their self-chosen diets.

Fasting Ox/Creat-ratios were similarly low in normal subjects and stone-formers whether in the presence or absence of hypercalciuria. In fact, they do not differ from Vancouver values using an identical protocol. This indicates that hyperoxaluria in Saudi Arabia is not an inherent state but most likely occurs secondary to dietary differences. In 24-h non-fasting urine samples, Ox was increased in both groups of stone-formers but not in normal subjects. This we also observed in Vancouver. However, the magnitude of the increase was greater in the Saudi Arabian stone-formers but similar to Vancouver values when we reduced dietary Ca intake in the Vancouver patients. This suggests that a low-Ca intake which many stone-formers are advised to eat in Saudi Arabia may contribute to hyperoxaluria in the non-fasting state since hyperabsorption of dietary Ca, which can also cause hyperoxaluria, is a rare finding in Saudi Arabia.

	Fasting	
	Ca/Creat.	Ox/Creat.
NS (33)	0.07 ± 0.01	0.022 ± 0.002
FNC (76)	0.06 ± 0.003	0.022 ± 0.002
FHC (47)	0.15 ± 0.01	0.023 ± 0.001
	Non-fasting	
	Ca/Creat.	Ox/Creat.
NS (33)	0.07 ± 0.01	0.027 ± 0.002
FNC (76)	0.11 ± 0.01*	0.036 ± 0.002*
FHC (47)	0.14 ± 0.01	0.043 ± 0.003*

Values are ion/creatinine (mg/mg) ratios (mean ± SEM); *n* in ( );
* $P < 0.001$ as compared to fasting values.

B25. Volume Control in the Desert – Stone-Formers in Saudi Arabia

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In Saudi Arabia, temperatures for most of the year can range between 40° and 50 °C and the relative humidity between 4% and 7%. As a result, considerable loss of salt and water occurs through the sweat glands and airways. In the desert on a dry, hot day, a man can lose 10–12 l of water. We have examined urinary salt excretion in a Middle Eastern population of 145 male idiopathic stone-formers (SF) and 33 normal subjects (NS) in the well-hydrated state following overnight fast and also in 24-h non-fasting collections where subjects were pursuing their normal activities indoors and out.

	Fasting		Non-fasting	
	NS	SF	NS	SF
Na/Creat.	0.10 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.10 ± 0.004
Cl/Creat.	0.10 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	0.10 ± 0.004

Values shown are mean ± SEM; ion/creatinine ratios are mEq/mg

In more temperate climates in the world, Na and Cl as well as dietary ions increase significantly in the urine in response to food. However, in Saudi Arabia this was not a consistent finding in normal subjects or stone-formers. In fact, urinary Na and Cl either stayed the same or decreased from well-hydrated fasting values despite the fact that salt intake in Saudi Arabia is considerably higher. Increases in other ions such as Ca or Mg, which share many common transport sites in the kidney and which also have a high concentration in sweat, were also blunted in our subjects in Saudi Arabia. Secondary to such salt and water loss, dehydration is commonly found in Saudi Arabia. This is associated with urine specific gravities as high as 1.080 and random urine pHs rarely above 5. Thus, climate in Saudi Arabia initiates a setting for increased risk of stone production in its population.

B26. Urinary Calcium Excretion in Saudi Arabia

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Urinary calcium excretion in Saudi Arabia is frequently reported as low when using Western values. Low dietary calcium, low vitamin D levels, loss of Ca in sweat, and a generally smaller body size in

Saudi Arabia may all be contributing factors in this assessment. We have examined urinary Ca excretion in male idiopathic Saudi Arabian stone-formers (SF) and normal subjects (NS) both in 2-h collections following 13-h overnight fast and in 24-h non-fasting collections, comparing values with those we found in Vancouver, Canada:

Ca/ Creat. (mg/mg)	Saudi Arabia		Vancouver	
	SF (145)	NS (33)	SF (45)	NS (15)
Fast	0.10 ± 0.01	0.06 ± 0.01*	0.11 ± 0.01	0.06 ± 0.01**
24-h	0.13 ± 0.01	0.07 ± 0.01**	0.18 ± 0.02	0.10 ± 0.01*

Values shown are mean ± SEM; n (in parenthesis);
* $P < 0.01$; ** $P < 0.001$ vs SF

In a well-hydrated state following fasting, Ca/Creat-ratios did not differ between the two geographic groups. However, lower levels were found for both stone-formers and normal subjects in 24-h collections taken in Saudi Arabia. The difference between the two stone-former groups can be accounted for in part by the fact that in the Ca-load studies we carried out in both countries, we found that only 13% of stone-formers in Saudi Arabia demonstrate hyperabsorption of dietary Ca. In Canada, this value was 50%. In Saudi Arabia, the 24-h Ca/Creat-ratio for the hyperabsorbers alone was 0.19 ± 0.02. If in the Saudi Arabian stone-formers the percentage of hyperabsorbes had been higher, the difference in non-fasting 24-h urine collections would have been narrowed. However, it does appear that hypercalciuria is less frequent in Saudi Arabia. Only 31% had 24-h Ca/Creat-ratios >0.15 *vs* 42% in Vancouver. The decreased incidence of hyperabsorption of dietary Ca ist one reason, but other factors previously noted must also play a role.

B27. Renal Handling of Oxalate in Normals and Patients with Primary and Secondary Hyperoxaluria and Idiopathic Stone Disease

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Adaptation of the oxalate oxidase method of Kasidas (Ann Clin Biochem 22:412, 1985) with a modified Kratos 757 HPLC spectrometer and a Kipp and Zonen BD41 recorder has allowed reliable direct determination of plasma oxalate (Pox). Renal oxalate filtration, reabsorption, and fractional excretion (FE) were determined in the following groups in a fasting state ($\bar{x}$ ± SD):

	(N) Normals (9)	(1°) Primary hyperoxaluria (8)
Pox (μm/l)	3.09 ± 1.09	6.91 ± 3.28**
GFR (ml/min)	151 ± 38	100 ± 40**
FEox	0.469 ± 0.282	2.74 ± 1.23**
Uox (mg/24°)	–	229 ± 112
	(2°) Secondary hyperoxaluria (4)	(I) Idiopathic (8)
Pox (μm/l)	4.18 ± 0.79*	2.01 ± 0.77***
GFR (ml/min)	47 ± 19**	137 ± 42
FEox	3.73 ± 1.31**	0.73 ± 0.288*
Uox (mg/24°)	125 ± 46	54 ± 20***

* $P = <0.05$ vs N; ** $P = <0.01$ vs N; *** $P = <0.05$ vs 1°

These data show Pox was significantly higher in 1° and 2° than in N. Pox was low in I. FE in N and I were <1.0, suggesting tubular reabsorption in these cases. In 1° and 2°, FE oxalate was >1.0, suggesting tubular secretion. In 1°, 2°, and I, FE was higher than normal, while Pox was up in 1° and 2° and down in I. In 1 patient with 1°, a liver/kidney transplant (Tx) was performed. Pox fell from 97 to 6.7 μm/l over 1 week with the FE rising from 2.01 to 3.23, and GFR increased from 17 to 67 ml/min. Over the next year FE fell to 0.76, while Pox fell to 3.0 μm/l. UV oxalate (mg/24°) fell from 289 mg pre-Tx to 184 mg at 1 week, and 31 mg at 1 year. FE in this patient clearly correlated to the amount of urinary oxalate rather than Pox (GFR remained constant). After an oxalate meal in 5 N, FE rose from 0.376 to 0.628 ($P < 0.05$), while Pox was unchanged at 3.38 and 2.78 μm/l. Pox is lower than normal in I, but increased in 1° and 2°. Normals have net tubular reabsorption of oxalate, whereas 1° and 2° secrete oxalate. FE is increased in all patient groups and correlates better to UVox than Pox. In I the high FE and low Pox is consistent with a "renal leak" of oxalate.

B28. Effect of Calcitriol Administration on Urinary Excretion of Nephrocalcin, a Calcium Oxalate Crystal Growth Inhibitor

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Nephrocalcin (NC) is an acidic glycoprotein that contains gamma-carboxy-glutamic acid (Gla), and inhibits calcium oxalate crystal growth in vitro. NC has been purified from urine and from human kidney tissue culture supernatants, and immunohistochemically localized to the proximal tubule. An analogous Gla-containing protein, osteocalcin, is found in association with the mineral phase of bone, and serum levels of osteocalcin rise in response to administration of 1,25-$(OH)_2$-D_3. In order to evaluate the effect of 1,25-$(OH)_2$-D_3 on urinary NC excretion, we studied six healthy men fed constant diets, before and during the administration of 1,25-$(OH)_2$-D_3, 0.5 μg every 6 h. Urinary NC was measured using a competitive ELISA assay, with antiserum raised in rabbits against human urinary NC. 1,25-$(OH)_2$-D_3 was measured by competitive protein binding assay. Values are means ± SEM.

	Control	Calcitriol	P
Serum 1,25-$(OH)_2$-D_3 (pM)	96 ± 16	163 ± 18	<0.001
Urine NC (mg/day)	144 ± 32	93 ± 34	<0.025
Urine Ca (mmol/day)	3.2 ± 0.5	9.4 ± 0.9	<0.001
Urine PO_4 (mmol/day)	31.5 ± 2.4	37.6 ± 2.1	<0.005
Urine oxalate (mmol/day)	0.46 ± 0.05	0.41 ± 0.04	NS

Urinary NC excretion fell during calcitriol administration. This decline may reflect suppression of NC production by calcitriol. Alternatively, since the hypercalciuria in these subjects may cause calcium oxalate crystalluria, NC may have been adsorbed to urinary crystals, thus preventing its measurement.

B29. The Value of Extensive Urine and Stone Cultures in Patients Undergoing Surgical Removal of Urinary Tract Stones

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It has recently been suggested that certain urease-producing microorganisms such as *Ureaplasma urealyticum* and some Corynebacteria could be involved in the formation of infection stones composed of magnesium ammonium phosphate and carbonate apatite. This study was initiated to investigate this and to evaluate how often infection stones are associated with the presence of urease-producing microorganisms in urine and the stone if extensive culture routines are undertaken. From 1 February 1986, stone cultures and cultures on preoperatively obtained voided urine have been performed on all patients operated upon percutaneously for renal and ureteral stones. The cultures have been performed under both aerobic and anaerobic conditions for at least 48 h and on specific media for *U. urealyticum*. By 31 October 1987, 257 patients had been operated upon. Thirty-nine patients were excluded due to incomplete data. Of 218 evaluable patients, 104 (48%) had negative stone and urine cultures. Positive cultures (urine and/or stone cultures) were most common in patients with infection stones (84%) while only 33% of the patients with calcium oxalate stones had positive cultures. Patients with calcium oxalate phosphate stones constituted an intermediate group, with infection in 51% of the patients. In 74% of the patients with pure infection stones and 58% of the patients with mixed stones (metabolic and infection stone components), urease-producing microorganisms could be cultured. Patients with calcium oxalate phosphate stones had a significantly higher prevalence of infections with non-urease-producing microorganisms than patients with calcium oxalate stones. Except for growth of *U. urealyticum* in 40 patients, the extended microbiological culture technique did not reveal any microorganisms not found with the standard culture procedure.

Conclusions: (1) Preoperative urinary tract infection was very common (52%) in patients who were operated on for urinary stones; 58% of the infections were stone-associated; (2) in most (74%) but not all of the patients with infection stones, an etiological cause in the form of urease-producing microorganisms could be identified; (3) the higher incidence of infections with non-urease-producing microorganisms in patients with calcium oxalate phosphate stones, compared to those with pure calcium oxalate stones, indicates that even such an infection could be of etiological significance in this type of stone.

B30. The Bacteriology of Renal Stones Removed by Operation

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The aim of the study was to investigate how often renal concrements are associated with an infection, how often they contain infection stone components (magnesium ammonium phosphate and carbonate apatite), how often the presence of infection stone components is linked to the presence of urease-producing microorganisms, and to evaluate the value of stone cultures. In 100 consecutive patients undergoing removal of renal stones during an 18-month period in 1982–1983, stone cultures and cultures from voided urine and pelvic urine were performed. Complete data were obtained in 80 patients (47 females) with a mean age of 52 years (range 18–80 years). The results from these 80 patients will be presented. The stones were extracted by nephropyelolithotomy in 58 patients, percutaneously in 19 patients, and in connection with nephrectomy in 3 patients. Forty stones (50%) contained infection stone components. Thirty-one of these were pure infection stones. The other 40 were metabolic stones. Sixty percent of the patients were infected. In only 44% of these patients was there complete accordance between voided urine cultures and stone cultures. In 7 (15%) a microorganism was cultured from the stone but not from voided urine. In only three-quarters of the patients with pure infection stones could a urease-producing microorganism be cultured from the stone or pelvic urine. The microorganism most commonly cultured from infection stones was *E. coli*, followed by *Proteus mirabilis* and *Ureaplasma urealyticum*. *E. coli* occurred both as a mixed infection with a urease-producing organism and, in 6 patients, as the only organism cultured, including growth within the stone. Infection stones apparently constitute a large proportion of the renal stones that require surgical removal. In a significant number of the patients, these stones were not linked to the presence of urease-producing microorganisms. *E. coli* was frequently cultured from infection stone patients where no urease-producing microorganism could be cultured. This suggests the possibility that *E. coli* might be involved in the stone formation. The correlation between stone and voided urine cultures was not high, and it is recommended that stone cultures be routinely performed.

B31. The Role of Infection in the Adherence of Urease-Induced Crystals to the Urothelium

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Apart from urine supersaturation with respect to magnesium ammonium phosphate and calcium phosphate, caused by urease-producing microorganisms, crystal retention is considered to be necessary for the formation of infection stones. This study was performed to investigate the role of the mucous coat, which lines the urothelium, in the adhesion of sterile urease-induced crystals and to determine to what extent the adhesion is influenced by infection. The adhesion of crystals was studied in rat bladders after incubation with a supersaturated slurry containing magnesium ammonium phosphate and calcium phosphate crystals for 1 h. Seven experimental groups were used: intakt rat bladders, rat bladders pretreated with 0.1 M HCl (which disrupts the mucous coat without destroying the underlying urothelium) and rat bladders preinocculated with *Proteus mirabilis, Escherichia coli, Enterococci* or *Ureaplasma urealyticum* for 2 h. In another group of rats, efforts were made to restore the function of the mucous coat with heparin after *E. coli* inoculation. Elimination of the mucous coat with weak acid increased the adherence of crystals six times compared to that in bladders with an intact mucous coat. Infection with *P. mirabilis, E. coli, Enterococci* and *U. urealyticum* increased the adherence 6, 5, 4, and 2 times, respectively. The increased crystal adherence caused by *E. coli* infection was completely eliminated by heparin treatment after the infection. An intact mucous coat prevents the adhesion of urease-induced crystals. Infection with urease as well as non-urease-producing uropathogenic microorganisms eliminates this protection. This indicates that urease-producing microorganisms can contribute to the formation of infection stones, not only by altering the urine composition but also by enhancing the retention of crystals in the urinary tract. It also suggests that non-urease-producing microorganisms, like *E. coli*, may play a role in the stone formation.

B32. *Ureaplasma Urealyticum*, an Etiological Agent for the Development of Infection Stones in the Urinary Tract

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At the Garmisch-Partenkirchen meeting 4 years ago, we presented our first results regarding the role of *Ureaplasma urealyticum* in the development of infection stones. Further culture results regarding *U. urealyticum* in patients operated on for renal stones will be presented. During the period 1982–1987, we have routinely performed cultures for *U. urealyticum* in patients operated on for renal stones. In 392 patients cultures have been performed on preoperatively voided urine and in 189 patients the stones have been cultured. The voided urine samples and the stones were cultured for *U. urealyticum* according to Shepard and Lunceford (J Bacteriol 93:1513–1520, 1967). Cultures were performed in a urease color-test fluid medium and on specific agar-culture plates. *U. urealyticum* was cultured from voided urine in 31 of 247 patients (13%) with metabolic stones (composed of calcium oxalate – phosphate or urate), compared to in 43 of 145 patients (30%) with infection stones (composed of struvite and carbonate apatite). *U. urealyticum* was cultured from 2 of 125 metabolic stones (2%), compared to from 10 of 64 infection stones (16%). These differences between metabolic and infection stones are highly significant ($P < 0.001$). The presented clinical data strongly suggest an etiological role of *U. urealyticum* in the development of infection stones. *U. urealyticum* is an urease-producing microorganism not identified by conventional bacterial culture methods, and special media and techniques are required for its isolation and identification. Its undetected presence may thus be one explanation for infection stone formation in the absence of urease-producing bacteria. It is therefore recommended that, besides bacteria, *U. urealyticum* be sought in all patients with renal stones in order to be able to give optimal treatment to avoid unnecessary stone recurrences.

B33. *E. Coli* – A Promoting Factor for the Development of Phosphate Stones?

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The aim of the study was to determine whether urine inoculation with *E. coli* prior to urease incubation influences the urease-induced precipitation of phosphate and magnesium. Synthetic urine and human urine were inoculated with *E. coli* for 20 h. After filtration with a 0.22 μm filter to remove the bacteria, the inoculated urine samples and control urine were incubated with urease for 4 h. The precipitation of phosphate and magnesium in the urine and on glass rods immersed in the urine was measured; pH and the ammonium ion concentration of the urine before and after *E. coli* inoculation and after urease incubation were also measured. *E. coli* inoculation of both synthetic and human urine was associated with urine alkalinization not accompanied by an ammonium ion increase. The subsequent urease incubation resulted in a more pronounced ammonium ion and pH increase in *E. coli*-inoculated synthetic urine compared to controls. This was not seen in *E. coli*-incubated human urine, however. The urease-induced precipitation of phosphate was more pronounced in both human and synthetic *E. coli*-inoculated urine. The precipitation on the glass rods was lower in *E. coli*-incubated synthetic urine compared to controls. For human urine, however, there was no difference in precipitation on glass rods between *E. coli*-inoculated urine and controls. The precipitation of magnesium showed a pattern similar to that of phosphate. There are clinical observations indicating that *E. coli* may be involved in phosphate stone formation, including that induced by urease-producing microorganisms. There are several plausible mechanisms through which this might occur. The results of this study support the possibility that *E. coli* alters urine in a way which augments urease-induced crystallization.

B34. Changes in Urinary Solute Excretion During Fasting in the Holy Month of Ramadan

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During the holy month of Ramadan, Muslims abstain (fast) from food and drink for about 15 h (dawn to sunset) daily for 30 days. The renal effects of this are largely unknown. Therefore, we studied the renal handling of calcium (Ca), phosphorus (P), uric acid (UA) and sodium (Na) in 14 healthy Middle Eastern men. Studies were conducted on 5 days – a control day 10 days before the start of Ramadan (day 1), the beginning, middle and end of Ramadan (days 2, 3, and 4, respectively), and a second control day 10 days after the end of Ramadan (day 5). Blood samples were collected at 0330 and 1830 hours each day, and urines were collected over four periods each day – 1830 to 0330, 0330 to 0700, 0700 to 1600 and 1600 to 1830 hours in that order. The results showed no significant difference in the daily excretion of Ca, P, UA and Na across the 5 study days. However, there was a significant difference in the urinary concentration of Ca (Uca) between the 1830 hours samples of day 1 and day 2 ($P = 0.007$). This difference gradually disappeared with time (i.e., day 1 vs day 3, $P = 0.02$, day 1 vs day 4, $P = 0.12$ and day 1 vs day 5, $P = 0.17$). This difference was not seen with *P*, UA, or Na. Similarly, the Ca excretion/100 ml glomerular filtrate $\left(\frac{UCa}{Ucr} \times P_{cr}\right)$ decreased significantly during the fasting period. However, this change disappeared by the next non-fasting study point. We conclude that the urinary concentration of Ca decreases significantly as a result of fasting in Ramadan. This decrease is, however, transient and appears to have no lasting effects.

B35. The Effect of Fasting During the Holy Month of Ramadan on the Risk of Calcium Oxalate and Uric Acid Stone Formation

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A study was conducted to determine the effect of prolonged fasting (15 h/day) during the holy month of Ramadan on the risk of forming urinary stones. The study was performed on 35 healthy male, Muslim volunteers, none of whom had a previous history of stone formation. Urines were collected on 5 days – a control day 10 days before the start of Ramadan, the beginning, middle and end of Ramadan and, finally, a second control day 10 days after the end of the month. Urines were collected over four periods on each day – 1830 to 0330, 0330 to 0700, 0700 to 1600 and 1600 to 1830 hours. These times were chosen to include the start (0330) and end (1830) of each day's fast. The results showed that for calcium oxalate (CaOx) stone formation the risk of this type of stone (which is high in Middle Easterners) was, in general, not increased during Ramadan, although the diurnal rhythm of CaOx supersaturation in Ramadan showed a steady increase from the commencement of each day's fast at 0330 until the end of the fast at 1830 because of increasing urinary concentration, presumably secondary to enhanced renal reabsorption of water. Uric acid (UA) supersaturation was, in general, lower during Ramadan than before and remained at a lower level after the end of Ramadan. This appeared to be mainly due to a reduction in the dietary intake of purines during the fasting period, a habit that persisted for

some time after the end of the month. Again, there was a steady increase in supersaturation between 0330 and 1830 during each study day in Ramadan as urine became more concentrated. It is concluded that the risk of urinary stone formation is not increased during the month of Ramadan in spite of the long fasting period each day and in spite of the hot, dry environment in this part of the world.

B36. Urinary Calcium After Oral Glucose Ingestion in Calcium Stone Formers – A Simple Test for Predicting Stone Recurrence?

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In an attempt to find a biological test of value for predicting stone recurrence, 7 control subjects (5 women and 2 men) and 37 calcium stone formers (13 women, 24 men) were submitted to an oral glucose load (100 g), followed by the infusion of 500 ml of normal saline within 90 min. Urine was sampled (30-min collection periods) immediately before and 30 and 60 min after glucose ingestion. In each sample the calcium-to-creatinine ratio (Ca/creat) was determined, and plasma glucose was measured before and 45 and 75 min after glucose intake. Among the calcium stone formers, 22 (6 women, 16 men) were classified as having an evolutive (E) lithiasis (3 or more new stones formed during the preceding 5 years) and 15 (7 women, 8 men) as having a non-evolutive (NE) lithiasis (less than 3 new stones in the preceding 5 years). Twenty-one stone formers had idiopathic hypercalciuria (urinary calcium output on their usual diet above 0.1 mmol/kg/day), 13 classified as having absorptive and 6 renal hypercalciuria. In controls as well as in stone formers a significant rise in Ca/creat was observed after glucose ingestion, but in the latter Ca/creat and the maximal rise in Ca/creat (ΔCa/creat) was significantly higher than in the former. No significant difference was noted between normocalciuric and hypercalciuric stone formers as regards Ca/creat and ΔCa/creat after glucose ingestion. In contrast, Ca/creat 60–90 min after glucose and ΔCa/creat were significantly higher in E than in NE lithiasis (Ca/creat: 0.339 ± 0.03 vs 0.235 ± 0.02, $P < 0.05$, ΔCa/creat: 0.209 ± 0.03 vs 0.127 ± 0.02, $P < 0.05$, in E and NE lithiasis, respectively). This significant difference persisted when matching the patients for sex (males: 0.179 ± 0.03 vs 0.009 ± 0.02, $P < 0.05$; females 0.290 ± 0.04 vs 0.160 ± 0.02, $P < 0.02$, in E and NE lithiasis, respectively). It is concluded that the oral glucose and sodium load described here could constitute a reliable index for predicting stone recurrence. However, additional work is necessary to prove the usefulness of the test on a prospective basis.

B37. Increased Interleukin-1 Activity and Decreased Bone Density in Patients with Fasting Hypercalciuria (FN)

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Since increased bone resorption and fasting hypercalciuria (FH) underlie some forms of idiopathic hypercalciuria (IH), we have measured interleukin-1 activity (IL-1) in the culture media of peripheral blood monocytes (PBM), blood BGP, urinary hydroxyproline (OHP) and vertebral bone density by QCT in 8 FH subjects, and in 13 control, non-hypercalciuric stone formers. IL-1 was measured with the D 10 G.4.1 T cell system and results expressed in units. QCT was expressed as z-score relative to the predicted value for age and sex. Blood PTH and $1,25(OH)_2D_3$ were also measured and found to be within normal limits in all subjects and similar in the two groups.

	IL-1 (units)	QCT (Z-score)	OHP (mg/24 h)	BGP (ng/ml)
FH patients	45.7 ± 15.9	-1.35 ± 0.15	65.1 ± 8.0	11.0 ± 3.7
Controls	6.0 ± 2.1	-0.37 ± 0.29	37.3 ± 6.1	6.1 ± 0.8
	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.07$

Conclusions: (1) these preliminary data demonstrate abnormal bone remodeling in FH; locally elaborated factors rather than a PTH or $1,25(OH)_2D_3$ dependent mechanism may account for the pathogenesis of "resorptive" IH and suggest that a clinically significant bone loss may complicate this condition; (2) although our data do not reveal the role of IL-1 in bone remodeling, they indicate that IL-1 is elevated not only in idiopathic osteoporosis, but also in other states of increased bone resorption.

C. Physical Chemistry – Inhibitors

C1. The Effect of Urinary Constituents of Low Molecular Weight on the Crystal Growth of Calcium Oxalate in Gel

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The automated gel crystallization method (GCM) was used to investigate the effects of major and minor small ionic constituents of human urine on the crystal growth rate (Vcr) of calcium oxalate (CaOx) in a gel matrix (0.5% agar-agar, 2 mM Na oxalate). The measuring device was a computer-controlled microphotometric system for transmitted light, equipped with rapid scanning stage for 96-well microplates (Zeiss, Oberkochen, FRG, Hewlett-Packard).
Results: From multiple variations of parameters in artificial urine, the relationship between Vcr and total concentrations of Ca, Mg, citrate, and pH could be quantified. For this purpose, a nonlinear regression model was used, starting from the point of "normal" urinary composition (Vcr = 1). The overlapping thermodynamic and kinetic effects of citrate on Vcr could be demonstrated. A series of minor urinary components were tested to evaluate their potential role in the inhibition of CaOx crystal growth. From these, pyrophosphate, isocitrate, glucuronic acid, and hippuric acid may each cause an inhibition of about 5% (or more) in artificial urine at pH 6.0 at its mean physiological concentration.
Conclusions: The effects and interactions of small ionic constituents of human urine, with respect to the crystal growth rate of CaOx, can be detected and quantified by the GCM. The effect of each single parameter depends on the constellation of all the others. Minor components might significantly contribute to the "overall inhibiting activity" of human urine. These results are of importance in evaluating the risk of stone formation or the efficacy of therapeutical measures in urolithiasis.

C2. The Effect of Urinary Macromolecules on the Crystal Growth of Calcium Oxalate in Gel

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Macromolecules of different types [chondroitin sulfates A, B, C, heparane sulfate (HS), hyaluronic acid, human serum albumin] and the macromolecular fraction of whole human urine with $M_r > 5{,}000$d

were tested with respect to their effects on the crystal growth rate (Vcr) of calcium oxalate (CaOx) in a gel phase (0.5% of agar-agar, 2 mM Na oxalate).

Method: Automated gel crystallization method (GCM) was used with computer-controlled multiple microphotometry in 96-well microtitre plates. The substances mentioned above were tested in 1.5 mM $CaCl_2$/50 mM MES buffer (test system I) and artificial urine (test sytem II) at pH 6.0.

Results: In the concentration range 1–1,000 mg/l, most substances showed small effect on Vcr (<5% inhibition), except for HS (about 70%/20% inhibition in I/II at 1 g/l). The inhibiting effects were much more pronounced in I than in II. Macromolecules from whole human urine were isolated by ultrafiltration and tested in II. Vcr was decreased by this fraction from 1.0 to 0.97 ∓ 0.03 (recurrent stone formers; $P < 0.05$) and to 0.95 ∓ 0.025 (normals; $P < 0.01$).

Conclusions: If stones grow in a gel-like medium, as demonstrated by others, *soluble* macromolecules should only play a subordinate inhibiting role in CaOx crystallization. However, as constituents of the medium (urothelium, stone matrix) itself, they might be of considerable importance in protecting the urinary tract from stone formation. This could be supported by additional experiments incorporating chondroitin sulfate into the gel phase.

C3. Kinetic Quantification of Crystal Growth in Gel Matrices: An Efficient Model of Urinary Stone Formation

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This paper reviews a new, highly efficient microtechnique (gel crystallization method: GCM) for the quantitative measurement of crystal growth kinetics in gel matrices and its recent applications in basic research, as well as in diagnosis and treatment of calcium stone formation. Relative crystal growth rates (Vcr) of calcium oxalate (CaOx) in gels are quantified by automated multiple measurements in 96-well microplates using a computer-controlled microphotometric system for transmitted light (Zeiss, Oberkochen). Efficiency: 120 kinetic measurements/h; nonprecision: <2% in standard solution; modes of measurement: dark and bright field, polarized light. The method was applied to the study of diverse crystal growth inhibitors (inorganic and organic phosphates, glycosaminoglycans, citrate, isocitrate, amino acids, etc.). New inhibitors (hippuric acid, glucuronic acid) were detected. Their contributions to the whole "inhibitory activity" of human urine were evaluated. From a series of experiments, the relationship between Vcr, pH, and total concentrations of calcium, magnesium, and citrate was mathematically quantified using a nonlinear regression model. The GCM has been employed to study the in vivo effects of different therapeutic measures on CaOx crystal growth. While the application of alkali citrates resulted in a >70% decrease of Vcr, no beneficial effects could be found for three different magnesium preparations. In conclusion, the method presented here has proven to be of high value with respect to a series of applications in the study of urolithiasis and may be applied to clinical routine measurements in undiluted urine. According to recent data in the literature, urinary stones grow in a gel-like state. Thus, the GCM also seems to be a useful model from a physiological point of view.

C4. Temperature Dependence of the Magnesium Oxalate Stability Constant

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Sodium oxalate (Na_2Ox) was titrated with acid in the presence of magnesium chloride ($MgCl_2$) at $T = 15°$, 25°, 38° and 45 °C. For analysis, the following model was assumed:

$$H^+ + Ox^{2-} \overset{K_1}{\Leftrightarrow} HOx^-$$

$$H^+ + HOx^- \overset{K_2}{\Leftrightarrow} H_2Ox$$

$$Na^+ + Ox^{2-} \overset{K_3}{\Leftrightarrow} NaOx^-$$

$$Mg^{2+} + Ox^{2-} \overset{K_4}{\Leftrightarrow} MgOx$$

$$MgOx + Ox^{2-} \overset{K_5}{\Leftrightarrow} MgOx_2^{2-}$$

$$Mg^{2+} + MgOx \overset{K_6}{\Leftrightarrow} Mg_2Ox^{2+}$$

$$Mg^{2+} + OH^- \overset{K_7}{\Leftrightarrow} MgOH^+$$

The univalent single ion activity coefficient was calculated with the Davies equation $ln\ f = -A[(\sqrt{I}/(1+\sqrt{I})) - BI]$. A is a temperature (T)-dependent parameter tabulated by Robinson and Stokes, I is ionic strength and $B = 0.286$ is a parameter with no temperature dependence in our range of interest. $K_1(T)$, $K_2(T)$, $K_3 = 13.4\ M^{-1}$, $K_5 = 4.75\ M^{-1}$, $K_6 = 5.90\ M^{-1}$ and $K_7 = 380.19\ M^{-1}$ were taken from the literature. K_4 was evaluated by nonlinear least squares fitting titration data to the assumed model. It was found from fitting the Van't Hoff isochore to estimates of K_4 that

$$K_4(38) = 4{,}088\ M^{-1}$$

$$\Delta H = (2.9 \pm 0.2)\ kcal/mol$$

The correlation coefficient for the fit is −0.993. This agrees well with a previous report of $K_4(38) = 4100\ M^{-1}$.

C5. Use of EQUIL to Estimate pH of Well-Defined Solutions

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EQUIL, the ion speciation program used in our laboratory, requires assignment of pH. An additional independent equation is required if pH is not stipulated and is calculated. Algebraic merging of the additional equation into EQUIL's large set of equations is currently not practical. Placing the additional equation into EQUIL's equation set without algebraic merging destroys the robustness of EQUIL's iterative convergence and in many cases works numerical harships not easily surmounted. However, the additional equation can easily be used interactively (which can be automated) by stipulating a pH, and invoking the water dissociation constant

$$K_W = (H^+)(OH^-) \tag{1}$$

and electrical neutrality

$$0 = \Sigma\ Z_i C_i \tag{2}$$

in which Z_i is the charge and C_i is the concentration of the $_i$th species. The amount by which calculated $\Sigma\ Z_i C_i$ fails to satisfy Eq. 2 indicates the amount of acid or base needed to obtain the stipulated pH. This is of great utility in planning the construction of experimental solutions. Conversely, by adjusting stipulated pH until Eq. 2 is satisfied permits pH estimation.

C6. COD Production

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Formal rules for weddelite (COD) production have not been enunciated. Systematic observation of the results provided by observing

well-defined initial solutions at 22° and 37 °C show that COD is favored by: (1) a high free calcium to free oxalate ratio; (2) low temperature; (3) low initial relative supersaturation. The reason for the first condition is not known. The reason for the second condition is that the apparent activation energy for COD nucleation is negative, ~−9.6 kcal/mol [apparent activation energy for whewellite (COM) is positive and very sensitive to initial concentration: 3.5 kcal/mol at relative supersaturation (RS) = 17.5 and 48.9 kcal/mol at RS = 12]. The third condition is probably explained by the high apparent activation energy at low RS for COM nucleation rate relative to COD. We were not able to produce "pure" COD without large amounts of citrate and magnesium. The resulting "pure" COD was unstable and underwent solution-mediated transformation to COM. Small concentrations of RNA delayed that transformation, most likely by poisoning COM nuclei.

C7. Surface Interaction Between Glycosaminoglycans and Calcium Oxalate

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Molecules and macromolecules are known to alter the process of crystallization, either through inhibition or promotion of nucleation, growth, and/or aggregation. One particular group of macromolecules, glycosaminoglycans (GAGs), has been of interest in our laboratory. The GAGs: chondroitin A, chondroitin C, heparan sulfate, dermatan sulfate, hyaluronic acid, and keratan sulfate have all been shown to be inhibitors of calcium oxalate crystallization. Heparin, the only GAG which is not naturally present in urine, is the most potent inhibitor of all GAGs. Using the method of Langmuir isotherm adsorption, we studied the adsorption of certain GAGs onto calcium oxalate crystals. Under standardized conditions, heparin, chondroitin C, hyaluronic acid and pentosan polysulfate (a synthetic polyanionic molecule similar to, but a weaker inhibitor than heparin) were adsorbed onto calcium oxalate. The total amount of GAG required to cover the crystal surface maximally was half-covered with GAG (inversely related to the desorption energy) were measured. Chondroitin C was adsorbed in the greatest amount, followed by heparin pentosan polysulfate, and finally hyaluronic acid. Using the method of fiducial limits, the only insignificant difference was between heparin and chondroitin C, and between hyalurolnic acid and pentosan polysulfate. Pentosan polysulfate required significantly higher equilibrium concentration than heparin and hyaluronic acid to cover half of the surface of the calcium oxalate crystals. The principle of Langmiur isotherm adsorption can be useful in predicting the effects of macromolecules on crystallization. Weaker inhibitors bind with less affinity than do stronger inhibitors. Further work is underway to characterize other inhibitors and promotors.

C8. Histochemistry of the Urinary Deposit

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Ordinary light microscopic (LM) study of urinary deposits only yields minimal information. Scanning electron microscopy and histochemistry identify structures not visualised by LM. This paper presents the findings of LM of 300 samples of urinary deposits of stone patients compared with the histochemical studies. Early morning urine (EMU) samples, random samples, and 24-h urine samples were collected from stone patients, centrifuged, and deposits taken on glass slides. One sample was studied as such and other samples stained with hematoxylin – Gommori's stain, eosin, methylene blue, methyl green, or crystal violet. Light microscopic studies showed 62% crystalluria among stone patients, whereas histochemistry identified crystalluria in 71%. Crystals not seen under LM were observed under histochemical staining. Such crystals included ammonium urates, brushite and apatite. Other than crystals, fibrous structures with branching and tendril-like entangling filaments were seen. These fibrous materials were significant in stone-forming patients but not in controls. The fibrous materials detailed above by histochemistry are probably the precursors of fibrous matrix identified in urinary stones. Different photographs of the deposits will be presented.

C9. Calcium Oxalate Precipitation in a Flow System

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The present study describes a new approach to simulate the generation of supersaturation and the early stages of CaOx precipitation in the distal convoluted tubule and collecting system of the kidney. A hollow-fiber reverse osmosis (RO) hyperfiltration model (operated at 30 °C) was employed to approximate a plug-flow device and to simulate the generation of high supersaturation levels as a consequence of the selective removal of water from metastable solutions [relative supersaturation (RS) = 0.67]. The results showed that the 3-min residence time (τ) spent by the concentrated solution in the crystallizer, which is approximately equal to the biological residence time of urine in the renal tubules, is sufficient to raise the CaOx formation product into the labile zone (log RS > 1), to reduce the induction time for spontaneous precipitation from about 100 min to 3 min, and to enhance the nucleation and growth of CaOx crystals. Crystal-size distribution measurements revealed that at these conditions about 1,500 particles/ml were formed with a mean size of 5.1 μm. SEM micrographs showed that the crystals had the "envelop" morphology, tended to aggrolomerate and identified as CaOx dihydrate by their X-ray diffraction patterns. When τ was shorten, supersaturation was generated but the respective yield of crystals was very small. The individual addition of heparin, glutamic acid, citric acid, and pyrophosphate to the feed stream significantly altered the crystal habit and reduced their agglomeration. $MgCl_2$ significantly reduced CaOx supersaturation.

Conclusions: CaOx crystals might be formed within the renal tubules, grow to a critical size, and then agglomerate to larger particles. Inhibitors reduce the yield of crystals in urine and, above all, reduce their tendency to agglomerate. In this context, the effect of duresis is very important in the preventive treatment.

C10. ^1H and ^2H Nuclear Relaxation Studies of Lyophilized Urines

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In order to gain a better understanding of the biological roles of water and macromolecules in the prevention/formation of kidney stones, ^1H and ^2H NMR studies were conducted on lyophilized urine samples selected from recurrent CaOx stone former (SF) patients and normal (N) individuals. Understanding the hydration characteristics is essential for defining the role of water at the molecular level in the activation of macromolecules for inhibiting stone formation. T_1 and T_2 relaxation times were measured on lyophilized urine samples and thereafter during gradual controlled rehydration. The composite proton signal was observed at 20 MHz at 37 °C. The results of ^1H NMR clearly showed a significantly ($P < 0.001$) enhanced prolongation of T_1 and T_2 of the rehydrated urines of SF than that of N groups. The calculated water com-

partmentalization showed that the hydration fraction and the fraction of bound water around compounds in the urine of SF are significantly smaller ($P < 0.01$) than those of Ns. When ^2H_2O was used, the water-suppressed proton relaxation signal of the rehydrated lyophilized urine is dominated by the ^1H of the urinary compounds. The results of ^2H NMR revealed that T_1 and T_2 of rehydrated urines of SF are significantly ($P < 0.01$ shorter than those of N. The results suggested that the compounds in the urines of SF and N differ in their conformation and proton content. It seems that hydrophilic compounds with a relatively larger active surface area characterize the urine of N individuals. It can be speculated that urinary macromolecules of SFs differ from those of Ns in their water-binding properties as a consequence of differences in the amount of hydrophilic polar groups and in their conformation. These differences could be reflected in vivo in the relatively reduced inhibitory activity in the urine of SF.

C11. Investigations of Formation and Dissolution of the Stone Minerals, Oxalate and Apatite

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Crystal growth inhibitors may play an important role in the regulation of urinary stone formation, depending on the supersaturation of the urine. From the results of these investigations, conclusions may be drawn regarding the treatment of patients with urolithiasis. The effects of an increase in the concentration of calcium, uric acid, pyrophosphate, oxalate, and citrate in the urine were investigated under standardized condition in relation to the formation of oxalate and phosphate stones. Precipitates were characterized by X-ray and scanning electron microscopy. The results revealed that there was an increase in the weight of oxalate stones under the influence of calcium depending on the formation of uric acid dihydrate, whereas under the influence of calcium there was a decrease in the weight of apatite stones depending on the pH value. The addition of uric acid also caused an increase in the weight of whewellite stones and crystal growth did not appear at all. dihydrate. Apatite maintained a proper balance between the process of growth and dissolution. There was no improvement in the dissolution of whewellite when pyrophosphate was added. The results when citrate was added are interesting. There was a decrease in the weight of whewellite stone and crystal growth did not appear at all. When twice as much citrate was added to the apatite, the weight decrease increased; here also no new formation of crystals did appear at all. The results show that one of the best kinds of medical prophylaxis is to increase the citrate concentration in the urine of oxalate and phosphate stone formers.

C12. Calcium Oxalate and Brushite Urinary Saturation in Calcium Stone-Formers

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Since urine from both stone-formers and non-stone-formers is supersaturated with respect to calcium oxalate (CaOx), the role of CaOx for originating the formation of calcium stones has been questioned and heterogeneous nucleation of CaOx crystals onto calcium phosphate seeds has been suggested. In addition, since the supersaturation with respect to CaOx is generally determined from concentration measurements made on 24-h urine collections, it is difficult to appreciate what happens throughout the whole day. To clarify the former point and avoid the problems resulting from the second one, we determined the supersaturations with respect to brushite (B) and calcium oxalate monohydrate (COM) in 6 urine samples collected at different moments of the day. This was made for 23 calcium stone-formers (SF) and 12 non-stone-formers (NSF). Both groups were exposed to a diet with 1 g calcium and 6 g sodium chloride. The urine volumes were equivalent for SF and NSF. Among the SF, 16 were hypercalciuric. The 24-h calciuria and phosphaturia were significantly greater in SF than in NSF whereas oxaluria, uricosuria, magnesuria, citraturia were equivalent in both groups. The calcium concentration in all the urine samples was higher for the SF. Urine from the SF was always supersaturated with respect to B, whereas that from NSF was often undersaturated with respect to this phase. Urine from both groups were supersaturated in COM in all samples. However, supersaturations of the only SF were high enough to be in the domain of spontaneous homogeneous nucleation of CaOx crystals we determined in a previous study. By contrast, supersaturations of the SF were always out of this domain. We conclude that calcium oxalate may form either by heterogeneous nucleation on brushite crystallites or directly by homogeneous nucleation since the urine of the SF are simultaneously supersaturated with respect to B and COM, which is not the case for the NSF.

C13. The Oxalate-Tolerance Method and its Application to Investigations on the Influence of Organic Macromolecules on the Growth of Calcium-Oxalate

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The aim of this study was to investigate the influence of organic macromolecules on the growth of calcium oxalate measured by turbidometry.
Material and methods: To a defined volume of undiluted urine, sodium oxalate is added under vigorous stirring. Precipitation of calcium oxalate is detected by turbidometry (700 nm, 37 °C). The concentration of sodium oxalate at which precipitation starts is called oxalate tolerance. Calcium concentration of natural urine determined by AAS is plotted against the oxalate tolerance. The value obtained is compared to a standard curve made from synthetic urine containing the main constituents of natural urine with different calcium concentrations. Organic macromolecules isolated from natural urine by gel filtration were added to synthetic urine. The presence and quantity of such substances in natural urine were tested by their surface active behavior in voltametric measurements.
Results: Concerning the oxalate tolerance no difference could be found between the 25 non-stone-formers and the 30 stone-formers. Investigations with urine equalized in conductivity before titration do not show better significance, but the urine of non-stone-formers. Investigations with urine equalized in conductivity before titration do not show better significance, but the urine of non-stone-formers showed a different pattern of organic macromolecules and, even at low concentrations, a significant enhancement of the oxalate tolerance of synthetic urine.

C14. Adsorbed Macromolecules as Promoters of Calcium Oxalate Monohydrate Growth

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Constant composition mineralization experiments have demonstrated the ability of immobilized films of human serum albumin (HSA) to nucleate calcium oxalate monohydrate (COM) when introduced into a supersaturated solution of this salt. HSA, when

absorbed on surfaces of hydroxyapatite (HAP), accelerates the nucleation of COM at apatite surfaces in calcium oxalate solutions of the same supersaturation. In contrast, when present in solution, HSA is capable of reducing the rate of COM crystal growth. Separated urinary macromolecules are also effective promoters of COM crystal growth, as monitored by the constant composition method. Clearly functional groups that are not involved in the surface binding are available to chelate calcium ions and induce formation of calcium oxalate nuclei.

C15. Magnesium Inhibits Octacalcium Phosphate and Apatite but Promotes Whitlockite and Brushite Formation

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Magnesium has been recommended for prophylactic use in urolithiasis as Mg inhibits in vitro apatite formation and stabilizes amorphous calcium magnesium phosphate (ACMP). However, Cheng et al. have shown that ACMP can transform to hydroxyapatite (HAP) or whitlockite (W; TCP) or brushite (B; DCPD), depending on the solution Mg/Ca ratio. It has been suggested that thermodynamic calculations would help us better understand the system and the various effects of Mg in urolithiasis. Initial Gibb's free energy (ΔG) was computed for each of various neutral solutions containing $[CaCl_2] = 1$ or 3 mM, $[MgCl_2] = 0$–9 mM, $[Na_2HPO_4 = 0.1$–90 mM and NaCl (total 300 mOsM), with a reiterative algorithm (local), using published stability constants for various calcium, magnesium, and sodium phosphates at 37 °C. Computed minimum ΔG values were different for different compounds, as summarized below (in kJ/mol):

	Heterogeneous nucleation	Homogeneous nucleation (via ACMP)
HAP	−7.00 ± 0.05 (via OCP)	−7.70 ± 0.05
W (TCP)	Not observed	−4.40 ± 0.05
OCP	−2.0 to −3.0	Not observed
B (DCPD)	−0.90 ± 0.05	−2.20 ± 0.02

In solutions supporting homogeneous nucleation, transformation of the initially formed ACMP was not governed as much by ΔG as by solution Mg/Ca. The results also showed that, at 37 °C and pH 7.0, while all four biological calcium phosphates would form, depending on ΔG values, when $Mg/Ca \leq 1$, B was the dominant phase regardless of ΔG when $Mg/Ca \geq 2$. W formation was favored in solutions supporting homogeneous nucleation and with $0 < Mg/Ca \leq 2$. It can be concluded from this and previous findings that Mg inhibits HAP and OCP but promotes W and B formation and that for studying calcium phosphates in urolithiasis, kinetic considerations are as important as, if not more important than, thermodynamic ones.

C16. Dietary Fiber and Urolithiasis – Part I. Physical, Chemical, and Hygienic Properties of Various Brans

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Before we started a test series about the treatment of idiopathic hypercalciuria with bran, some different sorts were analyzed for their physical, chemical, and hygienic properties. The following parameters were analyzed for rice, rye, soy, and two sorts of wheat bran (t1, t2): calcium-binding-capacity, distribution of the particle size, water-holding capacity, content of dietary fiber and phytic acid, contents of heavy metals, contamination with aflatoxins (B1), and chlorinated hydrocarbons. Big differences were found between the various brans in the calcium-binding capacity, which in wheat bran (t1) – dependent on the pH value of the solution – was up to three times higher than in rice bran. Similar differences could be seen in the distribution of the particle size which, moreover, showed an influence on calcium-binding capacity. The highest water-holding capacity was found in rice bran. It was twice that in wheat bran, which showed the lowest. The highest content of dietary fiber was found in soy bran, the lowest in rice bran. In the latter, the highest concentration of phytic acid was measured. A great variance was found for the concentrations of calcium and magnesium. There was no contamination with aflatoxin (B1) in any bran, whereas in all brans – except for soy bran – chlorinated hydrocarbons, especially lindan, could be found. Heavy metal concentrations were different in all brans, particularly cadmium and lead. From our results we draw the conclusion that the highly divergent statements concerning the effect of a high intake of bran on the mineral metabolism might be considered with regard to the above listed differences between brans and their physical and chemical properties.

C17. Dietary Fiber and Urolithiasis – Part II. Effects of a High Dietary Fiber Intake on the Urine Composition in Humans

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After the investigation on various brans with regard to their physical, and hygienic properties (part 1), we wanted to find out how much a high intake of bran might influence the urine composition, since other research groups have found controversial results. Our 15 test subjects twice received a standardized diet with a high Ca-intake (1,800 mg/day) for 5 days each, first without and then with a bran intake of 36 g/day. Between these two periods a 12-day period with individual diets but bran intake was inserted to adapt the test subjects to the bran. During the test period 24-h urines were collected daily and analyzed for lithogenic and inhibitory substances. So far, rice, soy and wheat bran have been tested. No significant influence of bran on the urine volume could be found, although it was slightly lowered. Calcium excretion was decreased by all brans, significantly by rice and soy bran. Oxalate excretion, however, we found to be increased by all brans and significantly in rice and wheat bran. As known, oxalate is the most powerful predictor of the supersaturation of the urine with CaOx. Thus, it is not astonishing that decreased calcium excretion and simultaneously increased oxalate excretion result in increased supersaturation for CaOx in all brans tested. In our opinion, one reason for the increased renal oxalate excretion must be the oxalate content of the brans and possibly higher intestinal absorption resulting from a decrease in the intestinal concentration of free calcium.

C18. Glycosaminoglycans and Urolithiasis

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Some discrepancies can be found in the literature about whether certain macromolecules act as promoters or inhibitors of calcium oxalate crystallization. The purpose of this report was to study and compare the inhibitory effects of several molecules containing sulphate groups, mainly glycosaminoglycans, under different con-

ditions. Studies of crystal growth were performed by potentiometric measurements, using a selective calcium electrode. Aggregation studies were carried out by measuring the number of each type of crystals obtained, using optical microscopy (400x). The kinetics of crystal growth of calcium oxalate monohydrate seed crystals were investigated in the presence of pentosan polysulphate, chondroitin sulphate, tartrazin, and chromotropic acid. An apparent rate order of 2 in all cases suggested a spiral growth mechanism. Pentosan polysulphate exhibited a higher inhibitory effect on crystal growth than chondroitin sulphate, whereas tartrazin and chromotropic acid manifested no effects. These facts suggest that adsorption of molecules containing sulphate groups is strongly dependent on the number of such groups that contain each particular molecule (the presence of only two or three groups in a discrete molecule is not sufficient to manifest important inhibitory effects). Application to a kinetic Langmuir-type model suggested that adsorption of the sulphate-containing macromolecules on the active growth sites is the cause of reduction in the crystal growth rates. Adsorption studies revealed that inhibition of crystal growth was enhanced at lower ionic solution strengths. This dependence on ionic strength indicates that the calcium oxalate-macromolecule interaction is mainly electrostatic in nature. Study of the influence of uric acid on the inhibitory capcity of pentosan polysulphate demonstrated that only when the uric acid was in a colloidal form did it diminish the inhibitory capacity of the glycosaminoglycans. Finally, it was found that in the presence of large amounts of pentosan polysulphate, agglomeration of calcium oxalate crystals was clearly favored. Consequently, it can be stated that the two processes of growth and aggregation, both being crystal-surface-related processes, may react in contrary directions upon the surface adhesion of the macromolecular substance.

C19. Combined Influence of Urinary Calcium and Oxalate Concentrations on Crystal Formation in Stone Formers

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As previous studies have shown that stones made of calcium-oxalate (CaOx) monohydrate (CO1) are mainly associated with hyperoxaluria, and those made of CaOx dihydrate (CO2) with hypercalciuria, we prospectively assessed the incidence of crystalluria and type of crystals in calcium stone formers (SF) with respect to Ca and Ox concentrations. We examined 1,200 first-morning urine specimens from 85 healthy controls and 250 SF by means of polarized microscopy and infrared spectroscopy on fresh samples and after 72-h storage at 4 °C. Overall, crystals were present in 13% of controls (mean diuresis 1,123 ml/day) and in 45.5% of SF (mean diuresis 2,089 ml/day) ($P < 0.001$). Incidence of crystalluria increased in SF in proportion to the molar CaOx product (pCaOx), as the percentage of urine specimens with crystalluria rose from 5% for pCaOx values under 0.5 $mmol^2/l^2$, up to 100% for pCaOx values over 3, with gradually increasing values between 0.5 and 3. However, for any given value of pCaOx between 0.5 and 3, the structural type of CaOx crystals was related to the molar Ca/Ox ratio (rCa/Ox). Only CO1 crystals were found in fresh urine specimens whose rCaOx was $<6 \pm 0.5$ (corresponding to high Ox concentration) and CO2 crystals in specimens with rCa/Ox >14 (corresponding to high Ca concentration), whereas CO2 alone or both CO1 and CO2 crystals were found in intermediate rCa/Ox range. As in most samples initial Ca/Ox ratio was >1, the proportion of CO2 crystals increased with time due to a spontaneous rise in rCa/Ox during crystallization. The combined influence of pCaOx and rCa/Ox on the frequency and structural type of crystalluria may be graphically depicted on an abacus, using the Ca and Ox concentrations determined in urine from individual patients. In conclusion, the risk of forming CaOx crystals mainly appears to depend on the molar product, whereas the type of CaOx crystals (either mono or dihydrate) depends on the ratio of Ca to Ox. The probability of forming CaOx crystals and the type of crystals may be better predicted from the combined analysis of Ca and Ox molar ratio and product than from separate consideration of their urinary concentration.

C20. The Effect of Low-Speed Centrifugation and Millipore Filtration on the Urinary Protein Content

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The preparation of urine used for the study of renal disease states frequently incorporates centrifugation and/or filtration steps. The aim of this study was to determine the effects of these procedures on urinary concentrations of total protein, Tamm-Horsfall mucoprotein (THM), albumin and on the urinary particle content. Urine specimens were collected from normal males and subjected to centrifugation at 1,000 g and filtration through 0.22 μm millipore filters. Total protein, THM and albumin were measured by a modified Lowry, electroimmuno- and immunoturbidimetric assays, respectively. A Coulter counter was used to characterize the particle content of the urine specimens, and associations between covariables were tested for using the Spearman rank order correlation. Both centrifugation and filtration of whole urine caused significant reductions (Wilcoxon matched pairs signed-rank test) in the total protein ($P < 0.01$) and THM ($P < 0.01$) concentrations. The concentration of albumin was reduced by filtration ($P < 0.02$) but not by centrifugation. THM and albumin did not account for the total protein losses caused by these procedures, suggesting that other proteins were also being removed. The particle content of the whole urine specimens correlated positively with the total protein ($P < 0.01$) and the THM ($P < 0.05$) concentrations. The loss of THM ($P < 0.01$) and total protein ($P < 0.05$) by filtration correlated positively with the reduction in particle content, but similar correlations were not found with centrifugation of the urines. SDS-PAGE gel electrophoresis of urines confirmed the reduction in THM, albumin, and other additional urinary proteins, which were identified and quantified by this technique. It was concluded that both centrifugation and filtration of urine should be avoided in the preparation of specimens where the use of whole urine is required for diagnostic or research purposes.

C21. Microlith Formation in Urine – New Evidence

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Various theories have been postulated about the genesis of stones in the human urinary system. Carr's postulates and Randall's plaques have proved to be convincing. However, recent investigations using scanning electron microscopy (SEM) have revolutionized the study of urinary calculus formation in modern times. This paper is based on SEM studies of urinary deposits of 100 stone patients. Proved urinary stone patients with crystalluria were selected for the study. Early morning urine (EMU) samples were collected and centrifuged deposits taken up for SEM examination under JEOL JSM 35c microscope. All crystals seen under light microscopy (LM) were seen in greater detail under SEM. Details of crystal aggregation and clumping were more pronounced. Crystals like apatite, brushite, and octo-calcium-phosphate, not usually noticed under LM, were observed under SEM. Over and above these uncommon crystals, fibrillar materials not seen under LM were also in evidence. Some of these fibrils had crystals entangled within these strands. Such

fibrillar materials were seen in 12% of the urine samples studied. They were not related to urinary infection. It is proposed that these fibrillar materials are precursors of the fibrillar organic matrix of the mature urinary stones. Such organic matrix thus initiates microlith formation. Histochemical studies have confirmed the presence of fibrous materials, which take up different strains. The various photographs that form the evidence to prove the new theory of microlith formation in the urine of the stone patient will be presented. The relationship between the matrix material described and crystals seen in urine and the stones formed by patients will be presented in detail.

C22. Early Morning Urine Versus Random Samples for Studying Crystalluria

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In studying crystalluria, some clinicians consider fresh random samples to be superior to early morning urine (EMU) samples. This study was undertaken to compare the differences between EMU and random samples in 200 patients with crystalluria. EMU samples were retained at room temperature (25–30 °C) for 3–4 h. Random samples were collected and examined immediately and after 3–4 h. Of the patients who showed crystalluria in EMU samples, 96% showed similar finding in random samples examined immediately and after an interval. Red blood cells (RBC), were seen in 96% of the EMU samples and random samples with crystalluria. Pus cells were present in 96% of the crystalluric EMU samples and 88% of the random samples. The size of the calcium oxalate dihydrate (COD) crystals was smaller in the random samples. There was no difference in the pH of the samples. Aggregation and clumping were equal in both groups. It is concluded from the study that the EMU sample is minimally better in determining crystalluria. Preservation at room temperature for a few hours did not alter the findings. EMU sampling is a probably better indicator of the calculogenic capacity of individual stone patients.

C23. Gentamycin Accelerates COM Nucleation

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Gentamycin nephrotoxicity triggers calcium oxalate nephrocalcinosis in rats receiving subtoxic doses of ethylene glycol. We examined urine from rats to see if some effect of gentamycin on calcium oxalate nucleation could be detected. Conventional nucleation theory gives

$$J = A \exp\left(-B/(\ln(RS))^2\right)$$

in which J is nucleation rate, A is a parameter, B is a function $B(v, T, \sigma)$ with v being molecular volume, T being temperature, and σ interfacial energy of the nucleus, and RS is relative supersaturation, $RS = 1 \rightarrow$ saturation. It is conventional to let $J \alpha t^{-1}$ where t is the time required for precipitation to be detected. A and B are evaluated by measuring $\ln(t^{-1})$ as a function of $(\ln(RS))^{-2}$. We have observed that making our calcium oxalate nucleation system 5% with respect to normal rat urine increases B and decreases the nucleation rate, whereas 5% urine from rats receiving nephrotoxic doses of gentamycin causes B to decrease and increases the nucleation rate. The cause of this qualitative difference is not known. Two possible explanations are: (1) submicron membrane fragments are catalyzing nucleation and (2) submicron calcium oxalate nuclei are present in the "gentamycin" urine.

C24. Changes in the Activity of Urinary Inhibitors in Calcium Oxalate Urolithiasis

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There are conflicting opinions in the literature about the effects of the urinary inhibitors of crystallization. We have developed a simple method of studying the inhibitory effects in real urine. The inhibitory capacity of pyrophosphate, citrate, magnesium, and chondroitinsulphate was investigated, using the urine of 20 stone formers. The main urinary lithogenic biochemical parameters of these individuals were also investigated. The inhibitory activity of the different substances studied was evaluated by a combination of nephelometric (light scattering) and optic microscopic measurements. The urine used corresponded in all cases to urine accumulated during a period of 2 h, following an overnight fast. The results obtained showed that citrate and magnesium manifested inhibitory effects in an important number of cases. Significantly less often did pyrophosphate and chondroitin sulphate exhibit notable inhibitory action. It was found that the group of individuals in whom citrate had important inhibitory effects had less calciuria than the other subjects. In the group of stone formers in whom magnesium manifested considerable inhibitory effects, there was more calciuria and less citraturia than in the others. Thus, the inhibitory effects on calcium oxalate crystallization of a given substance markedly depends on the type of urine used in the corresponding study. These facts can be explained: the inhibitory effects must be related to adsorption processes and a great number of circumstances can affect them (pH, ionic strength, presence of other active substances, etc.). Therefore, as the mean composition of urine varies from one person to another, the factors that affect the adsorption processes of a substance also vary and cause variation in the inhibitory effects. Consequently, the best inhibitor for a given person must be determined by a study using that person's own urine.

C25. Urolithiasis Inhibitors and Calculus Nucleation

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Some controversy exists about the so-called inhibitors of nucleation. In this regard two types of nucleation inhibitors should be well distinguished: inhibitors of homogeneous nucleation and inhibitors of initial heterogeneous nucleus growth. In this comunication, we study the effect of several products on the calcium phosphate or uric acid heterogeneous nucleation of calcium oxalate. The inhibitory capacity of each substance was evaluated in urine by addition of calcium and oxalate, measuring the type and number of crystals obtained (using optical microscopy: 400x) in the presence and absence of heterogeneous nucleation and using different quantities of the inhibitor assayed. The results obtained when uric acid acted as the heterogeneous nucleation agent showed that in this circumstance, magnesium, diphosphate and citrate exhibited very weak effects, while chondroitin sulphate manifested important inhibitory effects. However, in the absence of this agent, all four substances had important inhibitory effects. In the presence of calcium phosphate as a heterogeneous agent, magnesium, diphosphate and citrate manifested important inhibitory effects, whereas chondroitin sulphate had practically no effect. These facts can be explained: magnesium, diphosphate, citrate, and chondroitin sulphate show important inhibitory effects on calcium oxalate crystallization because of their adsorption to the calcium oxalate crystalline surface. However, in the presence of calcium

phosphate heterogeneous nuclei, magnesium, diphosphate and citrate have good adsorption to the calcium phosphate crystalline surface, causing important inhibitory effects. In the presence of uric acid as heterogeneous nuclear agent, only the chondroitin sulphate experiments showed good adsorption to the uric acid crystalline surface, manifesting great inhibitory effects. Consequently, it is clear that we must distinguish inhibitors of calcium oxalate growth from inhibitors of initial heterogeneous nucleus growth (inhibitors of heterogeneous nucleation).

C26. The Effect of Tamm-Horsfall Mucoprotein on Calcium Oxalate Crystallization in Urine – Two Methods Compared

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The role of urinary macromolecules in calcium oxalate (CaOx) stone disease is poorly defined. Tamm-Horsfall mucoprotein (THM) is the major macromolecule in human urine. Using two different methods, it has been reported both to inhibit and to promote CaOx crystallization in ultrafiltered (UF) urine. The aim of this study was to compare the effect of THM on CaOx crystallization in the same UF urines, using these two different methods. Urine was collected from ten healthy men and ultrafiltered (10,000 Da). Each sample was divided and human THM was added to one-half sufficiently to give a final concentration of 35 μg/ml. Crystallization was induced in the samples by addition of an oxalate load [1] and by evaporation [2]. Using the evaporation technique, THM significantly increased the deposition of CaOx as determined using ^{14}C-oxalate, from 9,772 cpm to 43,652 cpm (Wilcoxon rank sum test: $P < 0.05$). Using the oxalate load technique, THM significantly increased the amount of crystalline material deposited from 30 to 38 $\mu m^3/\mu l \times 10^{-3}$. This was attributed to an increase in crystal surface area caused by inhibition of crystal aggregation. In keeping with this, the crystals were significantly smaller ($P < 0.05$) in the presence of THM (9.2 μm) than in its absence (12.6 μm). These findings were confirmed by scanning electron microscopy. It was concluded that in UF urine, exogenous THM inhibits CaOx aggregation. Concentration of the same UF urine by evaporation causes polymerization of exogenous THM and consequent promotion of crystallization.

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C27. Quantitative Studies of Calcium Crystalluria

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The study examined calcium crystal concentrations, crystal incidence and chemical composition in 1,100 urine samples from calcium stone formers due to various causes and 79 samples from normals. Light microscopy was used to examine centrifuged deposits from fresh urine before and after evaporation at 37 °C to constant osmolality. A new chemical assay allowed direct determination of calcium oxalate crystal concentrations as μmol/l and calcium phosphate crystalluria was also measured as mmol/l. Calcium oxalate crystal concentrations increased with urinary oxalate levels, a sharp rise appearing at levels over 2 SD from the mean normal concentration. The frequency with which envelope-type calcium oxalate crystals were seen followed a similar pattern, and the incidence of aggregated and dumbbell crystals of this material was also proportional to urinary oxalate. Calcium oxalate precipitation also correlated with urine dilution (measured by osmolality) and with urinary calcium. In the latter, however, no further increase in crystalluria occurred when urine calcium levels exceeded 2 SD from the mean normal level. No correlation was present between urinary urate and calcium oxalate crystal concentration. The study also revealed coprecipitation of significant amounts of calcium oxalate with calcium phosphate in alkaline urines. Hence with rising urinary pH, calcium oxalate crystalluria increases rather than decreases as previously thought. Idiopathic hypercalciuric patients treated with bendrofluazide showed calcium oxalate crystal concentrations significantly lower than those treated by diet alone. The hazard of high urinary oxalate levels is demonstrated by assays of calcium oxalate crystals. Calcium is a lesser but not negligible factor. The significance of calcium oxalate coprecipitated with calcium phosphate requires further inquiry. Bendrofluazide is effective in reducing calcium oxalate crystal concentrations in hypercalciuric patients to near normal levels.

C28. Functional Heterogeneity of Tamm-Horsfall Glycoprotein Isolated from Urines of Normals and Idiopathic Calcium Stone Formers and from Human Calcium Kidney Stones

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Tamm-Horsfall glycoprotein (THP) has been described either as a promoter on an inhibitor of crystal aggregation. We have asked whether urinary THP from normals (nTHP) and idiopathic calcium stone formers (sfTHP) and THP isolated from human kidney stones (stTHP) differ in their ability to inhibit calcium oxalate monohydrate crystal (COM) aggregation in vitro. We isolated THP from urines of six healthy men and six men with severe idiopathic calcium stone disease (>20 stones) and from the supernatant of calcium kidney stones desintegrated by EDTA. COM aggregation, induced by slow stirring of a COM slurry with 200 mM NaCl, was measured spectrophotometrically by the rate of decrease of turbidity (T_S) during particle sedimentation that reflects the radius-dependent terminal velocity. Surface zeta potential (ZP) of crystals incubated with THP at pH 5.7 was measured by a Zeta-meter. Viscosity of THP was measured at pH 5.7 (10 mM Na-acetate) and 200 mM NaCl by an Ubbelohde-type viscometer. Without THP, T_S was 31.9 ± 1.4 at pH 7.2 and 29.6 ± 0.9 at pH 5.7. 5×10^{-7} M nTHP (3.2 ± 0.4) and sfTHP (4 ± 1) inhibited COM aggregation at pH 7.2; pH 5.7 reduced inhibition of nTHP (8.5 ± 2.1), but more of sfTHP (15.4 ± 2.7), $P < 0.05$ vs nTHP. 5×10^{-7} M stTHP (40.1 ± 1.7) appeared to promote COM aggregation at pH 5.7. ZP (in mV) of COM with 1×10^{-7} M nTHP was −18.5 ± 1.1, not different from sfTHP (−17.2 ± 0.8); ZP values were not correlated to COM aggregation inhibition by individual THPs. Introducing additional carboxyl groups to the THP molecules increased the negative ZP of all THPs ($P < 0.005$), but decreased aggregation inhibition ($P < 0.005$). Viscosity increased disproportionally with decreasing (THP) between 80 and 35 mg/l in 4/6 nTHP, 5/6 sfTHP and stTHP. S-200 chromatography, performed on four individual THPs, indicates conformational changes at lower (THP) in THP molecules with an abnormal viscosity behavior. The (THP), at which viscosity increased sharply, was strongly correlated to the COM aggregation inhibition by 5×10^{-7} M THP ($P < 0.001$). Thus, at pH 5.7 and 200 mM NaCl, COM aggregation inhibition by THP is reduced, more markedly among sfTHP; stTHP even promotes aggregation. The loss of inhibition is not related to a reduced ZP, but to an increase of viscosity, which is most probably due to conformational changes of the THP molecules.

C29. Nephrocalcin Isolated from Human Kidney Stones is a Defective Calcium Oxalate Monohydrate Crystal Aggregation Inhibitor

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Although it lacks γ-carboxyglutamic acid and forms unstable air-water interfacial films compared to normal urinary nephrocalcin (nNC), nephrocalcin isolated from human calcium kidney stones (sNC) inhibits calcium oxalate monohydrate (COM) crystal growth in vitro as well as nNC. But since kidney stones can form by aggregation of small crystals whose growth has been retarded, sNC could predispose to stones by failing to inhibit crystal aggregation normally. Aggregation of COM crystals was induced by slow stirring (500 rpm) of an equilibrated crystal slurry (0.8 mg/ml) for 180 s and measured spectrophotometrically during particle sedimentation by the rate of decrease of turbidity at 620 nm (turbidity slope, T_S) that reflects the average terminal particle velocity (v_t). Since v_t (for spheres) is proportional to the square of particle radius, higher T_S values reflect larger particle sizes. Surface zeta potential (ZP), a basic force of particle repulsion, was measured at pH 5.7 by a Zeta meter after incubation of COM crystal suspensions with nNC or sNC for 4 h at 25 °C. Mean T_S (in s^{-1}) for COM control crystals was 30 ± 1 (± SEM, $n = 55$). Both monomeric NC (14 kD) inhibited crystal aggregation in a concentration dependent manner; nNC was inhibitory between 1×10^{-8} M ($T_S = 25 \pm 1$, $n = 5$) and 1×10^{-6} M ($T_S = 1.2 \pm 0.3$, $n = 3$), sNC only above 1×10^{-7} M. At 2×10^{-7} M, a physiologic NC concentration, T_S was 3.1 ± 0.3 ($n = 5$) for nNC and 21 ± 2 ($n = 3$) for sNC, $P < 0.001$. In the absence of NC, COM crystals had a ZP of +13.8 ± 0.3 ($n = 7$); 5×10^{-8} M nNC changed ZP to −16.5 ± 0.6 ($n = 9$) vs −7.8 ± 0.5 ($n = 11$) for sNC, $P < 0.001$. Corresponding values for nNC vs sNC each at 1×10^{-7} M were −18.2 ± 0.6 ($n = 6$) vs −12.8 ± 0.8 ($n = 6$), $P < 0.001$, and at 5×10^{-7} M were −18.2 ± 0.6 ($n = 9$) vs −13.3 ± 0.6 ($n = 5$), $P < 0.001$. Measured in the absence of supersaturation, sNC is a defective inhibitor of COM crystal aggregation in vitro, allowing formation of larger aggregates during slow stirring of a COM crystal slurry. The defect may arise at least in part from a reduced effect upon ZP when sNC is bound to the crystal surface.

C30. A New Spectrophotometric Method for Measuring Calcium Oxalate Monohydrate Crystal Aggregation in the Absence of Supersaturation

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Based on the fact that aggregation in stirred suspensions of calcium oxalate monohydrate crystals (COM) has been found to be more prominent at lower stirring rates, a method to measure COM aggregation in the absence of supersaturation has been developed. COM slurries (0.8 mg/ml) were equilibrated overnight under constant fast stirring (1,100 rpm). Particle concentration was determined by measuring OD at 620 nm; between 0.4 and 1.0 mg/ml, OD_{620} was linearly correlated to the amount of COM/volume ($P < 0.001$). Aggregation was induced by slow stirring (500 rpm) for 180 s; thereafter, particle sedimentation was monitored for 300 s. Rate of decrease of turbidity (turbidity slope, T_S) was taken as a measure of aggregation, as it reflects average terminal velocity (v_t), which is (for ideal spheres) proportional to the square of particle radius (r^2). Knowing the sedimentation distance L, v_t of particles settling out within time t can be calculated as L/t. From long-term experiments (135 min), the percentage of settled particles at various times, that have a $v_t > L/t$, $P>$, was derived using the equation $P> = P - [dP/d(\ln t)]$ [J Phys Chem (1942) 46:903–910]. From there, cumulative frequencies of various particle diameters could be obtained, as v_t is proportional to r^2. Scanning electron microscopy (EM) was performed on COM slurries before and after slow stirring, and aggregates ⩾15 μm and ⩾20 μm were counted on photographic enlargements of entire EM fields. At pH 7.2 (10 mM Tris-HCl)/90 mM CaCl, T_S after slow stirring was 30.1 ± 0.5 ($n = 55$), compared to 7.1 ± 0.3 without slow stirring ($n = 11$), $P < 0.001$. T_S was neither different at pH 7.2/200 mM NaCl (31.9 ± 1.4, $n = 11$) nor at pH 5.7 (10 mM Na-acetate)/200 mM NaCl (29.6 ± 0.9, $n = 9$). Using 3 mM Na-citrate at pH 6.5/100 mM NaCl/200 mM urea, T_S was 29.4 ± 1.5 ($n = 8$); 1×10^{-6} M *L*-polyaspartic acid (PAA, MW 15 kD) lowered T_S to 4.8 ± 1.1 ($n = 3$), $P < 0.001$ vs control. The cumulative frequencies of particles ⩽2 μm diameter were 58% before and 10% after slow stirring; corresponding values for particles ⩽4 μm were 96% and 59%, respectively. There were more aggregates ⩾15 μm and ⩾20 μm in all samples after slow stirring ($P < 0.001$). COM aggregation, induced by slow stirring of a COM slurry, can be measured by T_S; it is inhibited by PAA, but not by citrate.

C31. The Use of a Pulsed Dye Laser for Identification of Urinary Stone Composition

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Various methods have been described and used to identify the composition of urinary stones. These are time consuming and expensive. With modern non-invasive methods for stone removal, a rapid method of stone analysis may be of benefit when planning treatment, as stone composition and hardness can influence disintegration by ESWL or laser lithotripsy. A pulsed dye laser has been used to create ionisation of the constituent atoms in urinary stones. This results in the emission of different wavelength light, each wavelength being specific to an individual constituent ion or atom. A 512 element solid-state scanning reticon connected to a storage oscilloscope and an X-ray plotter gives instantaneous recording of the wavelength emissions. The technique will be described in detail, showing how an accurate qualitative analysis of urinary stone composition can be obtained in under 2 min from stones in vivo or in vitro. The simultaneous use of the same laser equipment for the disintegration of the stone offers an added advantage.

C32. At Last, a Non-Iterative Program to Calculate Growth and Aggregation Rates!

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Until now the determination of growth and aggregation rates from seeded batch crystallization tests has been a slow and tedious process. Further, the current technique of calculating the extents of growth and aggregation produces results that are difficult to compare in a quantitative way. The problem here is how to convert the crystal numbers, N_i, available from the Coulter counter into values for the growth rate, G, and the aggregation kernel, β_0. The heart of the new program, BATCH, is a novel transformation of the complex and intractable population balance that describes the experimental system. The transformation yields $\dot{N}_i = f(G, \beta_0 N_i)$ which is rearranged to give

$$G = \frac{f_1(\dot{N}_i)}{f_2(N_i)} \quad \text{and} \quad \beta_0 = \frac{f_3(\dot{N}_i)}{f_4(N_i)}$$

$f_1(\dot{N}_i)$ and $f_3(\dot{N}_i)$ are simply deduced from the slopes of total number volume plots. Values of G and β_0 along with supersaturation are then calculated for each time interval. The time taken for this process? On a Apple Macintosh, less than 5 s! Preliminary investigations have been carried out for crystallization in buffered saline solutions. Comparison of results for tests dosed with 1.0 mM Mg, 0.5 mM citrate and 1% urine show very clear variations in G and β_0. In each case, growth is characterized by the McCabe ΔL law and aggregation by a random coalescence kernel. It is found that both growth rate and the aggregation kernel are directly proportional to supersaturation. The key features of this work are: (1) the population balance approach has been extended to describe batch experiments, providing a rigorous basis for investigation and a remarkably good model for aggregation and growth of calcium oxalate systems; (2) a very fast technique for calculating the fundamental kinetic parameters has been established.

C33. Calcium Oxalate Crystal Formation in Urolithiasis

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Urine samples from stone-forming patients and healthy persons were examined for calcium oxalate (CaOx) crystal amounts and urinary levels of various substances related to crystals. The effect of various substances on the CaOx crystal formation was investigated for the purpose of clarifying the mechanism of CaOx crystal formation in urolithiasis. The subjects were 43 CaOx stone formers and 12 healthy controls. Urine samples were collected from them under conditions of constant diet and fluid intake. The subjects were also given diets loaded with calcium (Ca), oxalate (Ox), or protein to facilitate crystalluria production. Urinary CaOx crystal amounts were determined using a model ZBI Coulter counter and the levels of Ca, Ox, magnesium, citrate, uric acid, phosphate, and sodium in the sample were determined. The results obtained were as follows: 1. CaOx crystal formation in the controls was dependent only on Ox levels while the formation in the patients was dependent on both Ox levels and Ca levels. 2. The crystals were formed in the patients even if Ox levels and Ca levels were low, and the conditions were more favorable for crystal formation in the patients than in the controls. This may be due to levels of citrate and magnesium that are known as crystallization inhibitors. 3. Multiregressive analysis of CaOx crystal amounts with the two factors of Ca levels and Ox levels revealed that the crystal amounts in the controls only correlated with Ox levels and that in the patients the crystal amounts also correlated slightly with Ca levels and more crystals were formed. These results clarified that the mechanisms of CaOx crystal formation are different between urine of stone-forming patients and urine of healthy persons.

C34. Calcium Oxalate Crystal Growth – Influence of Natural Inhibitors

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An investigation of naturally occurring inhibitors was conducted with respect to their ability to influence the crystal growth rate of calcium oxalate monohydrate (COM); their contribution to the inhibitory activity of urine was also assessed. The "constant composition method," originally introduced by Nancollas et al., was modified as follows. Approximately 5 min after crystallization of COM is started by the injection of COM seed crystals, the inhibitor solution to be investigated is added. From the change in growth rate the inhibitory activity is calculated. Polyacrylate is used as an inhibitory standard. From the urinary constituents investigated, RNA turned out to be the most effective inhibitor. On a molar basis, heparan sulfate, keratan sulfate, chondroitin sulfate A, and chondroitin sulfate C exhibit only one-tenth of the inhibitory activity of RNA. The results agree with the findings of Robertson et al., which were obtained by different methods. The values of the urinary concentrations of the substances investigated show great discrepancies in the literature: estimations of their individual contributions to the total inhibitory activity of urine range from 10% to 90%. In addition, interferences of the various inhibitors competing in the same system may play an important role.

C35. Assay of Urinary Citrate by High-Performance Liquid Chromatography

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Urinary citrate has been conventionally assayed by the fluorometric method. Although this method has a high level of accuracy, it requires 2 days and is a complicated procedure. In view of these shortcomings, we have developed a new technique using high-performance liquid chromatography (HPLC), and it is presented in this report. The equipment used was Waters 206D compact model. The column used was Shodex KC-811 (manufactured by Showa Denko), filled with highly acidic cationic exchange resin. The eluent used was 0.1% H_3PO_4. The assay was performed at a flow rate of 0.7 cm^3/min, a temperature of 60 °C and an ultraviolet absorption of 214 nm. Urine was diluted four-folds with 0.5% H_3PO_4 and passed through Waters SEP-PAC C_{18} under the above-mentioned conditions. It was then injected in a volume of 20 μl into liquid chromatography. The peak delineated at the retention time of 8 min and 30 s was measured as citrate. Recovery rates after addition of citrate at 200 μg and 400 μg to urine were 101.5 ± 6.3% and 96.0 ± 2.6%, respectively. Furthermore, variation coefficients in the triplicate assay were in an acceptable range of 1.7% to 4.3%. A comparison with the results of an assay obtained on the same sample by the conventional fluorometric method of Hori et al. for assay of citrate demonstrated a very good correlation, with a correlation coefficient of 0.931.

C36. The Effect of Glutamic Acid and Aspartic Acid on Calcium-Containing Stone

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The aim of this study was to evaluate the effect of glutamic acid (Glu) and aspartic acid (Asp) on calcium-containing stone. First, the effect of addition of L-Glu and L-Asp, respectively, to synthetic urine was evaluated, using a mixed-suspension mixed-product removal (MSMPR) crystallization system and scanning electron microscopy (SEM). Both Glu and Asp were found to decrease the growth rate, the nucleation rate, and crystal volume (suspension density) of calcium oxalate crystal formation, the effect increasing in proportion to the concentration of the amino acids. Glu had a more powerful inhibitory effect than Asp. SEM examination showed that the predominant crystal was calcium oxalate trihydrate, and the average crystal size and number became smaller by the additional concentration of Glu and Asp. Second, the amino acid composition of urinary stones was examined. The largest amounts of amino acid were Glu and Asp in calcium oxalate dihydrate, calcium phosphate and struvite, but not uric acid. Finally, the

concentration of glutamic oxalacetic transaminase and glutamic pyruvic transaminase, which convert alanine and Asp, respectively, into Glu in stone formers, was less than that in control subjects. Only Glu and Asp are acidic amino acids in more than 30 and are negatively charged in ordinary human urine (pH 5.5–6.5). Both amino acids and oxalate have a carboxyl acid group and are suspected to act competitively on calcium-containing stone formation. This study supports these facts and suggests that Glu and Asp, as well as protein containing Glu and Asp, suppress the formation of calcium-containing urinary stone.

C37. Promoting Effect of Urine from Patients with Primary Hyperparathyroidism on Calcium Oxalate Crystal Aggregation

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We examined the promoting effect of stone-forming urine on calcium oxalate crystal aggregation by using 19 stones of hyperparathyroid urine specimens. A large volume of healthy urine specimens were collected with 0.02% sodium azide. After freezing three times, remealing and filtration, we obtained particle-free, pooled healthy urine as an experimental solution. Five milliliters of 0.005 M sodium oxalate and 0.5 ml of 1 M calcium chloride were added simultaneously to the mixture of 90 ml pooled urine and 10 ml test specimen. The mixture of 90 ml pooled urine and 10 ml distilled water was used as a control. After 3-h incubation under 37 °C, particle distribution assay was performed by using a Coulter counter, Model TAII, fitted with a population count accessory. As a result, the particle distribution revealed a similar pattern in pure pooled urine and the control in this whole-urine system. It is suggested that the lack of inhibitors of test specimens did not affect the particle distribution because enough inhibitory activity in the 90% volume of pooled urine masked the lack of inhibitors. In contrast, 15 of 19 hyperparathyroid urine specimens showed larger particles more frequently than that of controls. This result suggested the existence of promotors or masking substances of inhibitors on calcium oxalate crystal aggregation in the hyperparathyroid urine specimens because the lack of inhibitory activity alone cannot change the particle distribution pattern, as mentioned above. The stone type of primary hyperparathyroidism must be a good human model of calcium stone formation, and therefore it is interesting that hyperparathyroid urines had a promoting effect on calcium oxalate crystal aggregation in an in vitro whole-urine system.

C38. The Relationship Between Crystal Agglomeration and Citrate in Urine of Stone Formers

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Urine specimens from 90 patients (174 samples) with calcium nephrolithiasis were tested in a seeded crystal growth system in which the effects on solubility, growth and agglomeration of calcium oxalate monohydrate crystals were independently measured. The specimens were examined at a 1:5 dilution and compared to those of a group of healthy subjects. Forty-four patients had hypercalciuria (14 on thiazides) and 39 had hypocitraturia, urinary citrate <2 mmol/day (12 were also hypercalciuric). Of all the physicochemical parameters tested, only inhibition of crystal agglomeration [expressed as (Tm) in min] was significantly lower in the stone formers, being abnormally low in 51 (57%) patients. The (Tm) was correlated positively with urinary citrate excretion ($n = 90$, $r = 0.692$, $P < 0.001$). All but one of the patients with normal (Tm) also had normal urinary citrate excretion. Conversely, all but one of the hypocitraturic patients had abnormal (Tm) values. In 13 patients a low (Tm) was associated with normal citrate excretion, suggesting that other factors, apart from citrate, may also play some role in this process. To examine further the role of citrate in the processes of crystal agglomeration, we studied 16 hypocitraturic patients before and during treatment with alkali. An increase in urinary citrate from 1.25 ± 0.1 to 3.02 ± 0.3 mmol/day was associated with restoration of the ability of the urines to inhibit agglomeration of calcium oxalate crystals; the (Tm) rose from 83 ± 8 to 180 ± 17 min. A positive correlation ($r = 0.80$, $P < 0.001$) was found between urinary citrate and (Tm) in this group. In conclusion, a single physicochemical parameter (the ability of urines to inhibit crystal agglomeration) was found to be disturbed in renal stone formers with variable biochemical abnormalities. Citrate is an important modulator of this process, which is in accordance with our previous in vitro studies. The results suggest that hypocitraturia promotes renal stone formation not only by increasing supersaturation by diminishing calcium complexation, as is currently thought, but also by having an effect on the crystal surface-related process of agglomeration.

C39. Modulation of Calcium-Oxalate Monohydrate Crystallization Kinetics in vitro

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Urinary and model compounds were tested in a seeded crystal growth system in which the effects of additives on the solubility, growth, and agglomeration of calcium oxalate monohydrate crystals were measured separately. Heparin, chondroitin sulphate, pentosan polysulphate, nephrocalcin, pyrophosphate, citrate, magnesium, and phosphate were tested. The solubility of COM crystals was increased only by citrate and magnesium. Crystal growth was inhibited by all compounds tested. However, the high MW compounds were the strongest inhibitors at low concentrations already (micromolar range). In contrast, inhibition of crystal agglomeration was achieved only by the low MW compounds and citrate was the most potent inhibitor at concentrations likely to be present in normal urine. The high MW substances, despite their potent effect on growth, had no effect on agglomeration. When heparin and citrate were tested together, crystal growth inhibition was exerted mainly by heparine, while agglomeration was inhibited exclusively by citrate. Combination of citrate with various concentrations of magnesium or various calcium/oxalate ratios showed that the inhibitory effect of citrate on agglomeration can be modulated by different calcium and magnesium concentrations. Thus, the processes of crystal growth and agglomeration, although both crystal-surface related, are clearly dissociated. This has also been confirmed in urine specimens from stone formers (Kok et al., this symposium). High MW compounds inhibit crystal growth preferentially while citrate (and pyrophosphate in high concentrations) are the most potent inhibitors of crystal agglomeration. In addition, the effects of citrate on agglomeration can be modified by compounds that form soluble complexes with it (magnesium, calcium).

C40. Matrix in Stone – Electron Microscopic Evidence

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Urinary calculi invariably contain 2%–5% (by weight) of organic matrix material. We studied the matrix in stone based on scanning electron microscopy (SEM) of the ultrastructure of urinary stones

after treatment with various acids for different periods of time. Thirty urinary stones obtained from human stone patients were studied. Treatment of stones in different concentrations of HCl and HNo_3 for different time intervals was followed by gold sputtering and SEM examination under a JEOL JSM 35c microscope. The photographs showed clear exposition of the fibrous protrusions in the stone substance, indicating the existence of protein matrix in the calculi. Following the removal of soluble inorganic substances, evidence of intracrystalline and intercrystalline matrix materials was found. It is concluded that protein matrix of unknown chemical composition is a definite integral part of most human urinary stones. It is possible that an initial matrix coating over the crystals produces adhesions with the adjacent crystals and larger fibrous matrix becoming interspersed between these crystal deposits. Photographs to confirm the observations will be presented.

C41. Investigations on the Crystallization Tendency in Urine with Frequency Response Analysis (Impedimetry) and Cyclic Voltametry

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Nucleation, growth, and aggregation of crystals are primarily determined by the electrophysical properties of the outer and inner phase-limit layers of an electrolyte. In our investigations, we tried to settle the question of whether it is possible to discriminate urine with a "high crystallization tendency" and urine with a "low crystallization tendency" by determining the complex impedance or using cyclic voltametry. We used electrochemical testing equipment for impedance analysis (frequency range 0.01 to 10^5 Hz) and cyclic voltametry (−1.5 V to +1.5 V). We examined urine of calcium oxalate stone-formers and stone-free persons as well as so-called artificial urine. Urine of stone-formers showed an obvious influence of DC polarization (DC = direct current) in the low-frequency range (0.01 to 10 Hz). Even polarization of −0.4 V led to a visible decrease in resistance capacity (reactance) of urine and to an obvious decrease in negative phase shift between current and voltage (transition from diffusion-controlled to charge-transfer boundary reaction); up to a polarization value of −0.8 V there was no apparent influence on urine of stone-free persons. That means that urine of stone-free persons has a lower crystallization tendency. Measurements with cyclic voltametry showed that in the more stable urine of stone-free persons there is only a slight current modification in a wide DC voltage range. The fact that comparable artificial urine shows quite different behavior makes it clear that important components for crystallization behavior in natural urine are absent in artificial urine. It shows, too, that not only each single component is important, but the interaction of all components as well. The status of our investigations enables us to say that it is possible to discriminate urine by measuring complex impedance and using cyclic voltametry in order to evaluate the risk of stone formation – also from the point of view of metaphylactic measures.

C42. The Effect of Uric Acid on the Inhibition of Hydroxypatite Crystal Growth

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Calcium-oxalate stone formation may occur in patients with no metabolic abnormaly apart from increased uric acid concentration in the urine. Treatment with allopurinol, resulting in normalization of uric acid excretion, is effective in prevention of further stone growth. The mechanism whereby excess uric acid results in growth of calcium oxalate calculi is not known. That hydroxyapatite (HAP) crystallization is important in the pathogenesis of calcium-oxalate stone growth is well known. We used a HAP constant-composition seeded crystal-growth system to study the effect of uric acid on the inhibition of HAP. We found that increasing concentrations of uric acid resulted in no effect on the rate of HAP crystal growth in the absence of urinary inhibitors. In the presence of an artificial urine containing the known inhibitors, magnesium and citrate, increasing concentrations of uric acid resulted in increased inhibition of crystal growth for each concentration. This supports the hypothesis that uric acid somehow deactivates the inhibitor or else prevents the inhibitor from occupying active growth sites on the crystal surface.

C43. The Inhibitory Effect of Sodium Pentosan Polysulfate on Calcium-Oxalate Crystal Formation in vitro and in vivo

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The purpose of this study is to evaluate the effect of sodium pentosan polysulfate on the formation and growth of calcium-oxalate crystals and to measure the number and volume of crystals quantitatively in vivo with a Coulter counter. We evaluated the inhibitory effect of this substance with the seed crystal method and the whole-urine system as an in vitro study. Calcium-oxalate crystals were induced in the kidneys of male Sprague-Dawley rats by intraperitoneal injection of 4-hydroxy-L proline and administration of 0.4% ethylene glycol by drinking water ad libitum for 7 days. The rats were injected intravenously with 5 mg sodium pentosan polysulfate. The kidneys were homogenized and digested in Soluen-100. After calcium-oxalate crystals had been collected, the distribution and characteristics of crystal size were measured by a Coulter counter. Sodium pentosan polysulfate had a strong inhibitory effect on the aggregation and growth of calcium-oxalate mono- and dihydrate crystals with the seed crystal method and a mild inhibitory effect on the formation and growth in the whole-urine system. Optical microscopic study and scanning electron microscopic study showed the deposition of calcium-oxalate crystals both in the control kidneys and in kidneys treated with sodium polysulfate pentosan. However, our quantitative study by the crystal counting method revealed that the number, volume and average size of the crystals statistically decreased in the rats with the administration of sodium pentosan polysulfate.

C44. Urinary Macromolecular Inhibitory Substances of Calcium Oxalate Monohydrate Crystal Growth

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To evaluate the inhibitory activities of urinary glycosaminoglycans (GAG) in natural form (free GAG or proteoglycan), urinary macromolecular substances (more than 10,000 M.W.) were fractionated and examined individually. Urinary macromolecular substances (UMM) were fractionated into five groups as follows: (1) THM (Tamm-Horsfall mucoprotein); (2) UMM1 (UMM except for THM); (3) UMM2 (UMM except for THM and GAG1); (4) GAG1 (GAG and protein); (5) GAG2 (only GAG). Control urine specimens were collected from ten healthy males. THM was prepared by the original method of Tamm and Horsfall. GAG1 was obtained using the usual method of isolation of GAG, the method of Di Ferrante and Rich. GAG2 was obtained after proteolysis of GAG1. The method of measuring the inhibitory activities of crystal growth and aggregation was the non-crystal seed system developed by Koide et al.

Results: THM and UMM1 had strong inhibitory activities. On the other hand, UMM2 had a very low inhibitory activity. This result indicates that GAG1 is the main inhibitory substance in UMM1.

The inhibition index of each fraction in the original urine was THM 17.5%, UMM1 22.2%, UMM2 0.9% and GAG2 4.2%, respectively. GAG1 inhibited mainly crystal growth, although THM and UMM1 inhibited crystal aggregation. The protein in GAG1 mainly consisted of one protein of about 30,000 M.W. This protein, perhaps glycoprotein, was considered the main inhibitory substance in GAG1.

C45. Polyanionic Inhibition vs Supersaturation in Male and Female Recurrent Calcium Stone Formers

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Calcium oxalate stones form because of an imbalance between the forces of inhibition and supersaturation. The main inhibitory activity is a function of a group of urinary polyanions (e.g. glycosaminoglycans), which acts by binding to the surface of calcium-oxalate crystals and increasing their net negative surface charge, i.e. zeta potential. Urine saturation is determined by urinary volume and pH and the 24-h excretions of calcium, oxalate, and possibly uric acid. In order to determine abnormalities in the functional availability of the polyanionic inhibitors and in saturation-risk factors and to assess possible differences between males and females, the following individuals collected a 24-h urine specimen while on their normal diet and activity: 61 male recurrent calcium-oxalate stone formers (MRSF), 25 male control subjects (MCS), 19 female recurrent calcium-oxalate stone formers (FRSF), and 13 female control subjects (FCS). Three cubic centimeters of urine were combined with 27 ml of a supersaturated solution of calcium oxalate, and the resulting zeta potential of the crystals was measured using a Zeta meter. Each urine specimen was also analyzed for volume, pH, calcium, oxalate, uric acid, and creatinine. The mean zeta potential measurement using the urine of the MRSF was -16.5 ± 0.4 mV (mean ± SEM) compared with -20.3 ± 0.8 mV for the MCS ($P < 0.0001$). MRSF excreted 7.0 ± 0.3 mmol of calcium per 24-h compared with 5.6 ± 0.5 mmol by the MCS ($P < 0.05$). Other risk factor comparisons were not significantly different. Mean zeta potential measurement using the urine of FRSF was -17.3 ± 0.8 mV compared with -20.2 ± 1.0 mV for the FCS ($P < 0.05$). FRSF excreted more calcium, 5.8 ± 1.3 vs 2.7 ± 0.4 mmol ($P < 0.0001$), and oxalate, 0.40 ± 0.05 vs 0.20 ± 0.02 μmol ($P < 0.005$), per 24-h compared with FCS. Other risk factor comparisons were not significantly different. MCS had significantly greater 24-h excretions of calcium, uric acid, and oxalate than FCS, but MRSF only had significantly greater uric acid excretions compared with FRSF. Other intersex risk factor comparisons were not significantly different. In conclusion, both MCS and FCS have less risk of stone formation due to greater polyanionic inhibitory activity and less supersaturation. There was no difference between males and females in terms of inhibitory activity, but saturation was more of a problem in the male groups.

C46. Effective Prevention of Calcium Oxalate Crystal Formation in vitro and in vivo by Pentosan Polysulfate

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Sulfated polysaccharides belong to a major group of compounds that are thought to inhibit crystal formation and crystal growth in the urine. Heparinoid pentosan polysulfate (PPS) was found to increase the inhibitory activity of the urine in healthy volunteers (Norman et al. 1984) and was suggested to reduce stone recurrence (Danielson et al. 1987). In the present study we wanted to examine and quantitate in reproducible models the ability of PPS to reduce calcium oxalate (CaOx) crystal formation in vitro and in vivo.

Methods: In vitro calcium oxalate crystal formation was studied using the continuous-flow crystallizer under steady-state conditions according to Robertson. CaOx crystals were formed in artificial urine and analyzed for size and density population by a Coulter counter. The in vivo CaOx crystal formation was induced in rats by feeding them a vitamin B_6-deficient diet and 1% ethylenglycol as drinking water for 3–5 days. Energy-dispersive X-ray microanalysis (EDX) of the kidney revealed that CaOx crystals were formed only without contamination with phosphate or sulfate. PPS was administered orally by a gastric tube, s.c., or by means of a subcutaneously implanted osmotic minipump.

Results: In the continuous-flow crystallizer, PPS reduced at 10 μM the crystal growth rate from 0.39 ± 0.02 to 0.26 ± 0.02 μm/min while the nucleation rate was not affected. In the rat model of CaOx stone formation, 100 mg/kg p.o. were administered. PPS starting 2 days prior initiation of the lithogenic diet showed little reduction in CaOx crystal formation whereas 30 mg/kg/24 h via the osmotic minipump reduced CaOx formation by more than 80%. Calcification of the kidney tissue expressed by peak-to-background ratio of the Ca signal after scanning the kidney section area with EDX was reduced from the control value of 0.97 to 0.19 by PPS.

Conclusion: Pentosan polysulfate is an active inhibitor of CaOx crystal formation in vitro *and* in vivo, thus confirming the concept of crystal-growth inhibition by urinary polyanionic macromolecules. The results provide an experimental basis in the search for exogenous inhibitors that are designed to imitate or substitute for the endogenous inhibitors of crystal growth in urine.

C47. Role of Pyrophosphate in Calculogenesis

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There is a relatively large amount of evidence in favour of pyrophosphate (PP) as an inhibitor of lithogenic process, as its excretion has been found to be lower in many stone former populations. Our clinical and experimental results do not support this contention. The PP excretion in fasting urine samples of 19 healthy controls (HC) and 13 stone formers (SF) on a controlled hospital diet was 39.1 ± 17.8 and 32.1 ± 20.5 μmol/l, respectively. There was no significant difference. Twenty-four hour urine samples were collected from 10 HC and 12 SF from the above group. PP excretion was 53.46 ± 25.24 and 42.93 ± 26.9 μmol/24 h, respectively. Again the difference was non-significant. The mineralization and demineralization activity of pyrophosphate (sodium salt) in the concentration varied from 10 to 100 μmol/l (within physiological limits) and was tested in vitro on calcium phosphate by the method of Singla and Jethi (1981) (Ind J Exp Biol 19:283–285). The PP did not influence the mineralization activity. Simultaneously, inorganic phosphorus excretion was also determined in all HC and SF. No relationship (r) was observed between inorganic phosphate and PP levels.

C48. The Mechanism of Shockwave Fragmentation of Urinary Stones – A Scanning Electron Microscopic Study

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Electron microscopy (SEM) of urinary stones has shown that they are polycrystalline structures formed by aggregation of crystals. Both in extracorporeal shockwave lithotripsy (ESWL) and in contact electrohydraulic lithotripsy (EHL) shockwave cause disintegra-

tion of stones into particles of varying size. In this study the morphology of fragmentation sites of stones fragmented by both ESWL and EHL were examined by SEM to study the mechanism of fragmentation. Stone fragments from 24 patients were used for this study. Twelve received ESWL and 10 patients had EHL. In 2 patients stones were extracted endoscopically without fragmentation and these were used as controls. Stones subjected to ESWL and EHL fragmentation showed two distinct features. The main feature was cleavage along the crystal interfaces. In some specimens the fracture of the stone occurred across the crystals. Clinical experience and comparison with plain X-rays of the abdomen suggest that stones that have a heterogeneous X-ray appearance, and therefore contain distinct cleavage planes, are easier to fragment than those that are homogeneous and dense. The findings and implications will be discussed.

C49. Nucleation and Growth Inhibitors of Calcium-Oxalate Crystallization

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The aim of this study was to examine the inhibitory activity of a number of potential inhibitors of calcium-oxalate crystallization and to classify such inhibition in terms of nucleation and/or growth. Several potential inhibitors of calcium-oxalate crystallization were added to a standard synthetic urine at 37 °C (pH 5), which was then evaporated in a rotary evaporator. Aliquots of the concentrated solution were filtered and crystals analysed by X-ray powder diffraction (XRD) and scanning electron microscopy (SEM). Further aliquots were analysed using a Coulter counter. Calcium-oxalate monohydrate (COM) and dihydrate (COD) were identified by XRD in all samples after evaporation. This was confirmed by SEM, although COM was not always detected. In some cases COD crystals were observed of smaller cross section than those occurring in the reference urine after evaporation. Chondroitin sulphate A, B and C, as well as magnesium oxide (MgO), methylene blue, teepol and human urine, each caused a decrease in the total particle count (upon evaporation) relative to the standard urine. The biggest decrease in particle number was caused by the real urine. These results suggest that the above substances are nucleation inhibitors. In addition, the particle size-volume distribution profiles of the solutions treated with MgO, teepol, methylene blue and real urine were characterized by a general shift of peaks to the smaller diameter ranges relative to the reference urine. This, together with SEM data, indicates that these substances may also be growth inhibitors. The curves for the evaporated chondroitin sulphate solutions were similar to that of the reference urine. Hyaluronic acid, allopurinol, and zinc chloride ($ZnCl_2$) caused an increase in the total number of particles (after evaporation) relative to the standard urine, thereby suggesting that these substances are promoters of calcium-oxalate nucleation. However, the particle size-volume curves for allopurinol and hyaluronic acid indicated that they might be acting as growth inhibitors.

C50. Experimental and Computed Inhibition of Calcium-Oxalate Precipitation

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This study was undertaken to examine the qualitative and quantitative effect of magnesium, citrate and oxalate ions on calcium-oxalate crystallization in synthetic urine and to compare the results with those predicted by computer modelling of an analogous system. Artificial urines were prepared in which the concentrations of the three ions under investigation were independently varied. Samples (pH 5 and 6) were evaporated in a rotary evaporator. Residual Ca^{2+} and Mg^{2+} concentrations were determined by atomic absorption spectrophotometry. Precipitates were examined by scanning electron microscopy (SEM). An equilibrium speciation computer model, based on the program MINEQL, was used to predict the nature of the precipitates. Significant inhibition of Ca precipitation at both pH values occurred at Mg concentrations greater than 4.0 mmol/l whereas at lower concentrations the inhibition was minimal. This observation was supported by MINEQL computations, which predicted the formation of large amounts of (insoluble) calcium oxalate and small amounts of (soluble) magnesium oxalate at low Mg concentrations. With increasing Mg concentration, the reverse trend was predicted. Although SEM revealed calcium-oxalate dihydrate crystals, no brushite was observed despite its predicted appearance. Citrate showed similar inhibitory trends as Mg but appears to be a stronger inhibitor. Although MINEQL predicted the formation of brushite, it was only observed by SEM at low-citrate concentrations. Calcium-oxalate trihydrate occurred at the higher citrate concentrations. Precipitation of calcium oxalate increased with increasing oxalate concentration. Computer modelling predicted an accompanying decrease in brushite concentrations. Calcium-oxalate mono- and trihydrate were observed by SEM. However, brushite was not detected. Although reasonable agreement between experimental and computed results were obtained, discrepancies involving brushite occurred. This might be due to kinetic factors which were ignored in the "long-term" equilibrium-state model used in this study.

C51. The Effect of Macromolecules on the Crystallization of Calcium Oxalate in Human Urine

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The role of macromolecules in the formation of calcium oxalate (CaOx) urinary stones is still a subject for debate. Recent findings have shown that significant loss of macromolecules, particularly Tamm-Horsfall mucoprotein (THM), occurs on low-speed centrifugation and/or filtration of urine. The aim of this study was to compare CaOx crystallization in untreated human urine with that in parallel samples whose macromolecular content had been reduced by centrifugation and filtration, or ultrafiltration, and to determine whether any effects observed could be compensated for by the addition of physiological concentrations of chondroitin sulphate (CS), human serum albumin (HSA) or THM. Urine was collected from normal men and pooled. One-third was sieved (50 μm), one-third was centrifuged (10,000 *g*, 30 min) and then filtered (0.22 μm), and the remainder was ultrafiltered (10,000 Da). Crystallization was induced by the addition of a standard load of oxalate in excess of the measured metastable limit and a Coulter counter was used to detect and monitor crystallization. The detectable metastable limit was not significantly altered by any of the treatment procedures. The amount of crystalline material deposited from the sieved urine was significantly less than that in the other urines, which did not differ markedly. SEM showed that crystals precipitated from the sieved urine consisted of small, exclusively single, calcium-oxalate monohydrate (COM), as were those from the spun and filtered urine, which were, however, considerably larger. In marked contrast, the COM crystals from the ultrafiltered urine were mainly clustered into large aggregates. This transition from small, single crystals in the sieved urine to large aggregates in the ultrafiltered urine was consistently observed in numerous samples. The formation of these large aggregates was only partly inhibited by the addition to ultrafiltered urines of CS (20 mg/l), HSA (20 mg/l) or THM (50 mg/l), with THM exerting the most

marked effect. In some urines, THM also altered the morphology of the precipitated crystals from the COD "envelope" habit to the smaller COM "coffin" type. It was concluded that urinary macromolecules with Mr > 10,000 Da inhibit the formation of large crystal aggregates in human urine, and this inhibitory effect cannot be ascribed to any one class of urinary macromolecules.

C52. Ability of Some Carboxylic and Phosphorylated Carboxylic Acids to Prevent Hydroapatite and Calcium Oxalate Crystallization

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Phosphocitrate (PC) strongly inhibits calcium salt crystallization. The inhibitory power seems to reside in the special stereochemical configuration of the molecule, in particular, the relationship between the PO_4 and COOH moieties. With a goal to formulating new anticalcifying agents, the structure-activity relationship of PC and several related compounds has been examined. Compounds tested were either obtained commercially or synthesized in our laboratory and included: citrate, tricarballylate (TCA), tetracarballylate (TETCA), 2,3-diphosphoglycerate (2,3-DPG), PC, phosphomalate (PM), phosphomethylglutarate (PMG), phosphoisocitrate (PIC), 3-methyleneaminophosphonate tricarballylate (MAPT), and 2,4-dimethylene tetracarballylate (DMTETCA). Inhibition of the transformation of amorphous $CaPO_4$ to hydroxyapatite (HA) was measured by base titration whilst the rate of Ca^{2+} depletion from a CaOx seeded metastable solution was measured to assess inhibition of CaOx crystallization. PC was superior to any other inhibitor tested. For compounds retaining a phosphate moiety, an order of potency against HA and CaOx was PC > PAT > PIC > 2,3-DPG > PM > PMG. The order of potency with reference to PC for a COOH series was PC > TETCA > MAPT > DMTETCA > citrate > TCA, with both CaOx and HA being affected in a similar fashion. TETCA showed more inhibitory power toward CaOx than HA. MAPT was weakly inhibitory in both systems, reflecting that an increase in the length of the side chain reduced inhibitory power. It is concluded that stereochemistry and charge are important contributors to ensuring full inhibitory potential. At least one PO_4 group in association with one or more COOH groups is desirable. PC is confirmed as the strongest inhibitor examined.

C53. Suppression of Struvite and Newberyite Bladder Stone Formation in Rats by Phosphocitrate

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We have observed previously the powerful anticalcifying action of phosphocitrate (PC) in blocking hydroxyapatite and, to a lesser extent, calcium-oxalate crystallization. However, since PC's potential to control crystalline magnesium-salt deposition (e.g. struvite and newberyite) is unknown, the present studies have examined this aspect using three stone-forming rat models. Model A: Rats on normal chow had a 5 mg sterile stone fragment inserted into the bladder and received PC (50 mg/kg body wt.) daily for 4 weeks by IP injection. Model B: Following stone implantation, rats received normal chow supplemented with 1% glycolic acid for 4 weeks, changing to 0.5% glycolic acid for a further 3 weeks. PC was administered daily for the final 3 weeks. Model C: Rats on normal chow received an infected stone implant (Proteus mirabilis, 10^8 cells/ml). Groups of Model C rats daily received amoxycillin orally either alone or together with an IP dose of PC for 4 weeks. Bladder calculi recovered after the experimental periods were dried, weighed and composition assessed by IR spectroscopy. Urinary bacterial counts and chemistry parameters were measured. PC treatment produced a marked reduction in daughter stone formation in all models, as evidenced by total weight and numbers of stones recovered. Model A rats grew stones rapidly with composition newberyite (61%) – struvite (39%). Model B rats initially produced oxalate stones but with 0.5% glycolic acid, rapid deposition of magnesium salts occurred. Model C rats, as anticipated, produced mainly struvite stones (80%). When treated with an antibiotic, the stone composition changed to a mixture of newberyite and struvite and PC was still effective. Conclusions reached are that PC controls formation and growth of magnesium salt crystallites under a variety of conditions. Combination therapy of PC and an antibiotic appears promising for infection stone control.

C54. Assessment of Agglomeration of Calcium-Containing Crystals

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The present study describes a new approach to test and assess the tendency of CaP and CaOx crystals to agglomerate. CaP crystals were precipitated from highly supersaturated solutions (Ca:P 3:30) and allowed to grow and agglomerate for 20 min. Then the crystals were disintegrated by means of sonification for 1 min and again allowed to reagglomerate. Particle-size distribution (PSD) measurements were taken before disintegration and thereafter at different time intervals. A wide and nongaussian PSD was recorded predisintegration whereas a relatively narrow gaussian PSD with significantly reduced mean size was recorded 2 min postdisintegration. PSD determination taken 20 min later revealed that the particles were close in size to those observed predisintegration and the recorded PSD was bimodal. The effect of several phosphonates on the reagglomeration process was examined. PSD was not affected postsonification when polyalklene-polyamino-polykis (methylen phosphonic acid) was added but was significantly altered when phosphocitrate or ethylene-dinitro-tetrakis (methylen phosphonic acid) (ENTMP) were individually added. Reagglomeration of CaP was totaly prevented for more then 2 days postdisintegration and CaP particles did not attain their previous sizes. In this inhibition process ENTMP was found to be more potent than phosphocitrate. X-ray diffraction patterns showed that amorphous CaP was initially precipitated and was gradually transformed to hydroxyapatite. The tested additives affect the depletion of Ca ions from the solution and probably alter the surface area and characteristics of CaP particles. NMR study of these particles revealed that the additives significantly affect the water content of the amorphous phase and consequently enhance CaP maturation to hydroxyapatite. The reagglomeration processes of CaOx were examined in whole urine. This process was prevented or significantly delayed in urines of normal individuals but was allowed in the urine of recurrent SF. This effect might reflect the reduced inhibitory activity toward agglomeration in the urine of SFs. In this context the effect of drugs containing phosphonates is very important in preventive treatment.

C55. Ultrafiltration Studies on Crystallization of Urinary Calcium Oxalate

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To examine the nucleation-inducing and growth-inhibiting capacity of urinary macromolecules on the crystallization of endogenous calcium oxalate in human urine, crystals formed in dehydrated urine

(pH 5.3, 1,200 mOsm) and fractionated by ultrafiltration were compared with those formed in the nonfractionated counterpart. Results are shown in the following table: a retentate: whole urine (R/W) or ultrafiltrate: whole urine (UF/W) ratio of >1 for population density of crystals indicates enhanced nucleation, and a ratio of <1 for crystal size indicates growth inhibition.

	Normal controls (n = 32)		Stone formers (n = 37)	
	Population density (10^{-5}/ml)	Crystal size (μm)	Population density (10^{-5}/ml)	Crystal size (μm)
Whole urine	1.3 ± 1.7	11 ± 4	2.8 ± 2.7	9 ± 3
Fractionated urine				
10 kD cutoff				
R/W	7.5*	0.5*	16.5*	0.6*
UF/W	0.3*	1.8*	0.2*	1.8*
20 kD cutoff				
R/W	2.8*	0.9	2.4*	0.8*
UF/W	1.0	1.5*	0.1*	1.7*

* Significantly different from the corresponding whole urine; $P < 0.02$

Among the normal controls, macromolecules (10–20 kD) that were retained by the 10 kD filter were capable of enhancing nucleation, but neither of those retained or filterable through the 20 kD filter were as effective. In contrast, the concerted action of macromolecules retained by the 10 kD filter were more effective than those retained by the 20 kD filter in producing the observed growth inhibition. Among the stone formers, the considerably enhanced nucleation in the retentate of 10 kD cutoff, above that in the retentate of 20 kD cutoff, is suggestive of the cooperative effect of macromolecules retained by the 10 kD filter. The reduced crystal size in the retentates and the increased size in the ultrafiltrates, irrespective of a 10 kD or 20 kD filter, suggests that macromolecules of 10–20 kD were apparently inert and only those of >20 kD, retainable by either filter, were important in limiting crystal growth. The results demonstrate that crystal nucleation and growth were separately regulated by interactions with macromolecules. The macromolecular involvement in the controls differed from that in the stone formers.

C56. Correlation of Urinary Excretion of Glycosaminoglycans and Uric Acid in Healthy Adults and in Renal Stone-Formers

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Glycosaminoglycans (GAGs) exercise an inhibitory effect on the crystallization of urinary calcium salts, which is believed to be blocked by the interference of uric acid. Urinary excretion patterns of GAGs (1) and uric acid were examined in 43 stone formers (SF) and 35 normal subjects (NS).

The urinary concentration of GAGs showed a positive correlation with uric acid concentration in NS ($r = 0.6697$; $P < 0.001$), as well as in SF ($r = 0.6069$; $P < 0.001$). In accordance with earlier findings [2, 3], calcium-oxalate stone-formers exhibited a significant decrease in GAGs excretion. Also, the subjects with hyperuricosuria excrete more GAGs, as observed by others [1, 4].

Group	GAGs		Uric acid	
	Excretion (μmol/24 h)	Concentration (μmol/l)	Excretion (mmol/24 h)	Concentration (mmol/l)
SF	16.11 ± 6.51**	9.55 ± 5.97*	2.93 ± 1.05**	1.63 ± 0.86
NS	20.24 ± 5.40	12.95 ± 5.74	2.26 ± 0.67	1.46 ± 0.69

* $P < 0.05$; ** $P < 0.01$ as compared to NS

References: 1. Hesse A et al. (1986) Urol Int 41:81–87 – 2. Baggio B et al. (1982) Clin Chim Acta 124:149–155 – 3. Robertson WG et al. (1978) Br J Urol 50:449–554 – 4. Caudarella R et al. (1983) J Urol 129:665–667

C57. Do Stone Formers Lack Inhibitors in Urine?

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Various urinary inhibitors have been described, but there is no concrete evidence of the lack of such inhibitors in the urine of stone patients. This paper presents the findings of a reduced inhibitory capacity in the urine of stone patients compared to normal urine when added to an in vitro crystal growth set-up. Crystals seen in human urine, namely weddellite, whewellite, brushite, octocalcium phosphate, apatite, struvite, and newberyite, were grown in silica gel medium. Urine samples were used from 190 stone patients, 82 colic patients, and 52 controls. Distilled water was added to the samples and crystal growth was set up. The rate of growth and size of crystals were studied and the groups were compared. The inhibitory effect of urine samples on the rate and extent of growth of different crystals compared to controls was as follows.

Crystal	Normal urine	Stone patients	Colic patients
Whewellite	75%	21%	0%
Weddellite	33%	12%	0%
Brushite	63%	35%	0%
Newberyite	33%	8%	0%
Octocalcium phosphate	79%	24%	13%

Inhibition of apatite and struvite crystals was not significant

It is evident from the studies that the urine of normal individuals contains inhibitors against the growth of whewellite, weddellite, brushite octocalcium phosphate, and newberyite crystals. This inhibition activity was lacking in the urine of stone formers. The almost total lack of inhibition of crystal growth in colic patients points towards a relationship between the absence of inhibitor activity and the progression of stone activity in these patients.

C58. Seasonal Variations in Urinary Crystalluria, Glutamate Oxaloacetate Transaminase, and Glutamate Pyruvate Transaminase Levels in Stone Formers and Healthy Controls

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In view of the reports that crystalluria is more severe in summer than in winter due to concentrated urine and that urinary glutamate oxaloacetate transaminase (UGOT) and glutamate pyruvate transaminase (UPGT) levels are lower in stone formers (SF), we examined crystalluria, GOT and GPT in fasting urine specimens of healthy controls (HC) and SF in the Udaipur region where prevalence of urolithiasis is high. Crystalluria was examined in 572 HC and 170 SF. In the former no difference was observed between winter (5°–19 °C) and summer (37°–39 °C), but in the latter it was higher in summer. In HC, moderate-to-severe crystalluria (2+ to 4+) was present in 8.4% and 7.3% subjects in winter and summer, respectively. On the other hand, it was much higher in SF – 14% in winter and 19.6% in summer respectively. A comparison between the rural and urban HC population revealed that prevalence of crystalluria was higher in the former, which appeared to be due to their poor nutritional status and a larger intake of oxalates. UGOT and UGPT levels were determined in 423 and 124 HC and SF, respectively. There was no seasonal variation in these enzymes in either groups. However, contrary to earlier findings, the excretion of both of these enzymes was higher in SF (8.96 ± 6.58 IU/l and 7.26 ± 5.32 IU/l) than HC in winter (6.74 ± 3.60 IU/l and 6.07 ± 3.40 IU/l).

C59. Isolation of Polypeptides from the Urine of Human Beings and Their Role in Renal Calculi Formation

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Male human beings have been found to excrete polypeptides in their urine which, depending upon the experimental conditions, can not only inhibit in vitro mineral-phase formation but can also stimulate the demineralization of the performed mineral phase. Supersaturated solutions of the reactants were used to induce in vitro mineral phase formation. Conventional biochemical procedures (dialysis, ion-exchange chromatography, molecular sieve chromatography, FPLC, electrophoresis, etc.) were used to isolate and purify the potent urinary polypeptides. Studies were restricted only to those polypeptides with molecular weights between 2,000 and 15,000. Normal males were found to excrete five polypeptides in their urine (molecular weights 2,700, 3,200, 4,000, and 6,800), which not only inhibited mineral phase formation but also stimulated the demineralization of the preformed mineral phase. Male kidney stone patients were, however, found to excrete only three potent polypeptides (molecular weights 6,600, 9,200, and 12,500) in their urine. Polypeptides isolated from the urine of normal persons were found to be much more potent than those isolated from the urine of kidney stone patients regarding the influence on mineralization and demineralization reactions. Comparison of the amino acid composition of two of the polypeptides between normal persons and kidney stone patients revealed that polypeptides isolated from kidney stone patients were rich in Ser, Ala, Tyr, and Arg but relatively poor in Asp, Glu, Thr, Tys, and His. The quantitative and qualitative differences observed above in urinary polypeptides between normal persons and kidney stone patients may have an important role in renal calculi formation.

C60. A Comparison of Inhibitory Potential of Urine Fractions

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In urolithiasis research, fundamental questions remain concerning the possibility of differences existing between non-stone forming (NSF) and stone-forming (SF) urine towards the inhibition of calcium oxalate monohydrate (COM) crystal growth in vitro. Constant composition (CC) seeded crystal-growth experiments have shown that the chromatographically separated macromolecular components of NSF urines exhibit inhibitory potential over a wide range of molecular weights. In a limited study of eight cases, it was shown that while NSF samples showed inhibition in the 31–60 k dalton range of molecular weight, two of four SF urines had little influence even though the relative protein concentrations in each case were quite similar. While the inhibitory potential of whole NSF or SF urines, measured using the CC method, at comparable dilutions (usually 1.2% v/v are similar, subtle differences among SF fractions and between the NSF and SF groups seem to exist.

C61. Simple Method for Determining the Metastable Limit of Calcium Oxalate

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The purpose of this paper is to evaluate the simple microplate method for determining the metastable limit of calcium oxalate in urine reported by Ryall et al. and to measure its limitations under various conditions, as well as to examine the effect of various inhibitors on the whole urine system using the Coulter counter. In the microplate method, 10/μl sodium oxalate, with a final concentration of 0 to 150/μmol, was added to each 200/μl of urine, and the minimal calcium-oxalate crystal-forming point was determined by the use of inverted microscope. A Coulter counter and image analyzer were also used and the results were compared. Using this method, the urine of normal subjects and stone formers were examined under various conditions such as hydration, dehydration, and thiazide therapy. In correlation with the Coulter counter method and image-analyzing method, the microplate method was shown to be valuable and useful. The metastable limits correlated well with calcium and osmolarity, but not with oxalate concentration in urine. Stone formers showed significantly lower limits than normal subjects. All subjects showed lower limits in early morning urine. Sodium-copper chlorophyllin, citrate, and pentosan polysulfate inhibited crystal formation and growth above the metastable limit.

C62. Effect of Temperature on Crystal Growth and Morphology

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Studying crystals is a mandatory investigation in urolithiasis. Studies of deposits, however, are performed after variable time intervals and at different room temperatures world wide. It is possible to grow urinary crystals in vitro, but the experimental temperatures are not similar to body temperatures. In this paper, the alterations in crystal growth rates in vitro and those in the extent and morphology of urinary crystals after varying time intervals at different temperatures are presented. Urinary deposits of stone patients and normal individuals were examined immediately after passage and after different time intervals, namely: 2, 4, 6, 24, and 48 h; the samples were kept at room temperatures (22°–35 °C) and in the

refrigerator (4 °C). The in vitro crystal growth set up in silica gel medium was retained at the above room temperatures and in the refrigerator. Red blood cells and pus cells seen in fresh urine disappeared after 24 h at both temperatures. Crystalluria increased significantly after 24 h. The increase was more in the refrigerated samples compared to the samples kept at room temperatures. Bicarbonates and phosphates, however, increased at room temperatures. In vitro crystal growth was different at the two temperatures, and this difference varied with the type of crystal. Correlations between the behavior of crystals in human whole urine and the in vitro grown crystals will be discussed. The absence of crystals after waiting in normal individuals will also be discussed.

C63. Urinary Excretion of Lithogenic Substances and Inhibitory Activity Towards Calcium-Oxalate Monohydrate Crystal Growth in Idiopathic Urolithiasis

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Calculus formation occurs in urine supersaturated with calcium-oxalate and lacks the normal inhibitory potential. Fifty male adult patients with idiopathic urolithiasis (calcium-oxalate type) and 25 age- and sex-matched healthy controls were selected to determine the urinary excretion of lithogenic substances (calcium, oxalate, inorganic phosphorus, and uric acid) and urinary inhibitory activity (IA) towards calcium-oxalate monohydrate (COM) crystal growth. Urinary IA towards COM crystal growth was determined in terms of decreases in ^{14}C-oxalate in solution following addition of seed crystals to a metastable solution of calcium chloride and sodium oxalate [1]. The IA in stone-formers (1.25 ± 0.11 units/μmol creatinine) was significantly ($P < 0.001$) lower than that of controls (2.73 ± 0.30 units/μmol creatinine). There was no significant difference in the urinary excretion of oxalate and inorganic phosphorus in the two groups, but the stone-formers exhibited a significant hypercalciuria ($P < 0.05$) and hyperuricosuria ($P < 0.001$) as compared to control subjects. A significant negative correlation was observed between the urinary IA and uric acid levels in stone-formers. It is concluded that hyperuricosuria may indirectly predispose to calcium oxalate lithiasis by decreasing the urinary inhibitory activity.

References: 1. Coe FL, Margolis HC, Deutsch LH, Strauss AL (1980) Miner Electrolyte Metab 3:268–275

C64. Crystalluria in Health and Idiopathic Calcium Stone Disease

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It is generally held that crystalluria represents microurolithiasis. Thus, monitoring the latter may be possible by microscopic investigation of phases such as calcium phosphate(s), calcium oxalate(s), uric acid or urate(s). We reevaluated crystalluria in male ($n = 20$) and female ($n = 20$) controls and stone patients, the latter classified as either normocalciuric (NC; $n = 40$) or hypercalciuric (I-HC; $n = 40$). Freshly voided fasting or postprandial urines were filtered at 37 °C using the Mayo Clinic technique [1], and the crystal quality was identified by petrographic microscopy. Spheroids (table) are suggestive of hydroxyapatite (anisotropic) and contrast with amorphous calcium phosphate (isotropic); uric represents birefringent crystals (uric acid or urates).

Results (see Table at the bottom of the page): Among a total of 240 filters, crystalluria was more pronounced in females (111x) than in males (86x). The most frequent phase was uric (♂ + ♀ = 131x), followed by spheroids (79), whewellite (60), and isotropic (56). However, when spheroids and isotropic were pooled, calcium-phosphate phases were most frequent (135x). Based on the score, male and female stone patients develop more spheroids and uric crystals (fasting and postprandial urine) than controls, whereas in female controls whewellite was absent.

Conclusions: (1) The predominance of uric acid and calcium-phosphatelike materials in crystals suggests that these play an initiative role in stone-forming processes, especially during fasting (males and females); (2) absence of calcium-oxalate may be characteristic for non-stone-forming females.

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C65. The Influence of Aminoacids on Nucleation, Crystal Growth, and Aggregation of Calcium Oxalate

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Glutamic acid (Glu) has been referred to as an effective modifier of calcium-oxalate crystallization while ornithine (Orn) and tryptophan (Trp) have been specifically detected in urines of stone formers. The aim of this work was to evaluate quantitatively the

	Sex	Fasting urine				Postprandial urine			
P/A[2]		Controls; 20[a]	NC; 20	I-HC; 20	all	Controls; 20	NC; 20	I-HC; 20	all
	♂	11/9	11/9	18/2	40/20	17/3	11/9	18/2	46/14
	♀	19/1	19/1	19/1	57/3	18/2	18/2	18/2	54/6
Spheroids	♂	0.05[d]	0.06	0.24**	18[c]	0.04	0.04	0.16	13[c]
Whewellite	♂	0.26	0.06	0.28	19	0.13	0.13	0.11	19
Uric	♂	0.08	0.13	0.16	22	0.11	0.06	0.20	23
Isotropic	♂	0.15	0	0.03	3	0.45	0*	0.10*	9
Spheroids	♀	0.07	0.24	0.31	25	0.08	0.11	0.18	23
Whewellite	♀	0	0.13	0.07	12	0	0.11	0.08	10
Uric	♀	0.23	0.20	0.35	45	0.30	0.16	0.24	41
Isotropic	♀	0.93	0.06	0.20	15	0.95	0.83	0.78	29

[a] Number of filters studied in each male and female; [b] ratio of filters with crystals present (P) or absent (A); [c] sum of filters (controls + NC + I-HC) with phase present; [d] mean score (2); * $P < 0.05$, ** $P < 0.001$ vs controls

influences of these aminoacids (AAs) on nucleation, crystal growth, and aggregation of calcium oxalate. Crystallization systems were prepared by mixing equal volumes of equimolar solutions of sodium oxalate (pH = 6.5, 298 K) and calcium chloride 0.3 mol dm^{-3} in sodium chloride. Solutions of different concentrations of Glu, Orn or Trp were added to the oxalate component. The mode of stirring ensured precipitation of calcium-oxalate trihydrate (COT) in the control system. Precipitation kinetics were monitored by particle-size analysis; in addition, TGA, X-ray diffraction and optical microscopy were employed. All investigated AAs inhibited precipitation at low concentrations and promoted it at high concentrations. As nucleation modifiers, they promoted the formation of calcium-oxalate monohydrate (COM) in mixtures with COT (approx. 80 wt% COT and 20 wt% COM). Quantitative evaluation of the kinetic curves shows that at low concentrations of AAs the intensity of crystal-growth inhibition increases with concentration. The specific course of the N_t vs time curves recorded in the presence of Trp indicates intensive inhibition of crystal aggregation. In contrast, Orn tended to promote aggregation of the crystals.

Conclusion: All investigated aminoacids affected nucleation, crystal growth, and aggregation of calcium oxalate. The quality and intensity of the effect dependend on the type and concentration of the aminoacid.

C66. The Influence of Additives on Nucleation and Crystal Growth of Calcium Oxalate Hydrates

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The purpose of this study was to explore the manifold influence that additives (aminoacids (AAs), surfactants, glycoproteins) can exert on the nucleation and crystal growth of calcium-oxalate hydrates. Precipitation was initiated from unseeded solutions by direct mixing of calcium chloride and sodium oxalate (pH 6.0 or 6.5) solutions, which were 0.15 or 0.3 mol dm^{-3} in NaCl. The respective additive [glutamic acid (Glu), ornithine (Orn), tryptophan (Trp), sodium dodecyl sulphate (NaDS) or Tamm Horsfall glycoprotein (THP)] was added to the oxalate solution prior to pH adjustment. Precipitation kinetics was followed by particle-size analysis or light scattering; phase analysis was performed by TGA or X-ray diffraction. It was shown that the *AAs* inhibit crystal growth at low concentrations and enhance precipitation at higher concentrations. Regardless of concentration, all AAs promoted the formation of COM in systems in which COT would normally prevail. *NaDS* inhibited crystal growth at all investigated concentrations but at and above the critical micellary concentrations, the molecule acted in addition as a nucleation modifier, e.g., promoted the formation of COD; in the control systems COM was the dominant nucleating phase. *THP* acted as an intensive crystal-growth inhibitor at low (urinary) concentrations, whereas at THP $\geqslant$ 0.05 g/l, the effect was much less pronounced. This result is in good correlation with the state of aggregation of THP as affected by the presence of NaCl and calcium ions.

Conclusion: Evidence is presented that in the precipitation of calcium oxalate, the same additive can act as (1) promoter of nucleation, (2) inhibitor of nucleation and crystal growth, and (3) modifier of the nucleating phase. The quality and intensity of the effect depend on the type of additive and its concentration.

C67. Undissociated Uric Acid in Nephrolithiasis

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It is generally accepted that hyperuricosuria is not the main cause of urinary uric acid supersaturation in uric acid nephrolithiasis (NU) where supersaturation is mainly related to a low urinary pH. However, quantitative evaluations of FUa are not readily available in patients with NU or other kinds of nephrolithiasis. Undissociated uric acid (FUa), evaluated by the Henderson-Hasselbach formula with a $pK_{Ua} = 5.345$, was significantly higher (350 ± 183) in 25 patients than in 56 controls (C: 99.9 ± 74, $P < 0.0005$); FUa was also higher than in C in 19 patients with mixed (CaOx) and Ua) nephrolithiasis (NCU, 275 ± 105, $P < 0.0005$), but not in 29 patients with CaOx nephrolithiasis (NC, 114 ± 65). FUa was higher than physicochemical formation product for Ua (>300 mg/l) in 12/25 NU and 6/19 NCU, but not in any C or NC. It was in the metastable region (100–300 mg/l) in the remaining NU and NCU, as well as in 19/56 C and in 17/29 NC. FUa was significantly correlated with urinary pH ($r = -0.78$, $P < 0.0001$) and with total uricosuria ($r = 0.65$, $P < 0.0001$) in all patients. Nevertheless, in the single groups, FUa was highly correlated with pH in C ($r = -0.82$, $P < 0.0001$, NC ($r = -0.85$, $P < 0.0001$), NCU ($r = -0.73$, $P < 0.025$), but only slightly in NU ($r = 0.48$, $P < 0.05$). In addition, FUa was correlated with uricosuria in NU ($r = 0.8$, $P < 0.001$) but not in C ($r = 0.18$), NCU ($r = 0.03$) and NC ($r = 0.12$). Urinary pH was lower than in C (6.09 ± 0.56) in NU (5.33 ± 0.27, $P < 0.0005$) and NCU (5.53 ± 0.36, $P < 0.0005$) but not in NC (5.85 ± 0.4, P = NS); total uricosuria was higher than in C (551 ± 137 mg/24 h) in NU (719 ± 345, $P < 0.05$) and NCU (749 ± 196, $P < 0.005$) but not in NC (478 ± 149, P = NS).

Conclusion: Values of FUa above the formation product are observed only in patients with NU and NCU; however, values in the metastable region are common in all NU, NCU, and C. A low urinary pH is an important determinant of supersaturation for FUa in NU and NCU, even though total Ua excretion is also an important determinant of FUa in NU. The absence of "absolute" supersaturation in some NU and NCU suggest that either promoting factor(s) may exist in the urinary tract of some patients, or that supersaturation may occur intermittently.

D. Medical Management

D1. Combined Hydrochlorothiazide and Magnesium Treatment in Calcium Oxalate Stone Formers – A Randomized Study

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A combination of thiazide and magnesium in the prophylactic treatment of calcium oxalate stone formers may result in more favorable urine composition than either drug alone. So far, no clinical results using this treatment have been reported. Fifty patients with recurrent calcium-oxalate stone formation and either high-calcium or low-magnesium excretion were randomly assigned to three groups: no medical treatment (group 1, 19 patients); treatment with hydrochlorothiazide (Esidrex), 25 mg x 2 (group 2, 15 patients); or treatment with hydrochlorothiazide, 25 mg, and magnesium-aspartate-hydrochloride-trihydrate (Magnesiocard), 1.23 g twice daily (group 3, 16 patients). The groups were stratified according to annual rate of stone formation as well as calcium and magnesium excretion. All patients were advised to increase their fluid intake and reduce oxalate intake. The patients were monitored for up to 3 years. Side effects causing withdrawal of therapy were common in groups 2 and 3. Patients with medical treatment had less stone recurrence than the control group. Serum magnesium decreased only in group 2 whereas urinary magnesium increased in group 3. Urinary calcium decreased in groups 2 and 3, and in all groups the urine volume increased. AP (CaOx) index, an estimate of the ion-activity product of calcium-oxalate in urine, decreased in

groups 1 and 3. In conclusion, thiazide or a combination of thiazide and magnesium seemed to be more effective than giving general advice on the prevention of recurrent stone formation, but at the price of considerable side effects. A combination of thiazide and magnesium resulted in uncharged serum magnesium, increased excretion and a favorable effect on the AP (CaOx) index. The effects on urine composition were more pronounced than with hydrochlorothiazide alone.

D2. Favorable Effect of Glycosaminoglycans on Cellular and Urinary Abnormalities in Idiopathic Calcium-Oxalate Nephrolithiasis

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In our experience, idiopathic calcium-oxalate nephrolithiasis is generally associated with an increase in oxalate self-exchange in red blood cells (RBC). This anomaly seems to be related to an alteration in the status of phosphorylation of the anion carrier (band-3 protein). We also have in vitro evidence that some glycosaminoglycans (GAGs), such as LMW heparin, heparan sulfate and dermatan sulfate, can inhibit band-3 phosphorylation and normalize oxalate self-exchange. Taking into account the above considerations, and in view of the possibility that the cellular transport defect could also be present in other cell lines, we set up a clinical trial to check the pharmacological effects of GAGs on lithogenic factors. To this end, we administered 60 mg by mouth of a mixture of heparan (80%) and dermatan sulfate (20%) to 40 idiopathic calcium-oxalate stone formers for 2 weeks. At the end of the treatment, we found: (1) a fall in urinary oxalate (from 29.16 ± 14.72 SD mg/day to 21.50 ± 8.23; $t = 5.64$; $P < 0.001$), as well as reduced relative supersaturation for calcium-oxalate (from 1.01 ± 0.15 to 0.90 ± 0.18; $t = 2.62$; $P < 0.02$); 2) a reduction in RBC oxalate self-exchange (from $2.68 \pm 1.64 \times 10^{-2}$ min^{-1} to 1.03 ± 0.71; $t = 6.50$; $P < 0.01$); (3) a decrease in the phosphorylation status of RBC ghosts (80.665 ± 7.026 cpm/mg protein to 62.250 ± 9.768; $s = 4.84$; $P < 0.001$). The study suggests that GAGs are capable of correcting the cellular abnormalities associated with idiopathic calcium-oxalate nephrolithiasis. Furthermore, it also suggests that GAGs are useful drugs in the prevention of calcium-oxalate nephrolithiasis.

D3. Spurious Stones

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As reports of factitious disease have increased, we reviewed the analyses of 5,565 specimens submitted as urinary tract calculi (1979–1987) for artifacts, i.e., those not formed of the accepted constituents of urinary calculi. A total of 168 artifacts (3.1%) were submitted as urinary stones by 102 patients – females twice as often as males. Laboratory diagnostic methods included infrared and wet-chemical analysis and X-ray routinely, X-ray diffractive spectrometry whenever these methods suggested a geological origin, histochemistry and microscopy when indicated, and occasionally polarizing petrographic microscopy and mass spectrometry. Forty-six artifacts were of organic origin, and many were undoubtedly submitted by accident as calculi, especially in women. Sixty-one patients submitted 109 specimens, which were of geological origin, mainly quartz (SiO_2) and/or feldspar (silicates of aluminium and other metals), which commonly occur together in granite, and 12 patients submitted 13 metallic artifacts; it is believed that the great majority were submitted for secondary gain (e.g., for drugs), or for psychiatric reasons. Eighteen patients submitted 2 to 15 artifacts. One or more artifacts were submitted by 23 patients who also submitted one or more true urinary calculi. Substance abuse may follow the pain and treatment of multiple calculi, and management decisions for such patients when they present with pain suggesting urolithiasis are difficult. Confounding specimens included: those whose composition included silicate (which may form true calculi in patients ingesting medication containing silicates), or calcium carbonate which we suggest is often an artifact; specimens of bone or teeth whose apatite is indistinguishable by the usual physical and chemical analytic methods from that found in calculi; and specimens of plant origin which contained oxalate. Artifacts, especially if multiple, suggest factitious disease, which usually requires different management. Artifacts were important in our laboratory because of their frequency and because multiple methods and a lot of laboratory time is often needed to establish the clear-cut diagnosis necessary for the physician to confront the patient confidently.

D4. Effects of Urine Composition and Crystallization of Calcium Oxalate with Different Doses of Alkaline Citrate

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The elimination of risk factors for calcium oxalate (CaOx) crystallization is of great importance in the prevention of recurrent CaOx stone formation. Attributable to a favorable effect on urinary citrate, calcium, and pH, alkaline citrate appears to be a suitable alternative in the prevention of CaOx stone formation. Four patients (2 men, 2 women) with recurrent CaOx stone disease with hypercalciuria, hypocitraturia, or raised calcium/citrate quotient were biochemically investigated. While on a standardized diet, different doses of alkaline citrate (Uralyt-U) were administered. Five urine fractions during a 24-h period were analyzed with respect to pH, crystallization risk, and relevant urine constituents for calculation of the AP (CaOx) index and calcium/citrate quotient. In addition, direct measurement of the crystallization risk (CaOx-CR) was performed. During treatment, increased pH and citrate excretion were observed, together with a reduction in calcium excretion and the calcium/citrate quotient. The risk of crystallization was slightly reduced. The effects of different doses and administration intervals of alkaline citrate on CaOx crystallization were analyzed. Our results confirm that there are favorable effects of alkaline citrate on the urine composition, as well as the risk of CaOx crystallization in patients with recurrent CaOx stone disease when hypercalciuria, hypocitraturia, or a raised calcium/citrate quotient is present.

D5. The Effect of Sulindac and Indocid R on Calcium Excretion and Vitamin D_3 in Idiopathic Urolithiasis

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Prostaglandin synthetase inhibition with NSAIDs has been shown to reduce urinary calcium excretion in idiopathic urolithiasis (Buck et al. 1981, 1983). However, reports that NSAIDs may have an adverse effect on renal function could influence the decision to use these drugs in the treatment of idiopathic stone formers. However, the drug Sulindac does not appear to inhibit endogenous renal PG synthesis. The aim of this study was to compare and

evaluate the effect of Indomethacin R and Sulindac on urinary calcium excretion.
Method: Thirty-six recurrent idiopathic stone formers were entered into a two-arm study to receive either Indomethacin R, 75 mg daily (18 patients), or Sulindac, 400 mg daily (18 patients), for 4 weeks. The following investigations were carried out before treatment, at 4 weeks and at 8 weeks: (1) 3 x 24-h urinary calcium, oxalate, phosphate and creatinine clearance (GFR), cAMP and urinary PGE2; (2) serum calcium, $1,25(OH)_2D_3$, PTH and TmPO4/GFR.
Results: With Indomethacin R, there was a significant reduction in urinary calcium excretion and serum $1,25(OH)_2D_3$ ($P < 0.01$). Urinary PGE2 fell significantly at 4 weeks and rose at 8 weeks. Urinary calcium excretion and $1,25(OH)_2D_3$ also rose significantly at 8 weeks. There was no change in GFR at 4 weeks. None of the parameters were significantly affected with Sulindac. These results suggest that the ability of NSAIDs to reduce urinary calcium excretion is dependent on renal PG synthetase inhibition. The influence of NSAIDs on vitamin D_3 levels has important implications for the management of absorptive hypercalciuria.

D6. Influence of Single-Dose Alkali-Citrate on Hypocitraturic and Hypercalciuric Healthy Persons

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The prevention of calcium-oxalate lithiasis by potassium-sodium-citrate (Oxalyt-C) is due to enhanced urinary citrate and decreased total calcium. The compliance of working patients to a certain extent depends on the daily dose and the frequency of drug intake. It whether or not was the aim of this study to elucidate a single reduced dose of Oxalyt-C can effectively correct hypocitraturia and hypercalciuria.
Methods: Twelve healthy persons began calcium loading (A) by drinking 1.5 l mineral water (578 mg Ca/day), which was continued for 3 weeks. Oxalyt-C was given for 5 days (B) in a single dose of 52 mmol base in the evening. For acid loading, 58 mmol cysteine/day was added (C) and Oxalyt-C was ingested for another 5 days (D). Urine was collected in 12-h fractions (4 samples/phase) and analyzed for pH, titrable acid, ammonium, sodium, potassium, magnesium, sulfate, total and ionized calcium, citrate, and creatinine.
Results and Conclusions: Ten persons revealed hypercalciuria above 5 mmol/day. The acid load did not increase hypercalciuria but significantly decreased citrate excretion beyond the normal level. In the nightly urinary fraction there was a highly significant increase in citrate excretion ($P < 0.001$) during Oxalyt-C intake in phase B and D. In the daily urine samples, citrate still was elevated ($P < 0.05$). The decrease in total calcium was not significant in either urinary fraction. In contrast the ionized calcium was significantly lowered during the day and night ($P < 0.01$). It can be concluded that a single daily dose of 52 mmol Oxalyt-C enhances citrate in hypocitraturic non-stone formers to normal levels with the inherent effect of a significant complex of ionized calcium. The simplified mode of drug intake definitely improves a patient's compliance.

D7. Alkalicitrate vs Diet for the Prevention of Calcium Urolithiasis

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Alkalicitrates have been introduced recently for the prevention of calcium urolithiasis. The encouraging results that are available so far rely on open long-term trials. The efficacy of this new medical treatment must still be proven by randomized trials with placebo or control groups.
Methods: In a multicenter 2-year trial 41 recurrent calcium stone formers (36 males, 5 females) were randomly assigned to a diet group and a medical group. The following selective criteria were obligatory: (1) at least two stone occurrences 2 years retrospectively; (2) hypercalciuria not exceeding 7 mmol/day; (3) no RTA; (4) no chronic urinary infection; (5) no severe hypertension. The diet rules included: mild calcium restriction, severe restriction of oxalate-rich foods and 1.5 l mineral water/day. The medical group ingested 87 mmol potassium-sodium-citrate (Oxalyt-C, 3 g tid). Urine and blood chemistry were controlled quarterly; X-ray and ultrasound examinations were performed every year.
Results and Conclusion: After 1 year of follow-up, 3 patients in the diet group had a relapse. Stone analysis revealed pure calcium oxalate. In the Oxalyt-C group, there was 1 patient with new stone formation (X-ray positive). The increase in urinary citrate excretion was highly significant ($P < 0.001$), but total calcium decreased ($P < 0.05$). In the diet group, citrate excretion remained unchanged whereas the excretion of total calcium was significantly lower compared to the pretreatment level. Urine pH and volume were significantly higher in Oxalyt-C patients. There was no influence on blood PTH levels in either group. The preliminary 1-year results seem to indicate the efficacy and superiority of Oxalyt-C for the prevention of calcium stone formation.

D8. Effects of 1-Alpha-Hydroxylated Vitamin D Metabolites on Intestinal Radiocalcium Absorption and Urinary Calcium Excretion in Short- and Long-Term Treatment of Postmenopausal Osteoporosis

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Synthetic 1-alpha-hydroxylated vitamin D metabolites, 1αOHD3 and $1,25(OH)_2D_3$, have been successfully used in the management of postmenopausal osteoporosis. Dramatic results have been obtained in terms of increases in intestinal radiocalcium absorption in short- as well as in long-term courses and, in prolonged treatments, in terms of increases in mineral bone content and decreases in the occurrence of vertebral fractures. We have studied more than 200 postmenopausal osteoporotic women. In these patients, continuous administration for periods up to 8 years of 1 μg/day of 1α-hydroxylated D metabolites in two divided doses, without oral calcium supplementation, only exceptionally resulted in transient increases in plasma calcium, but constantly produced hypercalciuria. This was not accompanied by any increase in urinary hydroxyproline excretion, so that it could be assumed that the hypercalciuria was of exclusive absorption origin and that the synthetic vitamin D metabolites did not affect bone resorption. Indeed, a significant positive correlation was observed between radiocalcium absorption and urinary calcium excretion. In our patients, renal function, as assessed by BUN and creatinine clearance determinations, was not impaired by the treatment. No patient developed urinary tract stones. The conclusion can be drawn that treatment with 1α-hydroxylated vitamin D metabolites is save, particularly with respect to renal function, and does not cause urolithiasis.

D9. Prognosis of Asymptomatic Urolithiasis

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A decision to treat an asymptomatic kidney stone by extracorporeal shock-wave lithotripsy (ESWL) *or* to intervene only when symptoms occur requires information about the clinical course of asymptomatic stones. Patients followed in the Urolithiasis Clinic at St. Joseph's Hospital between January 1984 and April 1987 were the study population. The inclusion criteria were: (1) at least one asymptomatic stone documented by X-ray or ultrasound and (2) follow-up radiologic examination. ESWL was not available in Ontario during this period, and no patient had elective removal of an asymptomatic stone. For each patient, the follow-up time was that between diagnosis and the final radiologic examination. The definition of asymptomatic stones becoming symptomatic was: (1) operative removal, including endoureteral procedures and percutaneous nephrostolithotomy (PCNL); (2) passage of a stone; (3) renal colic, without stone passage, requiring analgesia. There were 104 patients followed for 1,905 patient months, an average of 18.3 months per patient. There were 8 operative removals or 1 per 238 patient months; 38 stones passed or 1 per 50.1 patient months; 19 renal colic episodes or 1 per 100.3 patient months. All events combined were 65 or 1 per 29.3 patient months. The relatively low rate of transition from asymptomatic to symptomatic status supports, in the setting of a urolithiasis clinic, the decision to defer intervention until symptoms appear.

D10. Effect of Different Therapeutic Treatments on Urinary Saturation and Urinary Inhibitors of Calcium Salts in Patients with Calcium-Oxalate Nephrolithiasis

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The therapeutic approach to calcium-oxalate nephrolithiasis is still controversial; various medical treatments have been proposed. The aim of our study was to evaluate the effect of four different therapeutic treatments on urinary saturation and the urinary inhibitors of calcium salts in patients with calcium-oxalate nephrolithiasis. During the study, lasting 12 months, 60 patients were treated: 15 with diet + hydrotherapy (group A); 15 with diet + hydrotherapy + hydrochlorthiazide (group B); 15 with diet + hydrotherapy + allopurinol (group C); 15 with diet + hydrotherapy + hydrochlorthiazide + allipurinol (group D). (See Table below).

Thus it may be deduced that: diet + hydrotherapy is useful in decreasing calciuria and has no effect on saturation or inhibitors of crystallization; allopurinol treatment increases inhibitory activity in patients with hyperuricosuria; and hydrochlorthiazide treatment reduces calciuria, too, but has no effect on saturation or inhibitory activity.

D11. Dissolution of Uric-Acid Stones

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We prospectively attempted to dissolve asymptomatic, lucent, non- or minimally obstructive renal stones in 21 compliant patients who could be followed up at 3-month intervals. There were 11 men and 10 women. Ages ranged from 25 to 75, average 58. At the time of entry, first-morning urine pH averaged 5.24. Seventeen of 19 patients had urine pHs ≤ 5.6. After treatment consisting of increased fluids, allopurinol (20 of 21 patients, usual dose 300 mg/d), and Polycitra (21 of 21 patients, usual dose 15 ml TID), the first-morning urine pH averaged 6.18. Initial 24-h urinary uric acid excretion averaged 480 mg; after treatment 373 mg. Only 2 of 15 patients were hyperuricosuric (>750 mg) pretreatment. The 24-h urine volume averaged 1,236 ml pretreatment; at last follow-up 2,211 ml. Urine osmolality fell on the first morning urinalysis from 619 to 548. Two-dimensional stone area measured on a KUB or IVP, using a drafting planimeter, averaged 3.23 cm^2 initially in 14 patients who had at least 1 stone over 1 cm^2. Six patients had a stone area too small to measure accurately (<1 cm^2) and one had a stone visualized only by CT scan. Of patients who returned regularly for follow-up, 10 patients dissolved their stones within 3 months while 3 dissolved within 6 months. Altogether, 15 of 21 patients (71%) eventually dissolved their stones. Two of 21 patients (10%) required urgent extracorporeal shock-wave lithotripsy during treatment because of colic. One of these patients had a potassium urate fragment recovered. Four of 21 patients (19%) failed to dissolve their stones. Two received extracorporeal shock-wave lithotripsy; in 1 of these, fragments showed 50% calcium oxalate. There was no drug toxicity requiring dose reduction or discontinuation. We conclude that our study showed 71% of suitable lucent stones can be safely dissolved by oral medication, 10% may cause colic, and 19% will fail to dissolve. A major reason for treatment failure is that the lucent stone may not be entirely uric acid.

	Ca (mg/24 h)	Uric a. (mg/24 h)	Ox (mg/24 h)	Citr. (mg/24 h)	Solf. (mmol/24 h)	Mg (mg/24 h)	GAGs (mg/24 h)	AP (CaOx)	RSR (CaOx)
A	357 ± 108	765 ± 164	31 ± 9	621 ± 437	24 ± 7	148 ± 54	8 ± 2	3 ± 2.5	6.6 ± 3
A_1	252 ± 91	672 ± 163	27 ± 11	576 ± 371	23 ± 10	119 ± 43	8 ± 2	2.7 ± 1.3	7.7 ± 3.2
	$P < 0.02$	NS	NS	NS	NS	NS	NS	NS	NS
B	416 ± 87	715 ± 164	36.6 ± 19	1,037 ± 488	30.6 ± 7.0	161 ± 78	8.1 ± 4.1	3.13 ± 0.8	9.1 ± 2
B_1	258 ± 88	581 ± 202	28.6 ± 9	821 ± 410	25.6 ± 10	162 ± 91	8.3 ± 4	3.23 ± 1.7	9.5 ± 3.8
	$P < 0.001$	NS	NS	NS	NS	NS	NS	NS	NS
C	362 ± 180	889 ± 291	30 ± 11	517 ± 22	28 ± 7	149 ± 55	5.8 ± 1.4	2.85 ± 1.4	8.5 ± 3.6
C_1	244 ± 68	461 ± 104	29 ± 11	516 ± 309	24 ± 8	135 ± 44	8.2 ± 2.3	2.1 ± 1	5.3 ± 2
	$P < 0.05$	$P < 0.001$	NS	NS	NS	NS	$P < 0.05$	NS	$P < 0.05$
D	407 ± 114	931 ± 272	4.1 ± 5	581 ± 291	34 ± 12	171 ± 69	5.7 ± 2.6	2.37 ± 0.7	7.16 ± 2
D_1	279 ± 72	543 ± 140	27 ± 15	516 ± 242	31 ± 8	151 ± 54	8.8 ± 3	2.76 ± 1.3	8.2 ± 3
	$P < 0.002$	$P < 0.001$	NS	NS	NS	NS	$P < 0.05$	NS	NS

D12. Prevention of Recurrent Calcium Oxalate Calculi with Hydrochlorothiazide Combined with Amiloride

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In order to assess the effectiveness of Moduretic (hydrochlorothiazide, 50 mg, combined with amiloride, 5 mg), we measured the metabolic and clinical outcomes after short- and long-term usage. Using a crossover design, we evaluated the metabolic effects of Moduretic (MOD) versus hydrochlorothiazide (HCTZ) alone in 18 subjects who had suffered recurrent calcium-oxalate calculi. Each subject received each agent for 6 weeks in a randomized sequence. Subsequently, these subjects and 32 others were given MOD daily, up to 3 years, or until a new calculous event occurred. In the short-term study, both agents showed equipotent hypocalciuric effects; mean reductions were 68 mg for MOD and 74 mg for HCTZ. Neither treatment produced significant alterations in urinary oxalate or uric acid. During the long-term administration of MOD (27.5 months), the hypocalciuric effect persisted (67 mg, on the average). A significant reduction in urinary uric acid was observed but oxalate was unchanged. New calculous events were observed in 19 of the 50 subjects (38%). Failure of treatment was due to passage of new calculi in 37%, appearance of new calculi on X-ray in 42%, and growth of preexisting calculi in 21%. The mean failure rate was 0.17 per patient per year. The likelihood of remission was not correlated with metabolic profiles at baseline evaluation or with biochemical changes observed during treatment. The degree of protection seen with MOD is less than we and others have observed with thiazides alone but is greater than usually observed with placebo. We conclude that the addition of amiloride does not increase the hypocalciuric effects of hydrochlorothiazide and does not appear to confer any additional protection against recurrent calcium-oxalate calculi.

D13. Short-Term Effects of Low-Dose Thiazide and Amiloride Administration Compared with Potassium-Citrate Therapy in Recurrent Calcium Nephrolithiasis

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We conducted a two-period self-controlled study in 15 adult male patients with recurrent calcium nephrolithiasis (RCN). During the pretreatment period, the RCN activity was 1.43 stones per year and 2.98 stones per patient. During the first period (19 months), the patients received low doses of hydrochlorothiazide (50 mg/24 h) and amiloride (5 mg/24 h). After a short washout, all patients were treated during the second period for 21 months with potassium citrate (60–80 mEq/day).

Results: During the thiazide period, in comparison with pretreatment, the serum levels of PO_4 significantly rose from 2.94 to 3.37 mg/dl. The urine excretion of calcium fell from 301.66 to 159.13 mg/24 h, that of Mg rose from 72.4 to 91.6 mg/24 h, and those of oxalate and glycosaminoglycans were unchanged. During citrate administration, no significant change in serum biochemistry was found. The only significant modification in urine biochemistry was an obvious increase in excretion of citrate. Urine glycosaminoglycans were increased, although not significantly (from 334.5 to 346.9 μg/24 h). During both periods of treatment no patient experienced new stone formation, as evaluated by history, radiographic, and ultrasonographic examinations. We conclude that both thiazide and citrate therapy are effective in slowing the rate of recurrence of RCN. The efficacy of citrate is probably linked to its effects on urine citrate excretion, perhaps on increased excretion, and perhaps on increased excretion of other inhibitors of stone formation.

D14. Biochemical and Clinical Studies After Parathyroidectomy in Primary Hyperparathyroidism

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We present a biochemical and clinical follow-up of 56 patients (32 females and 24 males, aged 19 to 65 years) with primary hyperparathyroidism, 1 to 11 years after succesful parathyroidectomy. In none of the patients was there recurrence of renal stones or persistence of hypercalcemia. Six months after surgery, all patients had a normal mean serum of Ca, Ca^{++}, PO_4, PTH, $1,25(OH)_2D_3$, 25(OH)D, ALP and normal mean urinary excretions of PO_4, OH-proline, AMPc and oxalate. Urine calcium became normal in 36 patients, whereas 20 patients (35%) had persistent hypercalciuria (>4 mg/kg per 24 h). They were treated with a daily dose of hydrochlorothiazide (50 mg) and amiloride (5 mg) for a mean period of 6 years. In this latter group, 7 patients remained hypercalciuric despite treatment. Serum $1,25(OH)_2D_3$ levels were directly correlated with persistent calcium leak, being slightly increased in 5 of these patients; the bone mineral content was within normal limits in patients with no metabolic abnormalities after parathyroidectomy and was slightly lower in those with persistent hypercalciuria, despite thiazide administration and in nonresponder patients. A reduced rate in the recurrence of renal stones after parathyroidectomy does not necessarily mean that all biochemical abnormalities have been excluded.

D15. The Effect of Calcium Antagonists and Thiazides in an Experimental Calcium-Oxalate Stone Model

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Hypercalciuria and hyperoxaluria are important risk factors in renal stone formation. Calcium antagonists have been reported to decrease urinary calcium and oxalate excretion in humans and to prevent experimental nephrocalcinosis. We have examined the effect of verapamil (a calcium antagonist) and thiazide on calcium and oxalate metabolism in a rat model of stone formation involving vitamin D_3 and ethylene glycol (EG) administration. Forty-eight rats were randomized into four groups. All groups received ethylene glycol, 0.5% daily. Groups 2, 3 and 4 received 1-alpha vitamin D_3, 0.5 μg, by mouth every other day. Group 3 received verapamil 10 mg/kg/day i.p., and group 4 received chlorothiazide 2 mg/kg/day i.p. The results were as follows:

	GFR (ml/min)	FENa (%)	FECa (%)	FEMg (%)	Oxalate (mg/24 h)	Citrate (mg/24 h)	Hypro[a] (mg/24 h)
1	1.85	0.67	1.23	22	5.50	89	1.47
	0.04	0.01	0.11	1.2	0.25	3.5	0.04
2	1.80	0.79	4.40	34	5.98	86.3	1.28
	0.12	0.02	0.77	1.5	0.70	5.7	0.06
3	1.60	0.77	3.40*	35	3.05*	74.6	1.08
	0.15	0.04	0.42	2.3	0.21	6.7	0.05
4	1.50	0.80	3.20*	37	8.92	70.1	1.02
	0.14	0.07	0.50	2.1	0.84	12.6	0.13

(M ± SEM) * $P < 0.001$; [a] Hydroxyproline

These results show that verapamil causes a significant decrease in urinary calcium and oxalate excretion. However, no difference was observed in the radiologic findings or in the calcium or magnesium content of the kidneys. We conclude that neither thiazide nor verapamil is effective in the prevention of renal stone formation in this model.

D16. Five Years of Experience with Oxalyt C (Sodium-Potassium Citrate) in Calcium-Oxalate Stone Formers

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The objective of our study was to prove the effect of a sufficient citrate concentration in urine in order to reduce the frequency of calcium-oxalate stone formation. Oxalyt C was used as a substrate. We carried out two studies (first study over 18 months, second study over 12-months of therapy), including a total of 20 patients. Oxalyt C was given in three dosages per day, according to the optimal pH value, 6.8–7.4. Clinical, biochemical, radiographic, and sonographic examinations were performed every 3 months during therapy. Oxalyt C was well tolerated, and no major side effects were seen. Eighteen out of 20 patients remained stone-free and only one (70 stone episodes) had three stone relapses under therapy and one patient after the end of therapy. Citrate excretion increased significantly and calcium excretion did not change. A moderate increase was seen in sodium and potassium excretion. In the plasma, no significant alterations in sodium, potassium, calcium, or uric acid took place. After therapy, all patients showed up for control checkups every 3 months. Six patients (33%) had to take Oxalyt C for 1 month to compensate for renewed decrease in citrate excretion. Oxalyt C therapy can be recommended for motivated patients with a relatively high rate of stone formation as well as for patients with hypocitraturia with or without hypercalciuria. Continuously reminding the patients of their stone disease during the alkaline period leads to their acquiring better liquid-intake habits.

D17. Importance of the Time of Furosemide Administration on the Diuretic, Natriuretic, and Kaluretic Effects

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To study the effect of the time of administration on the efficacy of drug action, furosemide (0.5 mg/kg, i.v.) was administered to 31 healthy adults who were active during the day (group I) at 0700 hours (study I) and urine output during the next 2 h was collected. Urine volume, urinary sodium, potassium and creatinine were determined. After 60 hours, furosemide (0.5 mg/kg, i.v.) was administered at 1900 hours (study II) and urine output during the subsequent 2 h (1900 to 2100 hours) was collected for estimation of the same parameters. Twenty-two healthy adults, active during the day (group II), who constituted the control group, underwent study I and study II and received 2 ml of normal saline instead of furosemide. There was no significant difference in urinary volume, urinary sodium, potassium or creatinine excretion levels between study I and study II in the control subjects (group II). In contrast, administration of furosemide at 0700 hours (study I) resulted in greater diuresis (247.1 ± 202.07 ml), more marked sodium excretion (42.66 ± 29.06 mmol), increased potassium excretion (5.14 ± 4.014 mmol), and greater creatinine excretion (0.415 ± 0.414 mmol) as compared to study II. In conclusion, the diuretic, natriuretic, and kaliuretic effects of furosemide are greater when administered at 0700 h than at 1900 hours. Therefore, in clinical practice the time of administration of furosemide can be chosen so as to achieve greater diuresis or to prevent marked loss of sodium.

D18.–D21. Withdrawn

D22. Treatment of Calcium Oxalate Nephrolithiasis with Alkali Citrate – Effects on Urinary Parameters in Patients on a Standardized and Free Diet

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The long-term effects of treatment with sodium-potassium citrate (SPC; Oxalyt-C) were investigated in 23 patients suffering from recurrent calcium oxalate stone formation. The average stone formation rate was 1.6 stones per year. The baseline urinary values employed were obtained in a previous metabolic study involving patients on a free diet and a standardized diet. While the patients were maintained on the same standardized diet for 1 week, they were concurrently given daily doses of 3 x 3 g of SPC. They were then allowed a free diet for 1 year while still receiving SPC, intended to prevent stone recurrence. At regular intervals (6 times per year) the following parameters were investigated in the 24-h urine: pH, Na, K, Ca, Mg, Cl, P, SO_4, uric acid, oxalic acid, citric acid, and creatinine. Seventeen patients were included in the evaluation since 6 subjects had withdrawn from the treatment schedule after only 6 months. As examples of the results obtained, we quote here the changes observed in urinary pH, citrate excretion, and the relative supersaturation of calcium oxalate (calculated according to EQUIL). For the duration of the standardized diet, treatment led to a statistically significant rise in pH, from 6.33 ± 0.9 to 7.06 ± 0.41; throughout the administration period it remained around 7.0 – significantly higher than the original value. Citrate excretion also increased significantly during the standardized diet period from 2.75 ± 0.97 mmol/die to 5.00 ± 1.59 mmol/die and stayed above the original values throughout the administration of SPC. The treatment also brought about a significant decrease in the values for relative supersaturation while the standardized diet was being given (4.18 ± 2.4 to 2.24 ± 1.65). These constantly remained below the baseline values during the free-diet period. During the course of treatment, the spontaneous passage of a residual calculus diagnosed prior to treatment was observed in only one patient. Sonographic and X-ray examinations after 1 year showed that during administration of SPC no new stone formation had occurred. This conforms with the urinary physiological and physicochemical changes induced by the treatment.

D23. The use of K-Lyte (Potassium Citrate) in Uric-Acid Lithiasis

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In an attempt to find a satisfactory urine-alkalinizing agent that is not associated with a high sodium load, K-Lyte (potassium citrate) was evaluated with respect to its ability to raise the urine pH, its effectiveness in dissolving existing uric acid stones, and in preventing further stone formation. Eight uric acid stone formers each collected two 24-h urine samples, one while taking no medication and one while taking 74 mEq K-Lyte daily. In each fresh collection the urine pH, ammonia, titratable acid, bicarbonate and net acid excretion was measured. In all patients, there was a significant rise in urine pH and a drop in ammonia, titratable acid, and net acid excretion levels. The urine pH rose from a mean of 5.32 off medication, to 7.08 on K-Lyte. Thirteen patients were given K-Lyte in

an attempt to dissolve existing uric acid calculi in eight, and to control stone formation in patients with metabolically active uric acid lithiasis in five. Increased fluid intake was recommended in all patients, and allopurinol was added in the eight patients in whom stone dissolution was attempted. Two of the 13 patients did not tolerate the K-Lyte due to gastrointestinal side effects. Stone dissolution was complete in six of the eight patients in whom it was attempted with the duration of treatment ranging from 3–22 weeks (mean 10.2 weeks). K-Lyte was administered as the sole agent for prophylaxis against recurrent uric acid stone formation in five patients. It was not tolerated in two, and in the remaining three patients stone formation has stopped with a minimum of 2 years follow-up. K-Lyte is an effective urine-alkalinizing agent, is well tolerated, and is an effective agent in the management of uric-acid stone disease.

D24. Clinical Effects of the Prophylactic Dietary Treatment of Renal Stones in Japan

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The purpose of this study was to evaluate the effects of dietary management in patients with renal stones in Japan. From an analysis of the dietary habits and the nutritional environment affecting renal stone disease in 315 renal stone formers (243 males and 72 females), we established the following general guidelines: (1) high fluid intake, especially after dinner; (2) correction of unbalanced diets; the diet should include all kinds of food, avoiding excessive intake of meat, and vegetables should be eaten every meal; (3) food intake should be divided between three meals and excess intake at dinner should be avoided; (4) the interval from dinner until retiring should be extended. Stone recurrence in the untreated patients and the patients treated by medication was significantly decreased by adding dietary treatment. The 5-year recurrence rate and stone episode rate in male calcium stone formers were: 54.3% and 1.16 stones/year in the untreated group ($n = 289$); 71.2% (3-year recurrence rate) and 1.24 stones/year in patients on thiazide therapy ($n = 69$); 36.7% and 0.22 stones/year in patients given dietary treatment ($n = 69$), and 46.5% and 0.14 stones/year in patients on thiazide therapy plus dietary treatment ($n = 127$). The 5-year recurrence rate and stone episode rate among patients after discharge from the outpatient clinic according to the type of treatment they received as outpatients were: 28.5% and 0.18 stones/year; 57.4% and 0.25 stones/year; 9.1% and 0.09 stones/year, and 22.0% and 0.08 stones/year, respectively. From these results, we conclude that individual dietary treatment is very important in Japan, especially after discharge from the outpatient clinic.

D25. Magnesium Therapy in Calcium-Oxalate Stone Patients

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The aim of the study was to investigate the influence of magnesium therapy in Ca-oxalate patients for a period of 5 years and to determine more parameters in urine and serum 4 times yearly. Twenty patients took 2 pills 3 times daily (335 mg each) of magnesium. Only 17 of these patients could be treated and observed for the whole experimental period (48 months). The data obtained were evaluated by means of transformation with analysis of variance, followed by the Schaffee test. The effect of magnesium therapy on the parameters in serum and urine are shown. Magnesium administration had a significant effect on calcium and oxalate excretion. It was surprising that there was a change in uric acid and creatinine excretion with a very small type-I error ($P < 0.0001$) following magnesium therapy. The course of urinary excretion of calcium, magnesium, oxalate, citrate, uric acid and creatinine is demonstrated.

Conclusion: All patients were free of recidive stones under this therapy, and we suggest that the stone formation was prevented by increasing the amount of citrate. It is known for sure whether magnesium therapy has a significant effect on citrate and magnesium excretion. There seems to be a slight increase in magnesium excretion.

D26. Calcium-Oxalate Stone Therapy with Thiazide and Allopurinol – A 5-Year Study

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In order to test the effect of thiazides and allopurinol treatment of calcium-oxalate stone patients on clinical chemical parameters, 35 calcium-oxalate stone patients (20–60 years of age) were divided into two groups: (I) patients with normocalciuria and hyperuricosuria ($n = 18$) received 300 mg allopurinol daily; (II) patients with hypercalciuria and hyperuricosuria ($n = 17$) received 50 mg thiazide, 16 mmol potassium, and 300 mg allopurinol daily. There were no patients with hypercalciuria alone. Serum Na, K, Ca and creatinine and urinary Na, K, Ca, P, uric acid, Mg, citrate and oxalate were determined every 3 months over a period of 5 years. The data were evaluated by descriptive and conclusions drawn from the statistics. Results of group I: Uric acid in serum, decreased markedly in the 1st year, remained unchanged in the 2nd year, and showed a slight increase in the time following. Uric acid in the urine decreased in the first 3 months of therapy. At the end of the 5-year study, uric acid in urine was 50% of the value before therapy. No effect on Ca in the serum and urine was noted. Results of group II: After 1 year of therapy, Ca in the urine decreased to 30% of the Ca value before therapy and remained unchanged to the end of the whole period. Uric acid in the urine showed a decreasing tendency and was about 50% of the value before therapy. Uric acid in the serum decreased slightly in the first 3 months and remained unchanged. No significant alterations were found for potassium in serum, and 2 patients suffered a recurrence.

D27. Role of Tamarind and Tomatoes in Controlling Crystalluria

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Controlling the extent of crystalluria has proved to be the most important factor in stone prophylaxis. Oxalate crystalluria is the most significant factor in idiopathic calcium-oxalate stone disease, and this type of crystalluria is influenced by dietetic alterations. In this study, 25 patients with calcium-oxalate crystalluria were prescribed different dietary regimes. The early response to the various diets, in terms of the extent of crystalluria, aggregation size and clumping, were studied. Administration of tamarinds and tomatoes produced a significant reduction in the number and size of calcium-oxalate dihydrate (COD) and calcium-oxalate monohydrate (COM) crystals in short-term and long-term studies. Of the patients, 52% showed complete disappearance of the COD and COM crystals after a tamarind diet. In the rest, the mean number of crystals per high power field was reduced from 8 to 2, and this difference was statistically significant ($P < 0.001$). Aggregation and clumping were significantly reduced. The effect of tomatoes was

less remarkable. Tamarind is very rich in potassium bitartrate and tartaric acid and is a strong chelating agent, which inhibits crystallization of calcium-oxalate in the urine. Tomatoes are known to be high in citrate. A minimal elevation in the citrate content of urine produces profound inhibitor activity. It is well known that the incidence of renal calculi is lower in southern India compared to northern India. Tamarind is one of the regular constituents of the southern Indian diet. This may be responsible for the variation in the incidence of stone disease in the two regions.

D28. Comparison of Two Different Types of Diuretics in Reducing Hypercalciuria in Recurrent Stone Formers

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The efficacy of thiazide in preventing renal stones regarding the capacity to reduce urinary calcium excretion, is well known. We therefore studied the efficacy of a new type of diuretic in calcium stone formers with hypercalciuria. Forty-two outpatients (pts) suffering from calcium stones and with hypercalciruia were randomly divided up as follows: group A (21 pts) treated with butizide 5 mg + potassium-canreonate, 50 mg; group B (13 pts) treated with hydrochlorothiazide, 50 mg + amiloride, 5 mg; group C (8 pts) treated with hydrochlorothiazide, 25 mg + amiloride, 2.5 mg. The mean follow-up was 7.1 ± 4.6 months for group A, 14.5 ± 8.3 months for group B, and 16.5 ± 8.3 months for group C. The urinary excretion of calcium, phosphate, oxalate, uric acid, magnesium and citrate was measured before and during follow-up. High values of urinary calcium before treatment were present in all three groups. A significant reduction in hypercalciuria was observed after 3 months of therapy in all three groups (group A, $P < 0.001$; group B, $P < 0.001$; group C, $P < 0.005$). It persisted after 6 months of therapy. Nevertheless, a slight increase in urinary calcium was found after 1 year of therapy in groups B and C. In contrast, a steady reduction in hypercalciuria was present after 1 year of therapy in group A. The values of urinary magnesium, oxalate, and citrate did not change in any of the three groups. The reduction in stone-relapse episodes was mainly observed in group A. We also observed that the lower incidence in side effects was due to a lower dose of diuretic, which also improved on patient compliance.

D29. Uralyt-U Maintenance: Optimal Urinary pH in Patients with Cytinuria

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Cystinuria is a rare hereditary disorder involving renal tubular reabsorption of cystine and dibasic amino acids. The predominant clinical problem in these patients is urinary calculi – related to the poor solubility of cystine in acid urine. Treatment is aimed at preventing and dissolving cystine stones, which is obtained by dilution and alkalinization of the urine. However, it is difficult to achieve a constant urinary pH higher than 7.4, and large frequent doses of drugs are needed. We measured the therapeutic efficacy of Uralyt-U (hexapotassium hexasodium pentacitrate hydrate complex, each measuring spoon containing 2.5 g of the granules), in achieving the desired pH in 11 patients with cystinuria (7 males and 4 females, 10 with the familial form). The patients' ages ranged from 23 to 65 years (mean 39.4); the beginning of the clinical symptoms was usually in childhood or the teenage years (range 8 months to 35 years); all suffered from recurrent kidney stones and 9 required recurrent surgery. Ten of the patients had been treated before by alkalinizing agents, drug A (330 mg potassium citrate, 300 mg sodium citrate per tablet) or sodium bicarbonate (0.5 g/tablet). The pH of each urine sample voided was measured for 2 weeks (1 on prior treatment and 1 on Uralyt-U). Eight of the patients were hospitalized in the Metabolic Ward and 3 were examined on an outpatient basis. On a daily dose of 3–4 measuring spoons (3.7 ± 0.6, mean ± SD) of Uralyt-U, the urinary pH ranged between 7.2 and 8.0 in 7 patients and 7.0–8.0 in 4. These results were superior to those with the previous treatment in 6 patients and were similar in 4. However, on prior treatment, the patients consumed daily 16–24 tablets (18.1 ± 2.2) of drug A and 10–16 tablets (14.7 ± 4.2) of sodium bicarbonate, in 4–6 divided doses. On treatment with Uralyt-U, the variation in the patients' urinary pH was smaller, and a low urinary pH was less frequent. No clinical or biochemical side effects were recorded. Three of the patients continued on Uralyt-U treatment, 3 spoons daily for more than a year; recurrent measurements of urinary pH ranged 7.4–7.8. In conclusion: 3–4 spoons of Uralyt-U maintain urinary pH in the optimal range for patients with cystinuria with no side effects. This form of treatment, in addition to high fluid intake, is well tolerated and shows better patient compliance.

D30. The Effect of Hirudo Extract on the Adherence of Crystals of Calcium Oxalate to Injured Bladder Mucosa

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The transitional cells of the surface of normal urinary bladders secrete and bind to their surface 1 or more glycosaminoglycans whose presence prevents the adherence of crystals of calcium oxalate to the bladder mucosa. We did the current study to determine whether extract of Hirudo prevents adherence of the crystals of calcium oxalate to the injured bladder mucosa. We used an in vivo adherence assay in which rats, after their vesical mucosa had been destroyed with 0.1 *N* hydrochloric acid for 2 min and then irrigated with normal saline, were divided into three groups: group 1 received nothing but a 0.25 ml suspension of calcium oxalate; group 2 received 10 mg heparin (0.05 ml) for 2 min, which was aspirated from the bladder, and then 0.05 ml suspension of calcium oxalate was instilled; group 3 received 0.05 ml Hirudo extract for 2 min, after which a 0.25 ml suspension of calcium oxalate was instilled. Finally, the bladders of all three groups were washed with normal saline, dried in a desiccator, pulverized. The calcium level was calculated using atomic absorption spectrometry. We found that heparin significantly decreased calcium-oxalate adherence in group 2, but in group 3 almost not calcium oxalate had adherent to the injured vesival mucosa. Based on these data, we suggest that the Hirudo extract showed best antiadherence effect in this study.

D31. Relapse Rate for Calcium Stones – Long-Term Results of Pharmacological Therapy

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Long-term pharmacological therapy (hydrochlorothiazide + amyloride and, in some selected cases, allopurinol) was performed in 137 stone-formers in order to reduce the relapse rate for forming calcium stones. The patients were divided in two groups (86 and 51 cases each), despite the fact that all showed the same clinical picture. Furthermore, as our experience suggested that 3 mg/kg per day is the upper limit of calcium urinary excretion, the two main

groups were divided into four subgroups per patients with hypercalciuria (Ia and IIa) and normal calciuria (Ib and IIb). The therapy was given during three different periods: 1st (32 months): group I with diet and drugs, group II with diet; 2nd (36 months): diet alone for all patients; 3rd (17 months): same as the 1st period. At the end of the 1st period, we obtained a strong reduction in the relapse rate: from 0.85 to 0.24 stones/patient per year in group I and from 0.84 to 0.31 in group II. At the end of the 2nd period, there was no significant change in group I (from 0.24 to 0.28), while group II remained having no change (0.31). In the 3rd period, group I showed a small reduction (to 0.22), while in group II the stone incidence did not change (from 0.31 to 0.32 stones/patient per year). Analysis of our data as regards calciuria showed an apparent discrepancy: in the 1st and 3rd period of therapy, in the subgroup Ia, the calciuria decreased from 370 ± 53 to 168 ± 60 mg/day and from 326 ± 70 to 170 ± 45 mg/day ($P < 0.01$), but the incidence of relapsing stones did not significantly change. These observations suggest the best and surest therapy is a careful diet (low calcium and high water intake) in all patients; in contract, we are not sure if drug treatment, even in the presence of hypercalciuria, is really indicated.

D32. Incidence of Urinary-Tract Stones in the Veteran Population of the United States

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The geographical distribution of urinary-tract stone disease in the United States veteran population was studied using a centralized computer data base, which contains the International Classification of Disease Codes for all hospital discharges at all VA medical facilities. These data allowed for accurate mapping of the stone-discharge rate in a population at risk for urolithiasis. There are 157 VA medical centers across the U.S. which, together, have approximately 8 million hospital discharges annually. Of those patients, 98% are male and their average age is 53.4. Stone discharge rate data have been compared with two previous studies on stone incidence in the U.S. conducted in 1952 and 1974. The stone discharge rate was 7.9 ± 3.4 in 1952, 9.97 ± 2.82 in 1974, and 7.58 ± 2.01 in the current study. The southeastern states continue to have a relatively high incidence but, compared to the 1952 study, the Rocky Mountain states show a higher incidence. These data indicate that the urinary-tract stone discharge rate in the U.S. has not statistically varied over the last 34 years, but there is less regional variation in 1986 compared to the 1952 and 1974 studies. The National Veterans Administration Crystal Identification Center was established in 1983 for the characterization of all crystal-containing veteran patient samples, using high resolution X-ray powder diffraction. To date, 139 of the 157 VA Medical Centers have utilized the Center. Of the samples, 98.6% were received from males and their average age was 59.0. An analysis of the percentage of occurrence of crystalline components indicates that approximately 65% of all stones analyzed contained oxalate. Whewellite was most frequently observed (55%), followed by weddelite (34%) and apatite (23%). When normalized to the percentage of occurrence per 1,000 hospital discharges, the geographical distribution of whewellite and weddelite paralleled the overall stone-incidence distribution. Apatite was equally distributed across the U.S. The geographical incidence of struvite stones did not correlate with the location of spinal-cord injury units at VA medical centers. The distribution of uric-acid stones indicated a slightly elevated level along the eastern seaboard and a decreased level along the western seaboard. Approximately 10% of the samples analyzed came from recurrent stone formers, and approximately 60% of these recurrent stone formers had changes in the composition of their stones. An analysis of the composition of the stones from recurrent stone formers over a 5-year period suggests that, compared to the single-episode patient, the recurrent stone formers had a significant decrease in the percentage of occurrence of oxalate stones, but the percentage of phosphate stones increased. The percentage of struvite stones was slightly increased, while the percentage of uric acid stones was identical to the single-episode occurrence.

D33. The Effect of Modified Diets on Urinary Risk Factors in Kidney Stone Disease

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It has been suggested that diet therapy can manipulate the following urinary risk factors for calcium oxalate stone disease: low volumes, hypercalciuria, hyperoxaluria, and hyperuricosuria. To address this issue a two-part study was conducted. Part 1 investigated the effect of a modified fluid/calcium/oxalate/protein diet (MD) on urinary risk factors and part 2 determined if the addition of fiber (MD + F) would provide further benefit. A daily 10-g supplement of dietary fiber was supplied by two Fibermed biscuits (wheat and corn bran). Twenty-one hypercalciuric patients with calcium-oxalate stone disease were included in the study. Four-day food records (analyzed for ten nutrients) and corresponding 24-h urine collections (analyzed for volume, calcium, oxalate, and uric acid) were assessed three times for each patient: on initial visit prior to any dietary intervention, following a 3-month period on the MD, and following a 4-week period on the MD + F. There were statistically significant decreases in the dietary energy, protein, fat, calcium, sodium and oxalate, and an increase in water content following implementation of the MD. This resulted in a mean increase in 24-h urine volumes of 800 ml/day ($P < 0.001$) and a decrease in the 24-h urinary calcium and oxalate (NS). The MD + F led to an average increase in dietary fiber from 5.9 g/day to 18.1 g/day ($P < 0.0001$), an increase in dietary iron ($P < 0.0001$) and a further reduction in dietary oxalate, but no other dietary changes occurred. The corresponding urine analysis showed a *reduction in calciuria* when compared to the initial visit ($P < 0.001$) and to the period of MD alone ($P < 0.05$). The 24-h urinary oxalate decreased from an initial value of 0.48 to 0.35 mmol ($P < 0.01$), and urine volumes remained at the levels reported for the period of MD. Urinary uric acid levels were unchanged following either diet.

Conclusions: (1) The major effect of the MD was a significant increase in 24-h urine volumes with only minor improvements in calcium and oxalate excretions; (2) addition of dietary fiber (MD + F) led to significant improvements in urinary calcium; (3) repetitive dietary counselling caused a significant decrease in urinary oxalate; (4) urinary uric-acid levels did not change with dietary manipulation.

D34. In vivo Effects of Dermatan Sulphate After Intravenous Injection in CaOx Stone Formers

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Dermatan sulphate (DS) – a polysaccharide with structural features in common with heparin – has been shown to inhibit calcium-oxalate (CaOx) crystallization, as well as to be the most active known endogenous glycosaminoglycan (GAG) in normalizing the cellular abnormalities associated with idiopathic CaOx nephrolithiasis. The aim of this study was to evaluate the effects of a single 100-mg intravenous dose of DS (from porcine intestinal mucosa) on CaOx crystalluria and the relative supersaturation of the salt. Six active stone formers with high-risk crystalluria of

CaOx mono- and dihydrate participated in the study. The urine samples of each patient were collected at fixed 4-h intervals over several 24-h periods. After collection, the urine was analyzed for crystalluria as well as for calcium, oxalate, creatinine, and uric acid. In addition, the urinary recovery of DS, determined as increase of the basal GAG output, was determined. Important modifications of the crystalluria were found in the urine samples collected after about 4 h following the administration of DS and lasting a maximum of 20 h. The most striking effect was a net reduction in the dimensions of the weddellite aggregates and, to a lesser extent, of the size of whewellite single crystals. The qualitative changes were more or less marked, depending on the individual patient. On the other hand, the circadian rhythm of calcium and oxalate excretion was unaffected by the treatment. The mean urinary recovery of DS was 48.5% (SD 8.2). Since the relative saturation of urine with respect to CaOx was unchanged following treatment, it can be reasonably assumed that the effects on crystalluria are due only to DS. The long-lasting and delayed effects with respect to the urinary elimination of the less degraded DS may possibly be explained by the fraction of DS captured and slowly released by the reticuloendothelial system.

D35. Significance of Urinary Excretion of Stone Components in Long-Term Follow-Up of Calcium Stone Patients

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Sixty-five patients with a history of recurrent idiopathic calcium-oxalate calculus were followed up for more than 3 years. Preventive medication, if necessary, was started after three initial determinations of 24-h urinary excretion of calcium, uric acid, sodium, oxalate, and citrate. Thiazide was given to patients with hypercalciuria and allopurinol to those with hyperuricosuria. To reinvestigate the usefulness of most commonly measured urinary components, i.e., daily volume, calcium and uric acid in the long-term follow-up of patients, we compared data obtained in the pretreatment period extending 3 to 6 months (P1) with those after the start of treatment up to 5 years (P2).

	Calcium (mg/day ± SD)		
No. patients	Ca ⩾ 250	Ca ⩾ 250	Ca < 250
16	325 ± 51	274 ± 21	
9	287 ± 20		210 ± 23
25			
No. patients	Ca < 250	Ca ⩾ 250	Ca < 250
9	213 ± 22	268 ± 8	
31	184 ± 39		182 ± 41
40			
Period	P1	P2	
	Uric acid (mg/day ± SD)		
No patients	UA ⩾ 700	UA ⩾ 700	UA < 700
8	752 ± 22	820 ± 132	
6	776 ± 102		571 ± 97
14			
No. patients	UA < 700	UA ⩾ 700	UA < 700
15	523 ± 95	807 ± 92	
36	494 ± 97		524 ± 92
51			
Period	P1	P2	

Initially, 25 patients were assigned to hypercalciuria. In the subsequent period 16 were still hypercalciuric despite thiazide therapy, although the urinary calcium level was reduced. On the other hand, 9 patients initially normocalciuric became hypercalciuric in the follow-up period. Of the 51 with initially normal uric acid excretion, 15 developed hyperuricosuria. All of these had thiazide, and in this group were included all 4 stone recurrences noted in the follow-up. In conclusion, hypercalciuria is not always revealed by the initial three determinations of 24-h urinary excretion. Therapy with thiazide should be discontinued when the urinary calcium level is not rendered below 250 mg per day, and especially when urinary uric-acid excretion has increased.

D36. Lack of Effect of Thiazide Therapy on Serum 1,25 Dihydroxyvitamin D in Idiopathic Hypercalciuria with Kidney Calculi

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Thiazide diuretics cause a persistent reduction of urine calcium excretion in normal subjects and in patients with idiopathic hypercalciuria. They have been reported to reduce 1,25dihydroxycholecalciferol serum levels and body-calcium turnover, both found significantly increased in unselected hypercalciurics with kidney stones. The aim of the study was to assess body-calcium turnover and vitamin D status separately in each hypercalciuric subgroup, namely renal and absorptive, and to look for modifications after thiazide therapy. Total body calcium clearance was investigated by analysis of the kinetics of an i.v. calcium load in 12 hypercalciurics, 16 absorptives and 6 renals before and after 3-month thiazide therapy (hydrochlorothiazide, 50 mg, plus amiloride, 5 mg). Total body calcium clearance was higher in hypercalciurics compared to normal controls, with no difference between renals and absorptives. Renal calcium clearance accounted for a small fraction of total body calcium clearance (5% approximately). 1,25dihydroxycholecalciferol serum levels were the same among hypercalciuric subgroups (renal 36 ± 4; absorptives 34 ± 2 pg/ml). After 3-months thiazide therapy, in spite of definitely reduced fasting renal clearances, the total body calcium clearances were still significantly higher in hypercalciurics compared to normal controls. Similarly, 1,25dihydroxycholecalciferol serum levels after thiazides were virtually the same as before treatment. These data indicate that in idiopathic hypercalciuria, the lowering effect of thiazides on urine calcium does not modify either calcium handling or vitamin D status.

D37. Correlation Between Recurrence of Nephrolithiasis and Persistence of Bone Resorption in Patients with RCN on Long-Term Thiazide

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We evaluated the effects of long-term (64.6 months) hydrochlorothiazide (50 mg) and amiloride (5 mg) therapy in relation to total

and ionized calcium, $1,25(OH)_2D$, PTH, GLA, ALP, bone mineral content, urine calcium, hydroxiproline and cAMP excretion in 32 patients with active calcium nephrolithiasis (1.15 stones/patient per year). Nineteen patients were selected from a larger group because they had formed at least one new stone during treatment. These stone-recurrence patients were compared with 23 patients who had no recurrence of nephrolithiasis. At the end of the follow-up period, no significant difference in S-Ca, $S\text{-}Ca^{++}$, GLA, PTH, ALP, or $U\text{-}PO_4$ was detected between the two groups. Patients with recurrent nephrolithiasis had significantly higher serum levels of $1,25(OH)_2D$ and urine excretion of OH-proline and Ca than those found in patients without a recurrence. Drug administration did not affect the excretion of citrate, glycosaminoglycans, sulfate, uric acid, phosphate, or cAMP.

	No recurrence of NL (19 patients)	Recurrence of NL (23 patients)	t	p
$1,25(OH)_2D_3$ (pg/ml)	35.5 ± 6.1	49.4 ± 14.6	3.1	0.01
OH-Proline (mg/ml)	47.2 ± 23	78.6 ± 21.3	2.6	0.01
U-Calcium (mg/ml)	179 ± 91	264 ± 154	4.2	0.001

Although obtained in a limited group, these data seem to suggest that the rate of recurrence of NL is affected by the status of bone during treatment and by slightly higher values of $1,25(OH)_2D$. It is likely that in some patients with RCN, long-term thiazide administration is not followed by a significant inhibition of bone resorption which, in turn, may explain the recurrence of NL in this particular subgroup of patients.

D38. Assessment of the Risk Factor Index in Stone Formers

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More than 2,000 patients had ESWL treatment for kidney stones at the Hadassah lithotripter unit. The urine of 243 randomized patients was examined and their risk factor towards stone formation (discriminant index, D.I. [1]) was determined. The D.I. test measures the inhibitory potential in the urine to Ca-oxalate precipitation. Values lower than 0.66 are obtained for healthy individuals whereas values higher than 1.08 are obtained for Ca-oxalate stone formers. Out of the 243 , 220 patients (90.5%) had values higher than 0.66. Only 9.4% had normal values; 149 patients (67.7%) had values higher than 1.08. During ESWL treatment, kidney stones are disintegrated into very small particles that are washed out in the urine, so that no stones are available for chemical identification. In spite of the fact that the D.I. test was performed on stone formers, without specific chemical identification of the stone, 90.5% of the patients had positive D.I. values, inidcating a high sensitivity of the test for stone formers in general.

References: 1. Sarig S, Garti N, Azoury R, Wax Y, Perlberg S (1982) J Urol 128:645–649

D39. Differences in Urine Specimens Collected from the Bladder and from a Stone-Forming Kidney

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Nephrostomy tubes were left postoperatively in four cases. Urine specimens from the nephrostomy and bladder were analyzed for calcium, uric acid, transaminase enzymes, and amino acid. Urine volume and creatinine clearance from both kidneys were determined. The overall potential for calcium-oxalate precipitation in vitro, as quantified by the DI value, was also separately measured. The DI value was found to be in the normal range for bladder urine excreted by the unaffected kidney, only when leakage from the stone-forming kidney was completely prevented. In all cases, the urines differed with respect to enzyme activity and the relative content of glutamic acid, as expressed by the amino acid factor. The calcium stone formers had low transaminase enzyme activity in the stone-forming kidney, while the activity in the bladder urines was in the normal range. Showing an excellent correlation, the amino acid factor in the urines of the affected kidney was extremely low, whereas it was medium to high in bladder urine. The results indicate some differences in several kidney factors between the two kidneys of the same patient.

D40. Potassium Citrate as an Adjuvant Post-ESWL Therapy

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Potassium citrate has been introduced by Pak in the prevention of stone recurrences with encouraging results. Urinary citrate reduces urinary saturation of calcium oxalate and calcium phosphate salts by forming complexes with calcium and retards nucleation and growth of calcium salts. We investigated the usefulness of potassium citrate in the period of fragment elimination after ESWL. Potassium citrate was routinely given at a dosage of 20 mEq twice per day to 30 patients without infection who had had ESWL. Potassium citrates reduced the time needed for stone clearance and the incidence of "steinstrasse". These effects seem to be related to the dissolution of calcium sand.

D41. Day to Day Variation in Urine Saturation and Risk Factors of Calcium Urolithiasis

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The assessment of patients suffering from calcium renal lithiasis has traditionally been based on a single measurement of the daily excretion of factors known to influence the degree of saturation of urine with insoluble calcium salts. The aims of this study were to determine (1) the reliability of a single 24-h urine analysis in the classification of subjects ingesting a diet of their own choice, and as a corollary, if a single analysis proved to be unreliable, (2) how many analyses would have to be performed in order to be confident of a person's categorisation as normal or abnormal before instituting corrective therapy. Complete 24-h urine samples were collected on 14 consecutive days from four healthy male controls and four men who had suffered at least three documented CaOx/CaP stone episodes. Measurements were made of 24-h urinary volume and the daily excretion of calcium, oxalate, glycosaminoglycans (GAGS), and urate. Daily urinary saturation with respect to calcium oxalate was also calculated using the program EQUIL. The daily fluctuations in these factors varied markedly with each person, but were as high as 370% for urinary saturation, 236% for calcium, 50% for oxalate, 200% for GAGS, and 135% for urate. Of particular note were the variations in calcium, the parameter conventionally regarded in the

past as the most diagnostically important. In one stone former, unequivocal classification of hypercalciuria was only possible by taking the mean of the values for 3 consecutive days, while in one normal subject, the average of 6 contiguous daily readings was required to arrive at an unambiguous categorisation of normocalciuria. If published normal ranges had been used, all subjects would have been classified as hypercalciuric on a number of the 14 days. These results highlight the necessity for using locally established, rather than published normal ranges, when classifying patients and seriously question whether any diagnostic, and therefore potential therapeutic value, is to be gained from a single determination of urinary risk factors in calcium-oxalate stone disease.

D42. Use of Risk Factors in Medical Management to Reduce the Recurrence of Calcium Oxalate Kidney Stones

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Risk factors for the formation of calcium-oxalate kidney stones were evaluated in urine specimens of patients who had undergone either surgical or ultrasound treatment. Two weeks after the start of therapeutic treatment, the risk factor was determined again. The method employed was the determination of the discriminating index (DI), which measures the overall inhibitory potential to calcium-oxalate crystallization in the tested urine [1]. The DI values form a scale: numbers higher than 1.08 were determined for stone formers whereas values lower than 0.66 were obtained for healthy controls and for successfully treated stone formers. Success of treatment is defined as cessation of stone formation during follow-up periods of 1–6 years. A dynamic scheme of therapy was developed. After surgical removal or stone disintegration the patient, as a rule with high DI, was treated with one of the three drugs: thiazide, allopurinol, or phosphates. About 10 days later, the DI was tested again. If the new value was below 0.66, the treatment was continued. If not, another drug was tried. When the suitable drug for an individual patient was found, the minimal dose necessary was adjusted. Reduction in stone formation calculated for 2.6 mean years of therapy was 98.6% [2]. Risk factors may be used to adjust drugs on an individual basis and to pinpoint the patients who need more detailed study of their underlying physiological derangement.

References: 1. Sarig S, Garti N, Azoury R, Wax Y, Perlberg S (1982) J Urol 128:645–649 – 2. Perlberg S, Azoury R, Garti N, Sarig S (1985) Br J Urol 57:500–504

D43. Preventing the Recurrence of Kidney Stones with the Bran Preparation Farnolith

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In the industrialized countries, lack of roughage in diet is one of the reasons for the high incidence of kidney stones. As attempts to change eating habits are not usually very successful, a mixed preparation consisting of wheat bran and soya bran and enriched with potassium, magnesium, iron, and zinc was developed to prevent the recurrence of kidney stones. Sixty-two patients suffering from frequently recurring calcium stones took 30 g Farnolith daily for a year. In addition to serum analyses and urine analyses (with urine culture), a 24-h urine specimen was examined 6 times. Under Farnolith treatment, the recurrence rate dropped to 0.46 stones per patient compared to 2.76 stones per patient in the year preceding treatment. In 90% of the patients, kidney stones did not recur. In contrast to earlier investigations, there was no reduction in calcium and oxalic-acid excretion. There was, however, a significant increase in citrate, magnesium, and potassium excretion, and the "danger index"

$$\frac{\text{Calcium} \times \text{oxalic acid}}{\text{Magnesium} \times \text{citrate}}$$

showed an improvement, falling from 0.099 to 0.065. As there is good tolerance and an almost complete absence of side effects, in addition to good compliance and a good prophylactic effect, bran preparations should be used in future as basic prophylactics for patients suffering from urolithiasis.

D44. Medicinal Plant Agents in the Treatment and Prophylaxis against Recurrence of Urolithiasis

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Herbal tea, infusions, and medicinal plant extracts were the first effective agents in the treatment of urinary stones. Even nowadays these phytopharmaca play a major role in urolithiasis. Mixed preparations containing *Radix rubiae, Semen amneous visnagae, Herba virgaureae, Radix Taraxaci* and *Aescin* have a spasmolytic antiedematous, diuretic, and stone-corroding effect. In a multicentric study, 5,415 patients suffering from acute colic due to urolithiasis were treated with 6–10 capsules of UROL per day. Tolerance was good and 83% of the patients reported that the effect regarding relief of pain was good or even very good. The discharge rate of urinary stones was over 90% after 10 days on average. Costs were reduced by half compared to treatment with other spasmoanalgetic agents. A study investigating prophylaxis against the recurrence of calcium-oxalate stones over a period of 2 years showed that the rate of stone formation per year and patient could be reduced from 2.6 to 0.13. Urinary citrate, phosphate, and magnesium excretion increased. However, the study did not confirm a decrease in urinary calcium, which had been observed by other investigators. The benefits of long-term phytopharmacon administration depend on good patient compliance.

D45. Citrate and Urolithiasis – Preliminary Results of Treatment with Two Oral Alkali Citrates

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Urinary citrate, an established inhibitor of crystal and stone formation, has a low level in idiopathic recurrent calcium urolithiasis (RCU), but the cause is unclear. Theoretically, several factors can account for this. Among others, dietary citrate, contained especially in fruits, soft drinks etc., may be deficient; impairment of intestinal citrate absorption or endogenous production, enhanced degradation, and non-ionic back-diffusion to renal tissue from tubular lumen (owing to lower-than-normal luminal pH) can all contribute. In this presentation, the oral intake of citrate and the interactions of citrate and calcium at intestinal absorption sites will be critically evaluated. In addition, the more important data are shown from RCU patients, when participating in two identically constructed trials employing either sodium-potassium-citrate (SPC; 1, 2) or potassium-citrate (PC; 3). All examinations followed our standardized laboratory program, with patients seen before intake of the drug (Before) and on drug at follow-up periods of three (On; months 1–3) and six (On; months 1–6) months' duration. Comparative consideration of available data (table; mean values) reveals that the recommended doses (SPC: 3–4 x 3 g/day; PC: 3 x 2 g/day) result in increased urinary pH, citrate, sodium, potassium, oxalate; with SPC calcium decreases during 6 months, with PC the decline is restricted to 3 months' treatment. With SPC, not with PC, blood pressure also falls, despite the extrasodium ingested with the former.

Groups	n	24-h urine[a]						Blood pressure (mmHg)	
		pH	Cit	Ca	Na	K	Ox	Systolic	Diastolic
Before SPC	32	6.28	276	140	123	35	16	132	88 (21)
On SPC; months 1–3	31	7.02*	461*	103*	134	58*	19	134	83 (26)
On SPC; months 1–6	19	7.14*	411*	113*	149*	59*	23*	123*	82* (16)
Before PC	37	6.11	260	178	115	40	17	129	83 (37)
On PC; months 1–3	28	6.76*	390*	131*	125	68*	21	129	83 (37)
On PC; months 1–6	13	6.57*	418*	167	143*	88*	23*	126	83 (13)

[a] Excretion of substances per g creatinine (Cit, Ca, Ox, in mg; Na, K, in mM); * $P < 0.005$ vs before; *n*: Number of patients (except where indicated in parentheses)

Conclusion: Some of the observations are unexpected but may advance to the present knowledge of the pathophysiology of RCU; others reflect the lesser known mechanisms of actions exerted by these drugs.

References: 1. Schwille PO et al. (1985) Urol Res 13:157 – 2. Schwille PO et al. (1987) Urol Int 42:81(1987) – 3. Pak C. Y. C. et al. (1985) J Urol 134:11

D46. Calcium Metabolism in Normal Subjects and in Hypercalciuric Patients on Farnolith

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In the present study, Farnolith (a granular powder consisting of different dietary fibers) was given to normals ($n = 5$), to patients suffering from absorptive hypercalciuria type I ($n = 6$), and to one patient suffering from renal hypercalciuria. Farnolith binds calcium and reduces calcium absorption from the intestine. In normals, the urine and serum parameters of calcium metabolism (total and ionized calcium, parathyroid hormone, and vitamin-D metabolites) remained unchanged. In patients suffering from absorptive hypercalciuria type I a significant reduction in hypercalciuria was found; oxalic acid excretion had decreased as well. Lowered parathyroid hormone values returned to normal; vitamin-D metabolites remained unaffected. In one patient suffering from renal hypercalciuria, parathyroid hormone and 1,25-dihydroxy-vitamin-D values increased; calcium excretion did not decrease, though. Our study demonstrates that Farnolith is suitable for the treatment of absorptive hypercalciuria. Calcium homeostasis is returned to normal by Farnolith; at the same time, it does not produce secondary hyperoxaluria (e.g., sodium cellulose phosphate). Patients with primary renal calcium loss should not be treated by Farnolith.

D47. A Computerized System for the Metabolic Evaluation of Renal Lithiasis Patients

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To select the proper therapy for renal lithiasis, a correct metabolic diagnosis is obviously needed. Such a diagnosis can be formulated based on the results of a complete evaluation of mineral metabolism. As a really large number of laboratory findings must be considered, computerized handling of these data could considerably speed up the diagnostic procedure, avoiding the boring phase of mathematical counts. Our group has recently elaborated on a computerized "expert system" for automated diagnosis of mineral metabolism pathologies linked with renal lithiasis. When patients' data are inserted as they come from the laboratory, this system simultaneously shows all the pathological findings; then all possible metabolic diagnoses are automatically proposed. The physician, globally considering the clinical features and the computer suggestions, can finally formulate his/her own diagnosis, on which selective therapy can be based. The software is contained on a 360K floppy disk and can operate on IBM-compatible computers with an MS-DOS operative system. The results of 170 patients are presented.

D48. Wheat Bran in the Selective Therapy of Absorptive Hypercalciuria – A Study Performed on 18 Lithiasic Patients

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Treatment of hypercalciuria with vegetable raw fiber has been already described by several authors. However, it seems that indications for this therapy have never been really selective according to the updated classification of hypercalciuric states. A group of 18 patients with a specific diagnosis of absorptive hypercalciuria received a dietary supplement of 14 g wheat bran at the two principal meals for 90 days. A complete assessment of mineral metabolism was performed after 45 and 90 days. Mean calciuria dropped from 350 to 245 after 45 days and to 240 after 90 days ($P < 0.001$). Calcium excretion remained constant in only 2 cases. No significant variations were noted in oxalate, phosphate, or sodium excretions. A slight reduction was noted in serum iron and urinary magnesium, which could be due to the nonselective binding properties of raw fiber. Wheat bran could therefore be proposed as an interesting alternative to the other effective treatments for absorptive hypercalciuria.

D49. Pharmacological Prevention of Renal Calcium Stones After ESWL

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With the advent of extracorporeal shock-wave lithotripsy (ESWL), the need for medical treatment in the prevention of renal stones might be questioned. In order to reevaluate the matter, we surveyed the long-term results of pharmacological treatment in our stone clinic. A total of 90 patients with metabolically active idiopathic calcium stones were treated and followed for more than 2 years. This population included 19 patients with hypercalciuria, 17 patients with hypercalciuria associated with hyperuricosuria, 10 patients with isolated hyperuricosuria, and 44 patients with normal excretion of calcium and uric acid. These patients were treated with

hydrochlorothiazide plus amiloride (HCTZ) and/or allopurinol (ALP). After 2 years, new calculous events occurred in 10% of the patients who were taking HCTZ (60 pts). HCTZ was effective in both hypercalciuric and normocalciuric cases, but the combined use of HCTZ and ALP showed no substantial improvement in stone prophylaxis. Progression of stone disease occurred in 30% of the patients with isolated hyperuricosuria taking ALP alone (10 pts). In contrast, the clinical effect of ALP alone in patients without hyperuricosuria (20 pts) was disappointing (60% treatment failures). We conclude that a 70%–90% decrease in stone events may be achieved by successful selective medical treatment, which is significant even when compared to a "stone clinic effect". On this basis, the prevention of recurrent idiopathic calcium stones is still cost-effective.

D50. Extended Investigations in 800 Recurrent Stone Formers – Methods and Results

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To obtain more understanding about the pathogenesis of recurrent stone formation, we performed a detailed and standardized metabolic investigation in 800 stone formers who, for this purpose, spent 12 days as inpatients. In each patient (343 females, mean age 42.5 years, and 457 males, mean age 43.8 years), the excretion of lithogenic and inhibitory substances in 24-h urine were determined under individual and standard diets during hospitalization. In the urine we determined the following parameters: pH value, spec.-grav., volume, sodium, potassium, calcium, magnesium, phosphate, sulfate, chloride, uric acid, citric acid, oxalic acid, and creatinine. Of the 800 patients, 78.4% (F: 71.9, M: 84.8) formed calcium-oxalate stones; 15.0% F: 22.4, M: 7.6) formed struvite or calcium-phosphate stones, whereas only 3.5% (F: 3.0, M: 4.0) had uric acid and 3.2% (F: 2.7, M: 3.6) cystine stones. We set the limits of pathological excretion for hypercalciuria at > 5 mmol/day, for hypomagnesiuria at < 3 mmol/day, for hyperuricosuria at >3 mmol/day, for hyperoxaluria at >0.5 mmol/day, and for hypocitraturia at < 2 mmol/day. With respect to these limits, 68% of the male calcium-oxalate stone formers had hypercalciuria under individual diets that persisted under a standard diet in 55%; the corresponding values for female stone formers were 48% and 46%, respectively. Hypocitraturia is influenced much more by diet. This was found on individual diets in 47.5% (M) and 46.2% (F), but on a standard diet in only 17.4% (M) and 18% (F). In the calcium-loading test, 40% of the male and 63% of the female calcium-oxalate stone formers produced pathological results. In 6.4% of the patients we found primary hyperparathyroidism. With respect to calcium-oxalate stone formation, we found one or more metabolic abnormalities in 83% of the women and in 92% of the men.

Conclusion: Through a detailed and standardized metabolic investigation on an inpatient basis, most of the recurrent stone formers revealed pathogenetic causes for their stone formation, leading to an improvement in the treatment of stone recurrence.

D51. Pyridoxine in the Long-Term Follow-Up of Crystalluric Patients

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Crystalluria is identified as being concomitant with calculogenic activity in the urinary stone patient. Oxalate crystalluria is known to correspond to the extent of hyperoxaluria. In this paper, the effect of pyridoxine on the extent of oxalate crystalluria and the process of stone initiation and growth are reported. One hundred patients with calcium-oxalate stone disease and oxalate crystalluria were studied. Pyridoxine at a dose of 40 mg per day was administered orally. The immediate changes regarding the extent of oxalate crystalluria, the size, shape, aggregation and clumping characteristics of the crystals, and the patient symptoms were studied daily for a week and later at varying intervals for up to 5 years. Clinical evidence of stone activity as demonstrated radiologically, (stone growth and extent of crystalluria) were studied on a long-term basis. Calcium-oxalate dihydrate (COD) crystalluria responded better to pyridoxine treatment. The disappearance of calcium-oxalate monohydrate (COM) crystalluria was slower. Long-term pyridoxine treatment cleared up both types of crystalluria. In 4% of the patients, increased doses of pyridoxine were needed of up/to 160 mg per day. No side effects were encountered. Stoppage of the drug resulted in recurrence of crystalluria and the symptoms. An increase in the intake of water alone did not relieve crystalluria. The studies confirm the efficacy of pyridoxine in the management of type I and type II hyperoxaluria-induced stone formation in the human urinary tract.

D52. High Dietary Magnesium and Thiazide Administration in the Prevention of Calcium-Oxalate Stone Formation in an Experimental Model

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Magnesium and thiazides are known to influence calcium-oxalate stone formation, the former by inhibiting crystal formation, and the latter by reducing urinary calcium excretion. In the present experiment we studied the effect of high-dietary magnesium (Mg) and thiazide (TZ) administration in a model of calcium-oxalate stone formation in rats. Kidney stones were induced in male Wistar rats by adding 0.5% of ehtylene glycol (EG) to their drinking water, and giving 1 alpha-hydroxy vitamin D_3, 0.25 g p.o., every 2 days (D_3). Rats were divided into four groups. All groups received EG + D_3. In addition, group II received a high magnesium diet (7.0 g MG SO_4/kg) and hydrochlorothiazide, 4 mg/kg i.p. dialy (CTZ). Group III were given a high Mg diet alone (as in group II), and group IV received CTZ only as in group II. The results were as follows:

Group		GFR (ml/min)	PCa (mmol/l)	FECa (%)
I	Cont	1.89 ± 0.04	2.57 ± 0.01	2.15 ± 0.01
	Expt	2.05 ± 0.09	2.81 ± 0.03	4.24 ± 0.04
II	Cont	1.98 ± 0.05	2.54 ± 0.01	1.97 ± 0.09
	Expt	2.06 ± 0.03	2.74 ± 0.07	4.99 ± 0.10
III	Cont	1.98 ± 0.05	2.56 ± 0.03	2.18 ± 0.16
	Expt	1.99 ± 0.11	2.84 ± 0.4	6.98 ± 0.20
IV	Cont	2.01 ± 0.04	2.58 ± 0.02	2.33 ± 0.09
	Expt	2.22 ± 0.12	2.88 ± 0.03	3.80 ± 0.05*
		PMg (mmol/l)	FEmg (%)	Oxalate (mg/24 h)
I	Cont	0.98 ± 0.01	23 ± 1	1.74 ± 0.05
	Expt	1.05. ± 0.03	25 ± 2	7.56 ± 1.04
II	Cont	0.97 ± 0.01	24 ± 1	1.71 ± 0.04
	Expt	1.19 ± 0.04	34 ± 2*	8.13 ± 0.76
III	Cont	9.99 ± 0.01	23 ± 2	1.77 ± 0.06
	Expt	1.20 ± 2.05	35 ± 2*	7.13 ± 0.76
IV	Cont	1.00 ± 0.01	24 ± 1	1.69 ± 0.04
	Expt	1.01 ± 0.03	25 ± 2	9.81 ± 0.75

A significant decrease in urinary Ca excretion was observed in the thiazide group (IV), and a significant increase in urinary Mg was noted in groups III and II as a result of the high dietary Mg. Renal stones were presented in all the groups, even though the amount of vitamin D given to the rats was less than in the original model. This model appears to be too sensitive to evaluate potential therapies. Therefore, a better experimental model is desirable.

E. Urological and Radiological Management

E1. Percutaneous Stone Removal Using a Combined Antegrade-Retrograde Access Technique

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Seven patients with renal calculi underwent percutaneous stone extraction using a new access technique. With the patient in lithotomy position, a 7 Fr end-hole ureteral catheter is advanced cystoscopically into the kidney and fluoroscopically manipulated into a preselected calyx, urins an 0.035-inch Bentson or torque wire. The wire is then exchanged for a wire basket, a Foley catheter placed, and the patient is placed in prone position. A modified blunt 18-gauge splenic needle is then used to perform the puncture fluoroscopically. The needle is advanced into the renal parenchyma just short of the targeted calyx and the obturator is removed. The previously positioned ureteral catheter is withdrawn, allowing the basket to form in the distal calyx. Next, a 22-gauge Hawkins needle guide is inserted through the 18-gauge needle and projected through the basket. The 21-gauge needle obturator is exchanged for an 0.018-inch J wire, which is advanced out past the needle tip. The 21-gauge needle is then retracted, leaving the guide wire in place. The ureteral catheter is advanced out over the basket, thus impaling the 0.018-inch guide wire. Subsequently, the basket and guide wire are retracted into the ureteral catheter, thus securing the tract. Tract dilation is performed in standard fashion. Using this method, two middle, three upper, and four lower calyceal targeted stones were removed successfully. One attempt to gain access failed when a stone impacted in an infundibulum prevented catheter manipulation into a stone-bearing upper calyx. Of all attempts at tract placement, 89% were successful and 89% of targeted stones were removed. In one case, bleeding that did not require transfusion prevented extraction at the time of tract placement; the stone was successfully removed 2 weeks later following clot dissolution. No other complications were observed. We conclude that this new combined, retrograde-antegrade access technique is a reasonable alternative to other methods and offers the advantage of tract placement at angles that may not otherwise be obtainable.

E2. "Lithostar" Extracorporeal Shock-Wave Lithotripsy (ESWL) Results with In Situ Upper and Lower Ureteral Calculi – Controversies and Current Recommendations

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Initial reports of treatment of ureteral calculi with the Dornier HM-3 waterbath lithotripter reported stone-free rates as high as 95%. Subsequent series suggested have had less success. Many series have emphasized the importance of ureteral stone manipulation and/or ureteral stenting as a prelude to treatment in all patients regardless of the presence of obstruction. This was easily undertaken prior to ESWL under the same general or spinal/epidural anaesthetic. With ESWL technology requiring only intravenous sedation or infiltration of local anaesthetic, the stone manipulation becomes the most uncomfortable part of the procedure for the patients. A preliminary review of the results of the first 28 ureteral stones treated in situ with 3-month follow-up revealed an overall success rate (stone free) of 75%. Further analysis showed that all patients treated without stents (7) had successful outcomes. The 5 complete failures were all in the stented group. In the absence of prospective or randomized studies, stone breakage rates may not be significantly different between comparable stented and unstented ureteral stones. Rather, factors such as the degree of impaction may be more important. With ESWL technology requiring little or no anaesthesia, treatment of ureteral stones in situ without stenting may be the best initial intervention.

E3. Piezoelectric Shock-Wave Lithotripsy – Experimental and Clinical Results

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The piezoelectric shock-wave lithotripter (Wolf Co.) was developed at the Urological University Clinic in Homburg/Saar and has been in clinical use since 1986. Animal experiments with dogs ($n = 14$) showed perfect stone disintegration without harm to the kidneys. Due to the small focus area, the procedure can be carried out without anesthesia and is free of pain. Maintenance costs are extremely low. Stone location by ultrasound is highly reliable and does not harm the patient or medical staff. Piezolith 2200 as well as the new model with two ultrasound probes (Piezolith 2300) permit stone location even with problem stones: calculi in gross obesity and ureteral stones. It is especially suitable in children. Up to October 1987, 797 patients with urinary calculi in 833 renal units had been treated: 212 pelvic and 480 caliceal stones, 64 staghorn, and 77 ureteral stones. With increasing stone size, the number of shock waves per session increases as well as the total number of sessions needed. It is a requirement in our clinic that stones above 1 cm are treated by ureteral stenting before shock-wave. Piezoelectric shock-wave lithotripsy has proven to be a very efficient method for stone disintegration in experimental and clinical studies. It is the method of choice in *adults and children.* Urinary calculi as well as gallbladder stones may be treated by the same machine. It is really a second-generation method and represents advancement in the urologist's equipment.

E4. In Vitro Dissolution of Infection-Induced Urinary Calculi

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Dissolution of infection-induced urinary calculi (struvite and carbonate apatite) was studied in vitro at pH 4.0. Whole calculi were embedded in a stainless steel die and then placed in a standardized dissolution apparatus that provided a constant surface for release. Temperature and stir speed were kept constant throughout each experiment. Rate constants were calculated by measuring the concentration of calcium released into the solution during dissolution. Various irrigating agents were tested, including 10% Renacidin, 2% acetylcysteine, Urologic G solution, and a combination of Renacidin and acetylcysteine. 10% Renacidin + 2% acetylcysteine was the most effective agent in dissolving infection-induced calculi ($r = 36.1$ mg/cm^2 per h). Urologic G solution and 10% Renacidin were equally effective in promoting calculus dissolution ($r = 26.6$ and 27.1 mg/cm^2 per h). 2% Acetylcysteine alone was relatively ineffective as a dissolution agent ($r = 13.6$ mg/cm^2 per h). Infection-induced urinary calculi are amenable to dissolution by irrigation techniques. Renacidin and Urologic G appear to be equally effective

in their ability to dissolve calculi. If Renacidin is to be used in the clinical setting, the addition of 2% acetylcysteine enhances calculus dissolution in vitro.

E5. Nonoperative Treatment of Staghorn Calculi

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Thirty-four patients with staghorn calculi were treated without open surgery. In group I, 16 patients underwent percutaneous lithotripsy alone. The success rate was 85.7% In group II, 4 patients underwent extracorporeal shock-wave lithotripsy (ESWL), but with only a success rate of 75%. In group III, 14 patients had debulking of calculi percutaneously prior to ESWL. Most of these kidneys had complex branch calculi. Complete stone removal was possible in 82% of cases. There was a marked difference in hospital stay ranging from 11.1 days for group I, 4.8 days for group II to 9.0 days for group III. In contrast to open nephrolithotomy, group II and III patients required more than two procedures (2.3 and 2.8, respectively) to accomplish a stone-free status. For patients treated with ESWL alone, the morbidity seemed to be higher due to the large number of fragments that had to be evacuated and, hence, obstruction was quite common. Uric-acid staghorn stones can be easily treated with ESWL combined with post-treatment sodium bicarbonate irrigation of the collecting system. As long as there was a large surface area exposed to an alkaline medium after shock-wave treatment, dissolution occurred rapidly. Since cystine stones did not fragment easily with ESWL, percutaneous lithotripsy was still the mainstay of treatment. Complications were uncommon in spite of the fact that almost one-third of the stones were struvite. Using a combination of percutaneous lithotripsy and ESWL, most staghorn calculi can be treated successfully with minimal patient morbidity.

E6. Analysis of Kidney-Stone Fragments Recovered from Patients after Shock-Wave Lithotripsy

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Since different stone compositions stem from different etiologies, successful prophylactic regimens to prevent stone recurrence depends on the definitive analysis of stones. In Toronto, analysis of kidney-stone fragments recovered from patients after extracorporal shock-wave lithotripsy (ESWL) involves both X-ray diffraction (XRD; for crystal phase identification) and analytical scanning and transmission electron microscopies (SEM and TEM; for elemental analysis and morphology). Routine XRD showed that most fragments are from mixed stones with various amounts of calcium-oxalate monohydrate (COM), dihydrate (COD) and apatite (Ap). Of particular interest is that Ap became more readily detected and some Ap gave much sharper XRD patterns than commonly observed in non-ESWL stones. Brushite, struvite, uric acid, uric acid dihydrate, ammonium acid urate, and cystine were also observed. Complicated mixtures (up to four phases), factitious stones (e.g., aquarium stones) and non-crystalline materials (e.g., proteinaceous precipitates) required the assistance of analytical SEM and TEM. Morphological observations under SEM showed some very interesting features: (1) some COD crystals were severely etched probably due to urine dissolution; (2) most COM fragments had intact crystal surfaces; (3) Ap fragments with diffuse XRD patterns had the common spherulitic appearance; (4) Ap fragments with sharp XRD patterns showed geodelike structures with large platelike crystals; a possible cause for the change in morphology and crystallinity could be the prolonged exposure to urine after ESWL. We concluded that both XRD and analytical EM are required for a complete analysis of urinary stones. Also, our results showed that after ESWL, the exposed mineral phases became more susceptible to urine dissolution or modification.

E7. Successful Concept for Anesthesia in Extracorporal Shock-Wave Lithotripsy

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For extracorporal shock-wave lithotripsy (ESWL), in general we apply a peridural anesthesia using a catheter. Up to now we have had experience in approximately 4,000 cases and favor this method because 90% of our patients are treated on an outpatient basis and transported back to the referring institutions after treatment. Neither side effects nor severe complications were observed. In order to use the machine up to its maximal capacity we checked on several drugs with respect to onset of reaction, analgesia and motoric block (drugs: Bupivacaine 0.5%-CO_2; Bupivacaine-HCL 0.5%; BU, Prilocaine 1.5%, P). In addition, hemodynamics and plasma levels were registered. The catheter was usually inserted 7–8 cm into the peridural space (level of puncture: L 3–4). The first onset of reaction using BU-CO_2 was seen 3 min 45 s after injection; extension generally reached D 7–8; motoric block score was Bromage 0–1. All patients were able to climb onto the carriage and did not require much help from personnel. Using BU, the onset was prolonged; Prilocaine caused a complete motoric bloc (Bromage 2). Anesthesia was excellent for each drug. The highest plasma levels for BU-CO_2 (0.4 μg/ml) were measured after 15 min. For patients suffering from spine diseases we used an intracutaneous local anesthetic administered to the broad kidney area (diameter 12–15 cm; Prilocaine 1.5%). This method was applied in 50 patients; in 49 cases excellent analgesia was achieved.

E8. Statistics of Stone Analysis in Childhood Urolithiasis

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Urolithiasis in children must be distinguished from urolithiasis in adults with regard to the composition of the urinary calculi as well as the cause. The material surveyed included 31261 urinary calculi, which were predominantly analyzed on the basis of X-ray diffraction regarding the qualitative and quantitative composition of their phases. Included were 383 urinary stones (1.22%) from children; 146 urinary stones were produced by girls and 237 by boys. More than half of the urinary calculi consisted of two components: about 30% of one component and about 15% of all three components. Whewellite, weddelite, apatite and struvite were, in this order, the crystal types found most often. In adults, uric acid and uric-acid dihydrate occurred more often than in children, in whom ammonium hydrogen urate and sodium hydrogen urate were found more frequently. Urinary calculi caused by an infection played a bigger role in the case of children than in adults. The older the children, the more the formation of infection stones decreased, whereas the rate of oxalate stones increased. Girls formed oxalates more often and boys more urinary stones caused by infection. In contrast, in the adult the picture is totally different. Even the location was different in boys, girls, and adults.

E9. A Scanning Electron Microscopic Study of Renal Stone Particles from Extracorporeal Shock-Wave Lithotripsy (ESWL) Treatment

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Particles in the urine of patients who had ESWL treatment were examined by scanning electron microscopy (SEM). The particles were identified by chemical and infrared analysis and by electron microprobe. Their morphology was also identified. Similar renal calculi were treated outside the body by ESWL and the results compared. Calcium-oxalate monohydrate stone cleaved into individual or clumps of crystals. Uric acid and urate stone broke into coarse fragments (3–5 mm), while laminations of calcium-oxalate monohydrate and uric acid or phosphate stones fragmented across the laminations (2–3 mm). The struvite and calcium phosphate broke into fine amorphous clumps of crystals. Cystine stone fragmented poorly. The in vitro and in vivo results were similar. The manner in which each urinary stone fragmented could be used to predict the size of the particles to be passed, which is of direct concern for clinical management both pre- and post-ESWL. This knowledge indicated the power required of the shock waves and the duration of the ESWL treatment.

E10. Extracorporeal Shock-Wave Lithotripsy of Ureteral Calculi

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From August 1987 to January 1988, 92 cases of ureteral calculi were treated in situ by ESWL. The cases could be classified into 47 in the upper third, 12 in the middle third above the pelvic brim, and 33 cases in the distal third of the ureters. The lithotripter used was the LT-01 by EDAP with an ultrasound guiding system and piezoelectric wave. For upper ureteric stones, the patients who received at most 50 mg of intramuscular pethidine lay supine or on their ipsilateral side; for distal ureteral stones, the patients lay prone over the generator bag. To obtain the best results, the patients must be well hydrated and drinking about 1 l of fluids before the treatment. Stone localization was in most cases good. The success rates were 95% in upper ureteric stones and 94% in lower ureteric stones. The technical aspects will be discussed.

E11. Technical Modifications in the Dornier HM3 – Preliminary Results from a Single Center

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Three technical modifications have been introduced into the Dornier HM3 since October 1986: (1) a low-pressure generator, reducing the pressure in the second focus by 30% (n = 149 patients); (2) a modified semiellipsoid with an increased aperture (17.2 cm) and altered geometric dimensions (n = 109); the twin-pulse technique in which two impulses are synchronized with one R-wave (n = 120). The data on 390 patients with a total of 489 treatments were evaluated. Since October 1986, our anesthesia technique has consisted exclusively of oral medication: an antianxiety drug given the night before and an analgesic (Tilidin-Naloxon) administered p.o. 45 min before treatment. The success rate of this medication is 94%. The data demonstrate that considerable pain reduction was achieved. Our preliminary experience with the Dornier Lithotriptor MFL 5000 will be presented (in cooperation with Dornier/Philips).

	1 old generator	2 low-pressure generator
Preoperative adjunct procedure (%)	22.9	28.9
Secondary and multitreatments	8.1	18.1
Mean no. of impulses	1,370	2,300
Mean operative time (min)	24	50
Postoperative ancillary procedure (%)	15.4	18.1
Successful stone disintegration (%)	93.0	96.0
	3 2 + new semiellipsoid	4 3 + twin-pulse technique
Preoperative adjunct procedure (%)	20.9	49.0
Secondary and multitreatments	15.5	19.7
Mean no. of impulses	1,650	2,100
Mean operative time (min)	28	18
Postoperative ancillary procedure (%)	11.9	14.3
Successful stone disintegration (%)	94.5	95.6

E12. Effect of High-Energy Shock-Waves on Bony Tissue

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In order to evaluate the effect of high-energy shock waves (HESW) on bony tissue, 24 rabbits were treated in the Dornier lithotripter HM3. The animals were exposed to 1,500 shocks each on the left anterior iliac spine and left distal femur. In 12 animals each, a generator voltage of 25 kV and 20 kV, respectively, was used. An equal number of rabbits was killed after 48 h, two weeks, and three weeks. Macro- and microscopic analyses were performed on bones, the overlying muscles, and the rectum. Fifty percent of the animals revealed mucosal and intramural rectal lesions (bleeding and ulcers), partly in a segmental pattern. These changes could also be confirmed via histology. In the 48-h group, intra- and intermuscular hematomas were found in all animals that occasionally healed with metaplastic cartilage formation. The surfaces of the bones exposed to HESW showed subperiosteal petechial bleeding, but were macroscopically unremarkable. On histology, however, after 2 and 3 weeks, aseptic bone-marrow necrosis, damage of osteocytes, and bone remodelling with incipient callus formation were found in all animals of the 25-kV group. The changes were most distinct on the shock-wave outlet side. Only minor histopathological changes were encountered in the 20-kV group. These experimental findings suggest that bony tissue reacts to HESW with the formation of new bone in a generator-voltage dependent manner. No pathological changes in the growth plate were encountered; however, there was no direct focus on this area.

E13. Extracorporeal Shock-Wave Lithotripsy (ESWL) – Increased Risk for Hematoma Formation by Acetylsalycic acid (ASA)?

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Subcapsular or perirenal hematomas were encountered in 0.3% of 4,000 patients treated with ESWL. The pathogenesis remains unclear. Clinical observations suggest an increased incidence in pa-

tients taking ASA. In an experimental lithotriptor made by Dornier, the left kidney of male Wistar rats was exposed to high-energy shock waves with 500 impulses and a generator voltage of 18 kV. For kidney location, a laser system was used. Barbiturates were used for the anesthesia. Twenty-four animals were examined: group 1: control group, no ASA; group 2: 1.5 mg ASA/kg body weight; group 3: 4.5 mg ASA/kg body weight; group 4: 15 mg ASA/kg body weight. In each group, 2 animals each were killed immediately as well as after 24 h and 7 days. Histological evaluation was performed with conventional light microscopy, transmission and electron microscopy. On systematic examination, glomerular and tubular changes were observed. Glomerular and perivascular bleeding was seen in medullary vessels. On transmission electron microscopy, the tubular epithelium displayed cytoplasmatic changes indicating degenerative processes. The incidence of perivascular bleeding and hematoma formation was not significantly different in the control and treatment groups and was independent of the ASA dosage used.

E14. Treatment of Ureteral Calculi with Anesthesia-Free ESWL

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There is an ongoing discussion on the management of ureteral calculi: in many centers, retrograde manipulation of stones in the upper ureter is routine prior to ESWL, whereas some clinics, including ours, prefer in situ treatment. With the "sitting" positioning technique, ESWL has become an alternative to ureteroscopy in the treatment of distal ureteral calculi; stones in projection over the sacroiilial joint can also be treated in situ with the patient in a prone ppsition. With the modified Dornier HM-3 Lithotripter that has been available in our department since May 1987, ESWL can now be performed without anesthesia in the majority of patients. We consider this new development to be an additional argument for in situ ESWL for ureteral calculi, as transurethral stone manipulation without anesthesia may cause considerable discomfort, particularly in male patients. To evaluate the efficacy of the modified lithotripter for the management of ureteral calculi, the results of patients treated before and after the introduction of anesthesia-free shock-wave lithotripsy were compared.

	Distal calculi		Proximal calculi	
No. of patients	41	64	51	74
Anesthesia[a]	38 (97%)	6 (9%)	49 (96%)	10 (13%)
(Session 1)				1)
Success: session 1	29 (71%)	47 (73%)	35 (68%)	56 (76%)
(In situ)				
Success: session 2	10 (24%)	11 (17%)	5 (10%)	6 (8%)
(In situ)				
Success: session 2	–	–	10 (20%)	10 (13%)
(Manipulation)				
Ureteroscopy	2 (5%)	6 (10%)	1 (2%)	2 (3%)

[a] Epidural or general anesthesia

The results indicate that there is no significant decrease in efficacy with the new system. As patient acceptance is high for anesthesia-free ESWL, we consider the primary "in situ approach" for ureteral calculi even more preferable.

E15. Clinical Experience with Intracorporeal Laser-Induced Shock-Wave Lithotripsy

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A Q-switched Nd-YAG laser (λ = 1,064 nm) with 8-ns pulse duration, up to 180 mJ pulse energy, and up to 50 Hz repetition rate is used for intracorporeal laser-induced shock-wave lithotripsy (LISL). The laser energy is coupled into highly flexible, thin quartz fibers (600, 400, or 200 μm), and the laser beam is focused at the fiber tip (20–70 mJ single-pulse energy).

Biologic effects: Direct irradiation of the kidney, pelvis, ureter, and bladder in pigs only caused a small rupture cone within the tissue (max. 40 μm depth). No necrosis or thermic effects could be observed.

Patient treatment: 41 patients with 43 calculi (ureteric, n = 39; kidney, n = 4) have been treated since June 1987. Only stones that did not pass spontaneously out of the ureter and stones not suitable for ESWL treatment were operated on with the rigid ureteroscope. The thin laser fiber was passed through the instrument and the stone irradiated under constant vision using an irrigation solution. Thirty-four ureteric calculi could be completely fragmented; 6 other stones were reduced to a size small enough to be taken out by forceps. Only 3 stones composed of very hard amorphous calcium-oxalate monohydrate could not be disintegrated. Laser-stone disintegration was done within 20-s to 5-min irradiation time (1,000–15,000 pulses), with an average of 25 min for the whole operation. Optimal conditions for stone fragmentation were recorded with 8 ns pulse duration, 35–45 mJ single-pulse energy at the fiber tip and 40–50 Hz repetition rate. (600 μm fiber). No harm was done to the ureteral wall by inadvertent laser irradiation into the urothelium (2 cases) or manipulation with the ureteroscope or the laser fiber itself. The patient was free of stones immediately following laser-stone disintegration, as the stone particles created were extremely tiny (less than 1 mm or stone powder) and flushed out through the instrument. Even extremely hard stones (calc-oxalate-mono-hydrate calculi), which can be fragmented only with difficulty using other methods (ultrasound, ESWL), could be disintegrated within a short time. Laser-stone disintegration with the ns Nd-YAG laser proved to be an effective and secure one-step procedure – no removal of the instrument from the ureteral orifice is necessary. The next step will be the use of LISL in combination with small flexible endoscopes.

E16. ESWL with Siemens "Lithostar" – The Manchester Experience

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The safety and effectiveness of the first-generation Dornier HM3 lithotripsy have been well established. Siemens "Lithostar" is a second-generation lithotriptor, which produces shock-waves by electromagnetic induction in a water-filled tube, thereby avoiding the need for a water bath. Stone localization is achieved by biplane X-rays. The "Lithostar" has been in operation in Manchester since June 1987, serving a population of over 15 million. In the first 6 months, 550 patients have been treated. (85% of the treatments were given under local infiltration anaesthesia (20 ml 0.5% Lignocaine or Marcain subcutaneously). General or regional anaesthesia was used only where a simultaneous endoscopic procedure, such as placement of a Double "J" ureteric stent, was performed. All types of stones were treated; stones in the ureter were successfully treated in situ and very large staghorn and partial staghorn stones were subjected to ESWL after preliminary percutaneous debulking. The

crude retreatment rate (stones of all types including bilateral and multiple sites) was 1.4 treatments per patient. The retreatment rate for solitary stones was only 15%. Our overall experience with this versatile machine will be discussed.

E17. Ureteric Stones: the Choice of Treatment

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The advent of extra corporeal shock-wave lithotripsy (ESWL) has revolutionized the treatment of renal stones. Until now, ureteric stones have been managed by ureteroscopy or by pushing the stone back into the kidney and then extracting it percutaneously or disintegrating it by ESWL. Ureteroscopy is not always successful and has a high complication rate in inexperienced hands. Pushing the stone back into the kidney requires general or regional anaesthesia. It is possible to disintegrate ureteric stones in situ by using the Siemens "Lithostar" Lithotriptor. Ninety patients with a total of 91 ureteric stones have been treated (63 males and 27 females with a mean age of 47 years). The stone size ranged from 4 to 40 mm (mean 10.5). Sixty-three stones were in the upper ureter, 6 in the middle ureter and 22 in the lower ureter. In each case, in situ ESWL was used as the first line of treatment. The treatment was given on a Siemens Lithostar under local infiltration anaesthesia. In 71 patients (78%), the disintegration was good after 1 session of ESWL; 19 (22%) needed 2 sessions; 2 patients required ureteroscopy and electrohydraulic disintegration after two failed ESWL treatments. To date, follow-up data are available on 34 patients, 30 of whom (88%) became stone free after treatment. We conclude that in situ ESWL should be the first line of treatment for ureteric stones. Other techniques should be reserved for stones that resist in situ ESWL.

E18. Thoracoscopy as an Aid to Safer Intercostal Percutaneous Nephrostomy

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Percutaneous access to the upper or middle calyces of a high-lying kidney may be necessary on occasion. Indications for this approach include upper-pole calculi with infundibular stenosis, failed ESWL for upper calyceal stones, especially cystine stones, endopyelotomy for UP junction obstruction in a kidney fixed by scar tissue from previous surgery, or occasionally antegrade ureteroscopy with the rigid ureteroscope. A supracostal puncture not uncommonly passes through the pleural cavity and, although complications are uncommon, they may be significant. A simultaneous thoracoscopy with a flexible nephroscope will allow visualization of any pleural puncture during percutaneous nephrostomy, and in this way injury of the lung can be avoided or a lower puncture site may be chosen in order to avoid traversing the pleura. Thoracoscopy (pleuroscopy) is a well-established diagnostic procedure performed by chest physicians and thoracic surgeons with minimal morbidity, and the insertion of a flexible scope is no more complicated than the insertion of a chest tube. We have now performed this procedure on eight patients, three with punctures above the 11th rib and five above the 12th rib. Seven of the nephrostomy tracts passed through the pleura and diaphragm with no complications; one passed below the level of the pleura. Two cases had transient, small pleural effusions which settled spontaneously. Intercostal nephrostomies tend to cause more pain, but the added thoracoscopy did not increase the morbidity in any case and protected against inadvertent injury to the lung.

E19. The Blood Pressure after Percutaneous Nephrolithotomy

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Ninety-four patients who underwent percutaneous nephrolithotomy with a minimum of 2 years' follow-up were reviewed. Preoperatively, 17 patients had a history of hypertension and 13 were taking antihypertensive medication. In addition, two patients were taking thiazides for control of stone disease. The mean blood pressure for whole patient group preoperatively was 97.2 mmHg, and this rose to 98.1 mmHg postoperatively ($P > 0.1$ NS). The blood pressure dropped postoperatively in 46 patients, 8 of whom started antihypertensive medication or thiazides for control of stone disease postoperatively. Postoperatively, 16 patients had systolic hypertension with a pressure of > 140 mmHg, 7 patients had a diastolic blood pressure of > 90 mmHg, and 8 patients had both systolic and diastolic hypertension. Of these 31 patients, 10 had a history of hypertension preoperatively; a further 5 had a drop in mean blood pressure postoperatively without starting antihypertensive medication; 8 of the remaining 16 patients had a preoperative systolic blood pressure greater than 140 mmHg or a diastolic blood pressure greater than 90 mmHg, or both. Two of the remaining 8 patients had urinary diversions and associated chronic pyelonephritis. In the remaining 6 patients no obvious predisposing cause for the increase in blood pressure could be identified. In addition, 5 patients were started on antihypertensive medications postoperatively. In 3 of these patients the preoperative blood pressure was normal. Nine of 94 patients (9.57%) have demonstrated an unexplained rise in blood pressure following percutaneous nephrolithotomy to levels ordinarily requiring drug therapy.

E20. Evaluation of Retrograde Nephrostomy in over 200 Procedures

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Retrograde techniques have been evaluated to establish their role in establishing a percutaneous nephrostomy tract for stone removal. A nephrostomy tract is established by passing a wire from the renal pelvis to the outside. Using retrograde techniques, we attempted to establish a percutaneous nephrostomy tract in 189 consecutive patients. Ten patients had bilateral nephrostomies established, and 7 patients had 2 nephrostomies established in the same side for a total of 206 attempted procedures. A nephrostomy tract was successfully established in 201 of 206 attempts for a success rate of 97.6%. Four failures were due to inability to pass a guide-wire into the renal pelvis, in two due to excessive tortuosity of the upper ureter, in two due to impacted calculi in the upper ureter, and one failure was due to excessive perirenal fibrosis related to previous renal surgery. The mean procedure time for all successful attempts was 27.8 min with a range from 10–195 min and a median of 22 min. The mean fluoroscopy time was 1.59 min. One patient sustained a perforation of the descending colon and in 2 patients intraperitoneal leakage of contrast was identified on postoperative nephrostograms. The Lawson system was tried first in all procedures and was successful in 178 of 201 successful attempts for a success rate of 89%. The Hunter-Hawkins system was used in the remaining 23 successful attempts. Retrograde nephrostomy is a safe procedure, within the capabilities of most urologists, and well suited to the undilated collecting system.

E21. Tissue Attentuation and Beam Refraction of the Shock Waves – Potential Reasons for Treatment Failures

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There is little information available on the properties of the focused waves generated by the lithotripter systems. The pulsed shape, penetration, and beam width should be optimized to improve the performance of treatment protocol. Of considerable concern is the large beam absorption by tissue and the potential of missing the stone due to acoustic refraction. The beam properties are detected by a 0.8-mm diameter piezoelectric film (polyvinylidene difluoride) hydrophone, which has a frequency response of up to 20 MHz. In a degassed waterbath, the hydrophone is scanned across the beam. In water, the rise-time of the shock wave is extremely sharp (<0.05 μs), and lateral beam-width of only 4 mm (full-width half maximum). This narrow beam-width indicates that considerable care must be made in directing the shock wave towards the stone. Due to the refracting properties of the shock wave passing at an acute angle from a fat layer (slow speed of sound value of ~1,450 m/s) to a kidney surface (higher speed of sound value of ~1,580 m/s), considerable beam steering is expected. Preliminary analysis indicates that beam steering of up to 5 mm could be obtained under certain conditions; thus the shock wave could miss the stone. A special ray-trace program has been perfected, and in conjunction with CT imaging, a more accurate assessment of the beam refraction will be presented. Experiments on the effects of frequency-dependent attenuation on the shock-wave pulse will be described as well, using phantom and in vitro samples.

E22. The Potential for Inter- and Intraobserver Variability in X-ray Review to Establish Stone-Free Rates after Lithotripsy

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Success rates of extracorporeal shock-wave lithotripsy (ESWL) rendering patients stone-free were initially reported to exceed 90%. Subsequent studies have recognized the significance of variables such as follow-up interval, stone size and stone location, but the reliability of imaging modalities used to define residual fragments has not been emphasized. Copies of standard plain films and plain renal tomograms from ten patients who had undergone ESWL 3 months before were individually submitted, unlabelled, to three diagnostic radiologists familiar with genitourinary imaging. A preliminary review of each patient's paired films suggested six had small residual fragments, two were negative, and two were equivocal. Observers were asked to report each film as positive, negative, or uncertain for residual stones. Complete interobserver agreement was obtained with only 30% of plain films but 70% of tomograms. Intraobserver agreement in the repeat duplicate films was 73% and 80%, respectively. This study highlights the potential variability of reporting and identifies the need for a "gold standard" to establish stone-free rates. The previously respected success rates of ESWL must be interpreted with caution and methodology should include exact imaging criteria.

E23. New Techniques and Developments in ESWL – Dornier HM4 and MPL 9000

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Since August 1986, a tub-free HM 4 lithotripter (Dornier Medizintechnik GmbH) has been in use. To guarantee energy coupling to the body, a water cushion that adjusts to the patient's anatomy is placed between the body and shock-wave source (electrode, ellipsoid). The X-ray location of the stone is observed via two monitors by request under computer-aided stone location by means of a light pen. The ESWL patient is positioned on a versatile stretcher. The treatment course is microprocessor-controlled. Technical modifications such as low-energy generator and larger ellipsoid enable anesthesia-free ESWL in more than 80% of the cases without any premedication. Using the water cushion (HM 4), clinical results of stone disintegration, auxiliary procedures, and long-term results are comparable to the well-known HM 3 ESWL data (bathtub). The new Dornier multipurpose lithotripter MPL 9000 with an electrode for shock-wave generation uses ultrasound image processing (inline ultrasound transducer and external scanner) for kidney and gallstone disintegration. Between October 1, 1987 and January 31, 1988, 29 urinary stone carriers between 3 and 85 years of age have been treated. Results: The ESWL on the MPL 9000 achieved complete disintegration in 28 out of 29 patients with a generator voltage of 16 kV and an average number of shock-waves of 1,200. A second treatment was necessary in 8 cases (28%). All but one treatments (girl aged 3) were performed anesthesia-free, including 15 of 29 cases without any medication at all. Patient positioning before treatment revealed that all kidney stones (pelvis, calices, staghorn stones), as well as proximal and prevesical ureteral stones, can be located without any problem.

E24. An Approach to Dissolving Kidney Calculi by Ion – Exchange Reaction in Aqueous Media

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Fundamental experiments to dissolve renal stones using the new method of an acidic cation-exchange resin (H-R) are presented. Three kind of renal stones taken from patients in a powdered and massive form were subjected to dissolution in an aqueous suspension containing H-R at a constant temperature. The alterations in the physical parameters of the supernatant fluid and in the mass weight were measured at appropriate intervals. CaP and MAP were similar as regards their solubility, and the powdered calculi could be dissolved in an aqueous suspension with the amount of H-R at ten times (wt/wt). Dissolution was not completed for the CaOX specimen. An appreciable amount of PO_4 ion was detected, accompanied by C_2O_4 ion, which suggests that CaOX contains a phosphate mineral as an impurity. Similar results were observed for the massive specimens. CaP and MAP calculi were readily dissolved in the suspension irrespective of their morphology, and it is believed that the technique can be applied for the removal of renal stones in clinical experiments. The method was useful in the case of powdered CaOX but was not as effective for massive specimens.

E25. Clinical Study on Recurrent Stone Cases after Extracorporeal Shock-Wave Lithotripsy (ESWL)

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A total of 1,710 ESWL (Dornier HM-3) sessions have been performed on 1,400 cases of urolithiasis at our hsopital for 3 yeats and 3 months, from September 1, 1984 to December 10, 1987. In this study, 860 cases were examined that had passed no stones for more than 3 months after the treatment. Of the 242 cases observed in our outpatient department, the recurrent stones occurred in 24 (9.9%). Of the other cases 413 were investigated by means of a question-

naire: recurrent stones occurred in 50 (12.1%). Overall, in 74 (11.3%) of 655 cases, stone recurrences were encountered the longest within 2 years and at 6 months.

E26. Renal Scintigraphic Follow-up after Extracorporeal Shock-Wave Lithotripsy Using Microexplosion

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Extracorporeal shock-wave lithotripsy (ESWL) is now widely used, and it is generally accepted that this method disintegrates stone selectively with no adverse effects on renal function. However, no long-term follow-up of renal function after ESWL has so far been reported in detail. We used microexplosion for an energy source of underwater shock-wave and developed microexplosive ESWL (mESWL). This method has been applied clinically since March 1985. As of May 1987, a total of 229 cases had been treated with 95% clinical success. Renal function, by ^{99m}Tc-dimercaptosuccinic acid renal uptake rate, was estimated in 92 cases. In most cases the relative uptake rate of the targeted kidney ranged from 80% to 110% compared to the pretreatment rate. The cases in whom the uptake rate decreased to lower than 80% were mostly related to stagnation of the fragmentated stone in the ureter, usually for more than 2 weeks, and to pyelonephritis. There was no correlation between the number of shots and the change in uptake rate. The results indicate that the main factor influencing renal function in mESWL is not the shock wave itself, but the period of stagnation of the stone debris inside the ureter, which causes hydronephrosis and pyelonephritis in the infected urine. Based on these results, we believe that the stagnant stone debris should be removed not later than 2 weeks in order to maintain kidney function after ESWL.

E27. Long-Term Results in ESWL-Treated Urinary Stone Patients

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We carried out follow-up studies one ESWL patients treated from February 1980 to December 1986. ^{131}I-hippuran clearance was studied separately in both kidneys. The first 18 patients showed no deterioration of renal function 7 years post-ESWL. There was no increase in the rate of hypertension in patients with a 1-year following-up post-ESWL (n = 518), but a (age-dependent ?) rise of 3% was observed within a mean of 40 months post-ESWL (n = 288). Follow-ups of 754 patients, treated between May 1982 and May 1984 with a wide range of indications for ESWL, showed that stones were detected at hospital discharge in 55%, 6 months post-ESWL in 16%, and on an average of 40 months post-ESWL in 25% of the cases. The portion of particles bigger than 5 mm increased from 4% (6 months post-ESWL) to 47% (40 months post-ESWL). Review of stone location showed an accumulation of residual stones in the lower calyces during the first few months after ESWL. In the long-term follow-up, the portion of pelvic stones increased. In follow-ups 12–60 months post-ESWL, 75% were stone-free; 25% showed recurrent or residual stones. Recurrent stones were found in about 8%. Between hospital discharge and $3^{1/3}$ years post-ESWL, further urological operations had to be performed, mostly ESWL, in 18%. In this long-term-follow-up we found no definite evidence of a risk of arterial hypertension or loss of renal function caused by ESWL. The problem of residual concretion requires further investigations.

E28. Comparison of Results and Morbidity of Percutaneous Nephrostolithotomy and Extracorporeal Shock-Wave Lithotripsy

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Two new therapies, percutaneous nephrostolithotomy (PCNL) and extracorporeal shock-wave lithotripsy (ESWL), have revolutionized the treatment of upper urinary tract calculi. We report the success and morbidity rates in 110 patients undergoing PCNL and 982 patients treated with ESWL. Staghorn calculi were excluded from this series. The overall success rate (free of stones plus small asymptomatic residual fragments) was comparable with both modalities (PCNL 98% and ESWL 95%), althought the presence of residual fragments was more common in kidneys treated with ESWL. Patient morbidity, as measured by temperature elevation, length of postoperative stay, pain, and blood loss, was significantly less ($P < 0.05$) with ESWL than with PCNL. Retreatment rates were similar with both procedures and tended to increase in relation to increasing stone size and stone number. Post-treatment ancillary procedures (cystoscopy and stone manipulation, and percutaneous nephrostomy) were used more frequently with ESWL. Because of its efficacy and low morbidity, we conclude that ESWL is the treatment of choice for upper urinary tract calculi < 2 cm in diameter. However, PCNL will continue to have a primary role in the management of stones larger than 2 cm and cystine stones, and it will be used as a secondary procedure after unsuccessful ESWL treatments. In addition, because of the complementary nature of these two new technologies, certain complex stones, such as staghorn calculi, may be handled best by a combination of the two techniques.

E29. Percutaneous Management of Calyceal Diverticula

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Controversy exists regarding the optimal management of patients with symptomatic stone-containing calyceal diverticula. Although open surgical excision of the diverticulum was required in the past, currently, newer treatment alternatives such as extracorporeal shock-wave lithotripsy (ESWL) and percutaneous nephrostolithotomy (PCNL) have become available. This report concerns 10 kidneys treated with ESWL and 13 kidneys treated with PCNL with fulguration of the diverticulum. No patient became free of stone following ESWL, a finding attributed to the narrow ostium of the diverticulum and the stasia secondary to its nonsecretory status. Percutaneous techniques yield much better results, with 11 of 13 patients becoming free of all stone material (2 patients had residual parenchymal fragments). Additionally, the diverticulum was absent on follow-up radiographs in 11 of 13 patients. One patient with a persistent diveticulum did not have fulguration at the time of percutaneous stone removal. The complex nature of access in these cases favors a single-stage approach. The special technical features of this approach critical to its success will be highlighted. These data suggest that symptomatic, stone-containing calyceal diverticula should be managed with PCNL in combination with fulguration of the diverticulum, as this not only allows complete stone removal in a high percentage of cases but also treats the underlying disease process by elimination of the diverticulum in most instances. ESWL monotherapy is very unlikely to produce a stone-free result.

E30. The Results of Current Management Options for Staghorn Calculi

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The optimal management of staghorn calculi is currently unsettled, with various combinations of extracorporeal shock-wave lithotripsy (ESWL) and percutaneous nephrostolithotomy (PCNL) proposed. Comparisons of the results and morbidity of ESWL and PCNL for the treatment of staghorn calculi is difficult, in part, because these stones vary greatly in size, complexity, and composition. Three hundred thirteen cases of staghorn calculi treted at our institution with either ESWL monotherapy or initial PCNL, followed by ESWL (combination therapy), were reviewed with the results being stratified by stone volume, renal anatomy (dilatation), and stone composi tion. Adequate follow-up was available in 296 cases (147 partial and 149 complete staghorn calculi). Overall, combination therapy achieved a significantly higher stone-free rate than ESWL monotherapy for complete staghorn calculi (91% vs 31%, $P < 0.0001$) and also for partial staghorn calculi (92% vs 67%, $P = 0.002$). When stratified by anatomy or composition, combination therapy achieved superior stone-free rates in all categories, although the difference was slight for partial staghorns in nondilated collecting systems (91% vs 84%, NS). Our data suggest that the morbidity of combination therapy is not greater than that of ESWL monotherapy, as both have similar lengths of hospital stay and number of procedures required per case. The complication rate was actually higher for ESWL monotherapy than for combination therapy (44% vs. 34%). However, complications tended to be more severe in the combination therapy patients. These results indicate that virtually all staghorn calculi are best treated with initial PCNL, followed by ESWL if necessary. This approach allows for chemolysis and secondary percutaneous procedures as well. Small-volume partial staghorn calculi in nondilated renal collecting systems may be considered for ESWL monotherapy with ureteral stenting.

E31. The Bioeffects of Shock Waves and the Risk of Hypertension Following ESWL

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Focused shock waves, as generated by the Dornier HM_3 lithotripter, have been documented to cause marked, acute effects on the dog kidney. These changes resolve rapidly, but some investigators have reported fibrosis and even gross renal scarring as sequelae. Changes in the dog kidney appear to be dose-dependent, but as yet there are no established parameters that define the safe limits of ESWL in humans. Clinical observations of shock-wave effects include liver and skeletal muscle-enzyme changes, pancreatitis, urinary lysosomal enzyme release, hematuria, perirenal bleeding, and extrasystoles. Hypertension was documented in 8.2% of patients 1 year following ESWL in a limited retrospective study at this institution. To evaluate further the risk of hypertension following ESWL and percutaneous nephrostolithotomy, an intensive survey of 1,500 case treated by these procedures is currently underway and the results will be reported. In addition, in an attempt to define further the biologic effects of shock waves, tissue from swine treated with ESWL was subjected to extensive microdissection and EM studies. The findings were compared to human renal tissue obtained by biopsy pre and within 24 h of ESWL.

E32. The Management of Ureteral Stones

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In the last few years, several new techniques have become suitable for the treatment of ureteral stones: ESWL, percutaneous approach by the flexible operative nephroscope and the electrohydraulic probe (5 or 3.5 Fr.), and ureterolithotripsy through the ureterorenoscope (9.5 and 8.5 Fr.). The indication for the correct use of each technique is given according to several features of the ureteral stones. The features are due both to the intrinsic structure of the stone (the size, shape, and hardness) and to the consequences produced in the urinary tract by the time the stone no longer moves, i.e., obstruction flogosis, infection. From January 1985 to April 1987 we treated 178 patients with ureteral stones: 107 had lumbar, 23 iliac, and 48 pelvic stones. ESWL treatment was carried out in 72 lumbar stones either in situ (42 cases) and after pushing them up (30). The success rate was 100% and 88%, respectively. Five stones failed to be broken by ESWL and the patients underwent surgery (3 cases) or PCNL (2 cases) by a flexible nephroscope. The stones treated directly by PCNL had a success rate of 87.5% and lithotripsy by URS had an 83% success note. In 3 patients with iliac calculi, the stones were pushed up and treated by ESWL. In 12 patients, URS was performed with 91% success. Surgery was the first treatment choice in 5 patients with large stones and obstruction. In pelvic stones, treatment by the Dormia basket had an 80% success rate, ESWL in situ 100%, and URS 94%.

E33. Renal Complications Following ESWL

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Obstruction caused by stone or debris is a common complication of extracorporeal shock-wave lithotripsy (ESWL), but it can be solved by auxiliary treatment. Parenchymal, subcapsular, or perirenal hematomas have been reported in only a few cases, but intrarenal edema and hemorrhage are much more frequent. These lesions can be shown using different imaging techniques (131 iodine-hippuran clarance, CT scan, NMR). Measurement of the variations of *N*-acetyl-glucosaminodase (NAG) levels is a much simpler method to demonstrate early renal damage. NAG is a lysosomal enzyme that is found in the renal tubules. Urinary NAG increase is a good marker of renal damage. In 24 patients, urinary NAG and creatinine were measured before and after ESWL. The urinary ratio of NAG to creatinine increased after treatment (5.7 ± 6.6 U/g vs 9.8 ± 8.9 U/g). In particular, a shock-wave count of more than 2,000 exposes the patient to risk of early tubular damage. Although the new-generation lithotripters involve lower pressures on the interposed tissues, cautious use of ESWL is recommended to minimize renal damage.

E34. Plane of Stone Cleavage in Extracorporeal Lithotripsy

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Extracorporeal shock-wave lithotripsy (ESWL) is being increasingly used for the retrieval of upper urinary stones. Study of the submicroscopic appearance of the line of cleavage is necessary to study the mechanism of action of the shock waves. This paper presents a scanning electron microscopic (SEM) study of the stone fragments passed by patients after ESWL. Samples from 30 stone patients were studied. Qualitative estimation and infrared analysis were done to assess the stone composition. After gold sputtering, the samples were studied under a JEOL JSM 35c scanning microscope. The sur-

faces and interior aspects were studied. The stones studied were mainly of the calcium-oxalate dihydrate or monohydrate variety. The latter showed concentric laminations and radial striations. Cleavage was predominantly observed in the intercrystalline plane. In places, the cleavage was seen across the laminations and striations. Broken crystals were rarely seen. Comparison of ESWL-fragmented stones with machine-fragmented stones will be made. SEM photographs will be shown. The correlation between the size of the original stone, number of shock waves needed, size of fragments obtained, and the SEM pattern will be presented.

E35. Comparative Value of Plain X-ray and Pyelogram in Ureteric Colic

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Clinicians in emergency departments respond differently to patients presenting with ureteric colic. Plain X-ray of the kidney, ureter and bladder region (KUB), taken immediately in a busy emergency radiology department, usually lacks the necessary precision due to lack of bowel preparation in the patient and lack of film clarity. Emergency intravenous urogram (IVU) usually shows evidence of acute renal shut-down on the colic side. This paper presents a comparative assessment of an emergency plain X-ray of the KUB region with a properly prepared and properly taken X-ray film of the KUB region and emergency IVU. Two hundred patients with ureteric colic were studied retrospectively; 78% of these had an emergency X-ray film done. Among these, 12% had an IVU done. Only 7% had a properly prepared and properly taken plain X-ray film; 22% had no X-ray at all; 81% of the patients needed a repeat X-ray KUB after proper preparation. The various X-ray pictures were compared. A good X-ray KUB deliniated the renal outlines, thereby identifying back pressure effects produced by radiolucent or small radio-opaque calculi. Knowledge about the anatomy of the kidneys obtained by IVU pictures could be obtained from good KUB-region plain X-ray films; 30% of the good plain X-rays showed radio-opaque shadows with kidney enlargement as against 7% of the poor-quality films; 12% of the good plain films showed enlargement of the kidneys without radio-opaque calculi. Half of these patients subsequently passed stones. We conclude that emergency KUB X-ray films should ideally be taken in a stable radiological set-up after proper patient preparation. IVU should be discarded as an emergency investigation in ureteric colic.

E36. ESWL plus Ureteral Stenting – A Suitable Treatment for Staghorn Stones?

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Recently published data indicate that morbidity after ESWL of large renal calculi (> 2 cm) can be significantly diminished by inserting a ureteral double-J stent prior to treatment. To evaluate whether or not this combination is also a suitable therapy for stones > 2.5 cm and staghorn calculi, we have designed a prospective randomized study comparing ESWL plus ureteral stenting (group 2) with the combination of PNL/ESWL (group 1). Currently, 97 patients have entered the study. Preliminary data on 53 patients are available.

Whereas complications (bleeding, septicemia, deterioration of renal function) were significantly higher in group 1, the results of group 2 with only 15% of the patients being stone-free at an average follow-up of 3 months, is disappointing. Currently, the patients are being reevaluated to see if improved results are obtained after a longer follow-up. These results will further clarify whether ESWL plus ureteral stenting is suitable for large calculi and staghorns. This question is of particular interest, as recently anesthesia-free ESWL with the Dornier HM3 lithotripter has been available.

	Group 1	Group 2
No. of patients	27	26
Age (years)	52	48 (Average)
ASA	2.2	1.8 (Average)
Mortality	0	0
Hospital stay (days)	22.6	16.3 (Average)
Complications	26%	4%
Auxiliary measures	11%	26%
Stone free (at discharge)	40%	0%
Stone free (average follow-up 3 months)	66%	15%

E37. Long-Term Follow-up of Renal Calculi Treated with Extracorporeal Shock-Wave Lithotripsy

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Although virtually any calculus in the upper urinary tract is amenable to fragmentation with extracorporeal shcok-wave lithotripsy (ESWL), the results that may be expected in the short and long term are not well established. One thousand nine hundred and ten renal units (in 1,840 patients), followed for 3 months after ESWL at the Methodist Hospital of Indiana, were analyzed with regard to stone burden (size and number), location, underlying renal anatomy, stone composition, and urinary tract infection. In addition, metabolic activity (new stone formation and growth of residual fragments) was examined in 653 patients with follow-up 1 year after ESWL and in 142 patients 2 years following ESWL. The likelihood of achieving a stone-free status (SF) was inversely related to the size and number of stones treated (stone burden). Stone location also influenced treatment outcome, with lower-pole calculi faring less well. The relative percentage of whewellite vs weddellite in stones did not influence treatment outcomes. However, hydroxyapatite and cystine calculi became stone-free in only 1/2 of cases. Factors associated with a stone-free state were: size < 2 cm (79% SF), solitary stones (78% SF), renal pelvis location (84% SF), and normal renal anatomy (71% SF). In patients becoming stone-free following ESWL, recurrent stones were less likely to occur than was fragment growth in patients not becoming stone-free following treatment (8.4% vs 21.6%, $P < 0.001$). Patients with multiple stones at the time of initial ESWL were more likely to demonstrate subsequently metabolic activity than patients presenting for treatment of single stones (20% vs 8%, $P < 0.001$). Because ESWL, when unsuccessful (defined as not stone-free), appears to be associated with an increased rate of new stone growth, emphasis should be placed on the aforementioned factors prior to recommending ESWL, so that the chances of a stone-free result can be maximized.

E38. Extracorporeal Shock-Wave Lithotripsy in the Pediatric Age Population – Short- and Long-Term Results

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Fifty-five stone events have been treated in 44 patients less than 18 years of age. Mean patient age at treatment is 11.3 years (range 1.1

to 17.5 years). Right renal calculi were treated in 22 patients, left renal calculi in 12 patients, and bilateral renal calculi in 8 patients. Twenty-three of the treated renal stone events involved renal pelvic stones only, while 20 treatments involved calyceal stones only. Seven treatments involved pelvic and calyceal stones. Five ureteral stones were treated in situ, 3 in association with renal calculi. A total of 62 ESWL treatments were necessary, as 5 patients required two treatments and 1 patient three. Treatments included a mean of 1,186 shock waves (range 250–2,100) at a mean of 19.6 kV (range 16–26). Associated procedures included placement of a ureteral catheter (10 cases), retrograde stone manipulation (7), placement of indwelling ureteral stent (3), ureteroscopy (1), and cystolitholapaxy (1). Three patients required percutaneous nephrostolithotomy in conjunction with ESWL. Stone analysis is available for 59% of the patients and includes calcium oxalate (50%), magnesium ammonium phosphate (19%), calcium phosphate (15%), uric acid (8%), and cystine (8%). Follow-up is available on 39 of 44 patients (89%), representing 48 of 55 stone events treated (87%). Of the 48 treated stone events with adequate follow-up, 40 (83%) were stone-free 3 months after treatment, and 43 (90%) eventually became stone-free. Retained fragments remain after 5 treated stone events, 3 of which are insignificant. The remaining 2 fragments (4%) represent failures, as 1 fragment is composed of struvite and 1 grew significantly and required retreatment after 28 months. Initial complications of ESWL therapy were rare. Two patients developed transient obstruction by a distal ureteral column of stone fragments. Both resolved spontaneously within 48 h. One small perirenal hematoma was noted on postoperative ultrasound. Renal growth after ESWL, as measured by IVP, will be examined in these pediatric patients.

E39. Ultrasonic-Enhanced Chemolysis of Struvite Calculi

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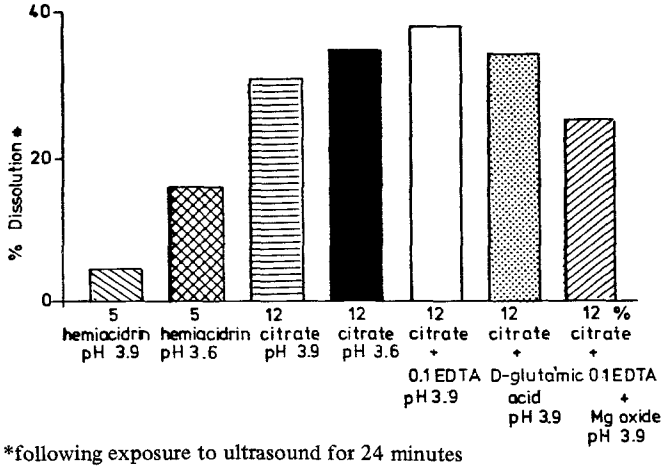
A computer-controlled broad-beam ultrasound system is presented, which in vitro accelerates struvite stone chemolysis. Hemiacidrin chemolysis was greatly potentiated and was linearly dependent on stone surface area and total ultrasound time. Dissolution was also dependent on citric acid concentration and solution pH, with only minimal dissolution above pH 4.2. Citrate concentrations of 12% provided maximal dissolution, which was further potentiated by 0.1 molar EDTA. With or without ultrasound application, the citrate-based compound demonstrated an eightfold activity over hemiacidrin. Magnesium was found to competitively inhibit dissolution activity.



*following exposure to ultrasound for 24 minutes

E40. Extracorporeal Shock-Wave Cholelithotripsy

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Extracorporeal shock-wave lithotripsy has revolutionized the management of upper urinary tract stones. Its success and safety have led to a proliferation of machines and of urologists trained in their use. Therefore, it is not surprising that at some institutions urologists have been asked to apply their expertise to fragment biliary calculi. We have successfully treated 11 patients with retained (post-cholecystostomy or -cholecystectomy) gallstones in the cystic (1), hepatic (1), or common bile ducts (9), using an unmodified HM-3 Dornier lithotripter. All patients were initially operated upon by a general surgeon and were subsequently evaluated by an interventional radiologist. Time from open surgery to extracorporeal shock-wave cholelithotripsy varied from 8 days to 22 years. All stones were considered inappropriate for percutaneous or endoscopic manipulation because of size or location (3), underwent unsuccessful sttempts at such procedures (4), or were impacted post-operatively (4). Stones varied in size from 6 to 20 mm; 1 was calcified and 10 were lucent. Imaging was possible by introducing dye through cholecystostomy (1), transhepatic (1), nasobiliary (3), or T-tubes (6). Eight patients were treated supine and 3 were treated prone. Two required repeat therapy. The average number of shocks required for fragmentation was 2,240 at 18–22 kV. All patients either passed the fragments (8) or had them reduced to a size that could be removed by percutaneous or endoscopic techniques (3). Morbidity was limited to transient elevation of LDH, transaminases, and alkaline phosphatase and the development of asymptomatic hemobilia and hematuria. No one developed pancreatitis. Because the number of situations in which this procedure can be used is limited, it may be difficult for other physicians to acquire and maintain the skills necessary to operate the lithotripter. Until specific biliary lithotripters become widely available, urologists may continue to be asked to assist in the management of these patients.

E41. Retroperitoneal Air and Extraporeal Shock-Wave Lithotripsy

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Over a 13-month period, 1,344 patients underwent extracorporeal shock-wave lithotripsy (ESWL). Plain X-rays (KUB) were done routinely 24 h post-ESWL to assess stone fragmentation. Six patients were noted to have retroperitoneal air (RA) on these films. All six had epidural anesthesia (EA) induced using "loss of resistance to air in a syringe" to identify the epidural space. Four patients had unilateral ESWL and two had bilateral. The number of shocks delivered to each kidney ranged from 500–3,800 at 18–22 kV. All stones were satisfactorily fragmented and post-operative courses were uneventful. Classically, RA is associated with colonic perforation or retroperitoneal infection; tissue damage to lung or colon by shock waves could lead to tracking of air along tissue planes, producing similar X-ray findings. None of these causes was apparent in our patients. A final possibility could be the introduction of air into paraspinal tissues or along spinal nerves during induction of EA. This is supported by the fact that the RA was not necessarily on the same side as the stone. It is further substantiated by a recent patient who developed RA on a KUB 30 min after EA but before ESWL. In conclusion, RA is found in a small number of patients undergoing EA and ESWL. It is likely introduced during induction of EA, and urologists treating these patients in the early post-ESWL period should be aware of this possible radiological finding and appreciate its benign nature.

E42. Four Years of Experience with Ureteroscopy – Techniques and Results in More than 500 Cases

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Since the introduction of ureteroscopy (URS) at our department in July 1984, 530 ureteroscopies for ureteral stones and other ureteral pathology have been performed. The technique used was originally described by Perez-Castro. For dilation of the ureteral orifice, four techniques were employed: (1) flexible metal bougies: (2) Teflon bougies: (3) a ureteral balloon catheter; (4) the ureteromat (Storz Co., FRG). In cases of extreme ureteral rigidity, insertion of a ureteral catheter with its softening effect proved to be advantageous. A 6 Fr. nephrostomy catheter was inserted prior to URS in cases of high-grade obstruction or pyonephrosis. With URS alone or in combination with ESWL or percutaneous nephrostolithotomy, 96.4% of the ureteral stones could be successfully removed. Ureteral perforation occurred in 4.8%, which usually healed with internal or external urine drainage. Three patients developed a ureteral stricture 3–9 weeks afte URS, necessitating endoscopic balloon dilation in one and a ureteroneocystostomy in two. Long-term follow-up after 9–14 months in 156 patients revealed ureteral narrowing at the former stone site; however, no further treatment seemed necessary. Our results indicate that complications usually occur 3–9 months after URS; later sequelae are exceptionally rare. For distal ureteral stones, ESWL in situ has emerged as an alternative. Our own experience in these cases reveals on overall success rate of 60%. Especially in cases of stone impaction or high-grade obstruction, the success rate was lower than with primary URS. Ureteroscopy is still indispensable for the treatment of ureteral stones and is associated with low morbidity and a low complication rate.

E43. Withdrawn

E44. Lithostar Extracorporeal Shock-Wave Lithotripsy – The Initial Experience

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Our initial experience with 340 patients with renal ($n = 297$) and/or ureteral ($n = 67$) calculi treated between October 1987 and January 1988 with the Siemens Lithostar bath-free lithotripter at the University of Toronto is presented. The Lithostar, a second-generation lithotripter, was introduced in North America in the spring of 1987, and we were the third center to use it. Neuroleptic anaesthesia (intravenous phentenyl, droperidol, and valium) was used. Treatment was well tolerated, suggesting that patients with low anaesthetic risk and no adverse stone parameters can be treated as outpatients. Ureteral stones, including those of the lower third, can also be treated, especially if previously stented. The average number of shocks per treatment was 3,804, and each treatment lasted approximately 105 min. Of the small sample with a minimum follow-up of 3 months, the first 35 patients (37%) had successfully passed all fragments while 63% had residual fragments, as determined by a plain film of the abdomen and plain renal tomograms. Also at 3-month follow-up, 66% of these patients were asymptomatic and were followed. Symptomatic patients with residual fragments ($\geqslant 5$ mm in diameter) were retreated, treated by alternate methods, or followed. The stone-free and symptom-free rate at 3 and 6 months follow-up of a larger group of treated patients will be reported. This constitutes the largest North American experience to date.

E45. Caliceal Stones with a Narrow Neck – The Real Problem in ESWL

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Caliceal stones can be successfully treated by ESWL, provided that the caliceal neck is unobstructed. A narrow neck does not represent an absolute contraindication but does account for an important percentage of unsuccessful cases. Stones that lodge in the calix break badly and do not migrate. We have treated these unsuccessful cases both by iterative ESWL after transurethral caliceal neck dilation and by percutaneous operative calicoscopy with a small instrument designed for this purpose. These techniques allowed us to resolve more than 90% of the cases after failure of the first ESWL.

E46. Percutaneous Debulking of Staghorn Stones – Indications, Technique, and Results

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A total of 122 staghorn stones in 114 patients were treated by combined percutaneous debulking and extracorporeal caliceal treatment. Out of 122 kidneys, 72 underwent dilation of the intrarenal collecting system. No complications related to the puncture occurred. Bleeding occurred in 4% of the cases during ultrasound lithotripsy, in 8.2% after withdrawal of the tube, and in 2.4% some after discharge. Early dislodgement of the nephrostomy tube was a common complication (10.6%). A new multipurpose nephrostomy tube is proposed to overcome these problems. Double-J stent reduced urinary complications. The complication rate is much higher in nondilated as opposed to dilated kidneys, so it should be preferable to treat staghorn stones in nondilated kidneys with staged ESWL monotherapy.

E47. Double-J ESWL – Technique, Results, and Complications

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We reviewed our experience with 337 double-J pre-ESWL ureteral stenting procedures. The indications for double-J stents were envisaged according to a computerized scheme that considered stone volume, infection, compliance of the urinary tract, patient cooperation, and the nature of the stone. In 15% of the cases, passage of the stent was difficult and could be accomplished by a retrograde angiographic technique using a cobra catheter (cobra stent) or by an antegrade route (percustent). In only 2% of the cases it was not possible to insert a stent. Clinical evidence of versicoureteral reflux (pain, fever) was present in 16% of the cases. In 3%, the stent had to be removed. Upward migration occurred in 0.3% of the cases, downward migration in 13% with a clear-cut prevalence of female patients. Obstructive complications (Steinstrasse) were 2%, in comparison to 9% in the pre-stent period. Long-term retained stents (more than 6 weeks) showed a considerably high rate of complications (occlusion, incrustation, colonization), so that a maximum period of 1 month is considered advisable.

E48. The Management of Staghorn Stones Using a Combination of Siemens Lithotriptor, PCNL, and Hemiacidrin Irrigation

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Although extracorporeal shock-wave lithotripsy (ESWL) is very effective in the treatment of the majority of renal and ureteric stones,

partial and complete staghorn stones continue to pose problems, and additional measures such as percutaneous debulking (PCNL) and/or irrigation of the kidney with Hemiacidrin solution may be required. Our experience with 45 patients treated between June 1987 and January 1988 will be presented. So far, 45 patients have been treated (21 complete staghorn stones and 24 partial staghorn stones, i.e. stone filling the whole of the pelvis and extending into one or two calyces). In 16 patients preliminary percutaneous debulking was undertaken. In 2 of these the kidney was cleared completely by this method (both staghorn stones), and in the remainder the residual peripheral fragments were subjected to ESWL. Twenty-nine patients were treated by ESWL alone afte placing a double "J" stent. In 3 patients it was necessary to irrigate the kidney with Hemiacidrin solution to dissolve whole or fragmented residual stones. To date, 27 kidneys are completely clear of stones, and in 10 the fragments are expected to pass within the next few weeks. In one patient, both PCNL and ESWL failed and in one with very large and dense calyceal fragments, ESWL failed to fragment the stones even after successful PCNL. In 8, further ESWL sessions are planned. Our success rate so far of 60% (expected rate of 95%) suggests that a combination of ESWL, PCNL, and Hemiacidrin irrigation of the kidney is necessary to achieve complete clearance and should be the treatment of choice for large stones in the kidney.

E49. ESWL Experience After 4,000 Treated Patients

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After $3\frac{1}{2}$ years of experience with the Dornier lithotripter HM3 (non-modified generator), we have now treated about 4,000 patients with a total session number of near 4,500. Eighty percent of our patients were treated in a semiambulatory manner, i.e., transfer to other urological clinics near their home on the day of treatment. Our report concerns the results of 2,500 fully documented cases. Serious complications, such as pyonephrosis or hematomas, were extremely rare (< 3%). A problem more often seen was renal obstruction (16.7%), requiring further auxiliary measures. Repeated treatments were necessary in 7.1% of stones with a diameter of less than 2 cm. Fractionated ESWL was performed in 36.7% of all treated stones with diameters above 2 cm. Of the patients, 90% showed good final results (stone-free or spontaneously passable fragments). Six percent had larger fragments; about half of these had not finished their treatment within 1 year of the first ESWL session. Four percent required open surgery, usually patients who had complicating factors, such as infectious stone disease, single kidney, or stones more than 2 cm in diameter. We conclude that cooperative treatment with peripheral urological clinics is desirable. Patients with complicating factors (anomalies in anatomy or physiology of upper urinary tract, children, etc.) should be followed up in a specialized ESWL center.

E50. ESWL under Local and Peridual Anesthesia

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This report comprises ESWL treatment with the Dornier lithotripter HM3 (non-modified generator) with the use of local anesthesia. Four hundred patients received local anesthesia of 30 ml 1.5% Prilocaine intracutaneously at the entry of the shock-wave cone. Ten percent of these patients felt pain and required further anesthetic measures, usually peridural anesthesia. A total of 296 patients treated under local anesthesia were compared with an equal number of patients treated with peridural anesthesia. Each group was comparable with regard to stone size, localization, patient age, and sex. We found that patients in the group treated under local anesthesia presented more difficulties (20.6%) than those treated with peridural anesthesia (14.5%). Auxiliary measures were required more often in the former group (17.9%) than in the latter (12.8%). However, with regard to the results 1 year after treatment, there is little difference in the final results (stone-free or spontaneously passable fragments in 91.9% and 92.6%, respectively). Local anesthesia is a possible form of anesthesia for ESWL treatment using the Dornier HM3. Our findings, however, with a higher rate of auxiliary measures, such as ureteral manipulations and repeated treatments in the locally anesthesized group, are indicative of the risks involved in treating stones under local anesthesia without judicious prior assessment.

E51. Complex Struvite Calculi Treated by Primary Extracorporeal Shock-Wave Lithotripsy and Chemolysis with Hemiacidrin Irrigation

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Extracorporeal shock-wave lithotripsy (ESWL) has become the primary method of treating most renal calculi. As stone size increases, so too does the post-treatment incidence of pain, ureteral obstruction, retained fragments, and the need for additional urologic and/or ESWL procedures. In an attempt to avoid the known complications associated with a large infected stone burden and to minimize the incidence of retained infectious fragments, we have begun treating patients with complex struvite stones by primary ESWL, followed by chemolysis. Ten patients with complex struvite stones were successfully treated by primary extracorporeal shock-wave lithotripsy, followed by chemolysis with 10% hemiacidrin renal irrigation. The average number of treatments per renal unit was 1.2. No patient required a blood transfusion. Ureteral obstruction did not occur in those patients receiving planned hemiacidrin irrigation immediately following extracorporeal shock-wave lithotripsy. At 6-week follow-up, 9 patients were free of residual fragments. The combination of extracorporeal shock-wave lithotripsy and hemiacidrin chemolysis represents a satisfactory alternative to the traditional surgical management of complex struvite calculi.

E52. Complications of Extracorporeal Piezoelectric Shock-Wave Lithotripsy (EPL)

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In the following study, we compiled the complications of extracorporeal piezoelectric shock-wave lithotripsy in the first 150 patients treated in our department. Since November 1987 we have used the "Piezolith 2300" to fragment renal, ureteral, vesical, and urethral stones. Piezoelectric-generated shock waves destroy the calculus within a focus of 3 x 8 mm. The focus can be moved in all three directions, and the treatment can be observed online by a 4-MHz ultrasound sanner. No serious complications or deaths occurred although high-risk patients were included. No patients needed general or spinal anesthesia; 2/150 received an oral sedative. Nearly all patients observed a transient hematuria without needing treatment. Ultrasound on the 1st day after EPL showed no hematoma formation. Our results indicate that: extracorporeal piezoelectric shock-wave lithotripsy does not require anesthesia; all urinary calculi that can be located by ultrasound can be treated by EPL; there is no need to fear serious complications.

E53. Flexible Urethrocystoscopy in Clinical Practice

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The advantages of direct visual examination by flexible fibrocystoscopy and the possibilities for instrumental manipulations are described.

E54. Initial Experience with the Second-Generation Lithotriptor Dornier HM4

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Besides the widely used first-generation lithotriptors (Dornier HM3), an increasing number of second-generation devices are now in clinical use. We report our initial experience with the Dornier HM4. Local coupling, modified shock-wave generation, and computer-assisted handling are the main improvements over the previous device. To date, 60 treatments in 46 patients have been performed successfully. There were 60% calyceal stones, 20% renal pelvic, and 20% upper and distal ureteral stones. Light analgesia and sedation (i.v. ketamin and midazolam) were sufficient to maintain a pain-free status in the patient during extracorporal shock-wave lithotripsy (ESWL). Obstructing upper ureteral stones were pushed back prior to ESWL. Patients with stones larger than 1.5 cm received a double-J stent initially. The posttreatment course was uneventful. One patient had obstruction and fever, which necessitated a percutaneous nephrostomy. At 1 month follow-up, 50% of the patients were already free of stones. The rate of secondary treatments and the number of impulses are higher than with the previous device (HM3). Overall stone disintegration, even cystine calculi, has been satisfactory so far.

E55. Renal Functional Alterations After Extracorporeal Shock-Wave Lithotripsy, Assessed by Measurement of Urinary Proteins

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From imaging techniques it is known that temporary renal malfunction is possible after extracorporeal shock-wave lithotripsy (ESWL). However, it has been difficult to assess the renal damage quantitatatively. In a total of 10 patients, urine samples were obtained before and after ESWL of renal stones, without prior manipulation, at regular intervals up to 4 days. To monitor glomerular function, IgM, α-2 macroglobulin, IgG and albumin, and to monitor tubular function, β-2-microglobulin and Tamm-Horsfall protein excretions were determined in aliquots of 24-h urine samples by routine laboratory radioimmunological and immunological methods and plotted over time. A pronounced increase in albumin excretion was found after ESWL which reached normal values after 4 days. IgG was also raised and remained above normal after 4 days. IgM and α-2 macroglobulin were not significantly elevated. Measurement of β-2-microglobulin also revealed an increase after ESWL with back to normal values after 4 days. Tamm-Horsfall protein which shows a rise after shock-wave exposure, does not come back to normal values after 4 days. The highest protein levels, obtained at 12 to 24 h after ESWL, revealed a pronounced increase over the upper limit of normal values. The increase of large-molecule urinary proteins is interpreted as an alteration in glomerular permeability. Small-molecule proteins are thought not to be reabsorbed adequately by tubular cells due to functional changes resulting from exposure to shock waves. As far as is known to date, these pathological protein determinations provide a reliable means of assessing temporary renal damage.

E56. Side Effects of Extracorporeal Shock-Wave Exposure on the Kidney in Dogs

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The effect of extracorporeal shock-wave (SW) exposure using a Dornier kidney lithotriptor HM3 on the kidney was investigated in dogs. The SW was generated by the spark discharge or 20 kV and was focused on the lower part of either kidney. During and just after an exposure of 1,000 shots of SW, the renal blood flow to the affected and contralateral kidney was measured by the microsphere method. Blood flow to the affected kidney decreased, and blood flow to the contralateral kidney was also decreased simultaneously just after the 1,000 SW shots. Renal scintigraphy using ^{99m}Tc-DTPA was performed before SW exposure, and at 30 min, 1, 2, and 4 weeks after exposure. The renograms were evaluated by the following parameters: T_{max} (time required to reach maximum radioactivity). RA_{max} ratio (maximum radioactivity of the affected kidney/ that for the contralateral kidney), and $T^{1/2}$ (the half-life of elimination). T_{max} was significantly prolonged for the affected kidney 30 min after exposure, while that for the contralateral kidney was not changed. The RA_{max} ratio was decreased 1 week after exposure for the affected kidney. $T^{1/2}$ was significantly prolonged 30 min after exposure, which was observed up to 2 weeks later. Scintigraphy also showed a slight enlargement on the affected side 30 min after exposure. The histological study showed hemorrhage of the peritubular space, indicating a rupture of the peritubular capillary. Intratubular hemorrhage was also noted but was not remarkable. In conclusion, the main effect of SW exposure on the kidney was rupture of the peritubular capillary, resulting in temporary and reversible deterioration of renal function.

E57. Results of Nonsurgical Uric Acid and Cystine Calculi Treatment

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Out of the 1,650 patients that underwent ESWL treatment for renal calculi at UCLA between March 1985 and September 1987, 48 patients (2.97%) had uric acid stones and 8 patients (0.48% suffered from cystine calculi. Treatment consisted of ESWL combined with urinary alkalinization. Prior to ESWL, radiolucent stones were focused as a filling defect on retrograde, pyelography and in some cases with renal ultrasound. At 3 months follow-up in the uric acid group, 34/48 of patients (70.8%) were free of stones and at 6 months, 40/48 patients (83.3%). In the cystine group 5/8 (62.5%) were stone-free after 6 months. Of 48 uric acid stone patients and 8 cystine stone patients, 36 and 6 respectively, required auxiliary procedures like percutaneous nephrostomy or double J stenting of the ureter. Although both groups were considered to have a low success rate at the beginning of ESWL treatment, they can now be successfully treated with excellent results, especially when using complimentary methods such as urinary alkalinization or ureteral stents.

E58. Staghorn Stone Treatment with ESWL – The Fate of Residual Stones

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Between March 1985 and September 1985, 82 patients with staghorn stones were treated with ESWL, PCN, or a combination of

both procedures. Review of the patients treated allowed modification of the treatment strategy. The most important determinants for a successful outcome of ESWL are overall stone burden, architecture of the renal collecting system, and stone composition. Staghorn stones filling a nondilated renal collecting system in the absence of any intrarenal stricture or distension of dependent calices responded well to ESWL monotherapy. The have a stone-free rate of 83% (struvite) and 86% (uric acid). This drops to 57% for calcium oxalate stones. In the presence of anatomical alteration of the collecting system, the stone-free rate cannot be expected to be higher than 42%. In these cases PCN or the combination of PCN and ESWL appears to be mandatory to achieve similar results. Staghorn stones, filling a grossly dilated renal collecting system, need to be debulked using PCN to achieve a stone-free rate of 80%. Of the 22 patients with residual stone particles whom we have followed for up to 2 years, 4 needed to be re-treated as they demonstrated regrowth of stones and 10 because of persisting obstruction and/or infection. The remainder is being followed conservatively, and the data obtained at 3 month-intervals indicate that the recurrence rate, as well as the symptoms of those patients with residual particles, compare favorably with those patients who had previously been treated with other procedures for staghorn stones.

E59. The Treatment of Urinary Calculi in Transplanted Kidneys

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This report outlines the noninvasive treatment, problems and success rate of the rare instances of stone formation in a transplanted kidney. From March 1985 to August 1987, four patients with opaque renal calculi measuring 1.5 cm (x 2), 2 cm, a staghorn and a ureteral calculus were treated by ESWL with a double-J stent, which was left indwelling for 2 weeks postoperatively. Two of the four patients wer positioned prone on the gantry, the other 2 supine. Three-quarters of the patients were stone-free after 4 weeks, as proven by ultrasound and KUB. The patient with staghorn calculus underwent several treatment sessions and is stone-free now as well. All four patients showed no signs of diminished kidney function (follow-up period: 2–18 months). No ureteral obstructions necessitating auxiliary procedures have occurred to date. Based on our experience, although transplanted kidneys require a different technique and handling, it seems that ESWL is technically feasible and advantageous in patients with transplanted or ectopic pelvic kidneys. No adverse side effects were noted during follow-up.

E60. Emerging Concepts in the Treatment of Ureteral Stones

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Since ureteral stones have been included in the range of stones routinely treated by ESWL, controversy as to whether ESWL should be preceded by ureteral stone manipulation has persisted. In this regard, we basically have noticed a continental shift reflecting the peculiarities of different health systems in Europe and the U.S. In the United States, most centers prefer stone manipulation by ureteral stents in a retrograde fashion. This is done in order to repsition the stone into the renal collecting system or to bypass it in order to create an artifical expansion chamber. We pursue a differentiated approach, which is as follows. Based on the radiographic appearance of an existing natural expansion chamber, approximately 10% of stones above the iliac crest and 25% of stones located in the pelvic window, that is in the true pelvis below the pelvic brim, are eligible for ESWL in situ treatment. All other stones that do not qualify for in situ treatment are still subjected to ureteral stone manipulation, utilizing stents and extensive ureteral lubrication. This differential approach has advantages over our previously described combination approach in that it does not change the success rate (97%) and overall hospital stay (1.2 days), but it does save ureteral manipulation for approximately 30% of patients.

E61. Comparative Experimental Study on the Disintegrating Effect of the Tunable Dye Laser and Ultrasound on Stones of Various Composition

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Most ureteral stones are amenable to ESWL therapy which, as a noninvasive or, in conjunction with ureteral manipulation, a minimally invasive procedure, has become the treatment of first choice. If large impacted stones cannot be manipulated, this is an indication that they should be disintegrated by other means. Recently, a 2.5 Fr wire probe and a 10.5 Fr rigid ureteroscope have become available (Storz), enabling us to break up all stones approached. The potential danger of this procedure includes ureteral damage from the ureteroscopy and thermal damage from the extensive use of the probe. The most innovative method of breaking up ureteral stones is the tunable dye laser (Candela), which has now been introduced as an investigational device. The 250-μm laser fiber takes up no space at all and can be guided to the stone using small-caliber flexible ureteroscopes, such as the 7 Fr, 9 Fr, or 10 Fr flexible ureteroscopes (ACMI, Reichardt, Storz). In animal experiments the safety of this procedure has already been demonstrated. A recent comparative study at UCLA on the fragmenting effect of wire probe ultrasound and the laser lithotripter has proven the same disintegrating efficacy in both devices when used under water and on stones contained in a Penrose drain held under tension. In routine clinical use, however, efficacy and reliability of the ultrasound wire probe have proved to be superior to the experimental laser. More recently, a commercial tunable dye laser unit has become available, which appears to be more reliable. In conjunction with suitable small-caliber flexible instruments, the use of the laser lithotripter obviates the need for ureteral dilation in many cases and can also be used for kidney stones via a retrograde approach, utilizing flexible instruments that can be guided.

E62. Ultrasound Ureterolithotripsy, Utilizing a 10.5 Fr, Rigid Ureteroscope and 2.5 Wire-Probe Transducer

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Between September 1985 and August 1987, 128 patients underwent ureterolithotripsy, utilizing a 10.5 Fr operating ureteroscope with a wire ultrasonic probe (Storz). On these 128 patients, 142 treatments for larger ureteral stones requiring disintegration were performed (64% for large solitary stones and 36% for management of Steinstrasse). Indications were: failed stone manipulation with or without ESWL for stones above the iliac crest; stones in the "stonecracker's no man's land" between the iliac crest and pelvic brim and large stones in the distal ureter that could not be manipulated. Of the stones treated, 9% were located in the upper third of the ureter, 8% in the midureter, and 83% in the lower third of the ureter. The operating time averaged 108 min (upper ureter) 120 min (midureter) and 56 min (lower ureter). Successful stone disintegration was achieved in 99% of all cases, and the average stone-free rate at a 6-week follow-up was 99%. The rate of periprocedural complications was low, as was the rate of auxiliary procedures (fever 3%, ureteral extravasation 10%, perforation 2%, percutaneous nephrostomy 6% (pre-URS 3%, post-URS 3%).

E63. Management of Post-ESWL Complications: Steinstrasse

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ESWL disintegrates human urinary stones contained in the upper urinary tract noninvasively. After successful disintegration, the resulting stone gravel is then eliminated spontaneously with the urine from the upper urinary tract. The rate of postprocedural complications during the elimination of gravel and the elimination time are directly related to the initial stone mass. Stones of a size of less than 2.5 cm require invasive auxiliary procedures after ESWL in approximately 10% (percutaneous nephrostomy, ureteroscopy) whereas in larger stones auxiliary procedures are needed in up to 60% when treated with ESWL alone. The most common complication after ESWL treatment is ureteral Steinstrasse, which presents as ureteral obstruction caused by passing debris. On ultrasound this can be detected at any time during the follow-up in approximately 60% of all stone patients. Usually, no therapy is necessary and the Steinstrasse resolves within days without any clinical symptoms other than occasional pain. In those cases were obstructive pyelonephritis is encountered, percutaneous drainage (5%) is required and long-standing obstruction without symptoms (2%) must be relieved as well. Ureteroscopy is performed less frequently (3%) since the liberal use of nephrostomy tube drainage allows for spontaneous passage in most instances. As a rule, increasing stone size leads to an increased risk of complications during the follow-up, such as pain, obstructive pyelonephritis, and urosepsis. Therefore, patients with large stones need to be followed-up with special diligence until they are free of stone debris. Our follow-up protocol is presented as are case demonstrations with technical hints.

F. Case Reports

F1. 2.8-Dihydroxyadenine Stones Mistaken for Uric Acid Stones

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2.8-dihydroxyadeniuria is an inborn metabolic error due to the absence of adenine phosphoribosyl transferase. Deficiency of this enzyme leads to accumulation of adenine, which is converted by xanthine oxidase to 2,8-dihydroxadenine. The latter is insoluble and forms stones. These are often mistaken for uric acid stones because of their similarity in the ultraviolet spectra [1]. We describe the first case reported from Iraq of this type of stone. An 18-month-old male child was admitted to hospital with 5 days anuria. X-ray of the abdomen and ultrasound showed moderate hydronephrosis, but no urinary tract stones. Ascending pyelography revealed a block at L5 on the right side and L4 on the left. Stones were removed operatively, analyzed by the method of Mekognost [2], and found to be of the uric acid type. The stones were also analyzed by the method of Simmons et al. [1] and found to be of the 2,8-dihydroxyadenine type, and not of the uric acid type.

References: 1. Simmonds, HA et al. (1976) Biochem J 157:485 – 2. Maurer C et al. (1976) Urology 16:226

F2. Ethylene Glycol Intoxication

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Plasma and urinary oxalate and glyoxylate, plasma creatinine, blood ethanol, and urinary formate were determined in a 16-year-old girl who was admitted to hospital 12 h after ingesting 250 ml of antifreeze [95% ethylene glycol (EG)]. She was treated with oral ethanol, 50 g, followed by intravenous sodium bicarbonate and ethanol infusion, 100 mg/h, 15 h/day over the first 3 days. Hemodialysis was performed 5 h/day, day 1–3, 3 h on day 4, and 4 h on day 5. Pyridoxine, 20 mg/day, was administered on days 1–3. Urinary and plasma oxalate were determined as previously described [1, 2]; urinary formate was determined with formate dehydrogenase [1] and glyoxylate with glyoxylate reductase. Urinary results are expressed as mm/l as only urine aliquots were received. The results are summarized in the table below.

	Plasma (μm/l)		Urinary excretion (mm/l)		
	Oxalate	Glyoxylate	Oxalate	Glyoxylate	Formate
Day 1	481.0	799	0.49	–	–
2	47.0	Undetected	0.07	0.36	0.75
3	–		0.18	0.61	0.96
4	18.3		0.32	–	–
5	33.0		0.20	–	–

Normal values: Plasma, oxalate 3.2 μm/l, glyoxylate < 1.0 μm/l; Urinary excretion mm/24 h, oxalate 0.29, glyoxylate 0.05, formate 0.33.

Plasma oxalate and glyoxylate were massively increased on day 1 and rapidly decreased with dialysis. Treatment with ethanol inhibited glyoxylate and oxalate synthesis from EG. Urinary glyoxylate was substantially raised at days 2 and 3 while only slight increases in urinary oxalate and formate were observed. The patient was discharged after 2 weeks with a plasma creatinine of 60 μm/l. We conclude that glyoxylate and oxalate are important, if minor, metabolites of EG in man and little formic acid is produced. The very high plasma concentrations of glyoxylate and oxalate produced in the first 12 h, although < 1.0% of EG ingested, suggest they are major contributors to the toxicity of EG in man.

References: 1. Costello J et al. (1976) J Lab Clin Med 87:903 – 2. Maguire M et al. (1981) Urolithiasis. Clin Basic Res 963

F3. Dietary Restriction of Sodium as a Means of Reducing Urinary Cystine

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Cystinuria is an autosomal recessive disorder manifested by impaired renal tubular reabsorption of cystine and frequent occurrence of urinary calculi. Medical therapy is based upon urinary dilution and alkalinization and reduction in cystine excretion. The latter two strategies may be ripe for conflict of interest because sodium bicarbonate is the most frequently used urinary alkalinizer, and there is evidence to suggest that cystine excretion may be sodium-dependent (NEJM: 315:1120–1123, 1986). To investigate the relationship between dietary sodium and urinary cystine further, we studied a 23-year-old male with cystinuria and a 10-year history of recurrent cystine stones. When first seen, he was taking 2 g of sodium bicarbonate daily to alkalinize his urine and was on an unrestricted diet. After two initial 24-h urine collections and a corresponding 4-day food record were obtained, he was started on a low sodium diet and the sodium bicarbonate was discontinued. Methionine intake was not altered. After 3 months, the 24-h urine collections and diet records were repeated. Mean results are summarized below:

Trial	Diet		Urine	
	Methionine (g/day)	Sodium (mmol/day)	Sodium (mmol/mg creatinine)	Cystine (mg/mg creatinine)
1	2.5	221	14.6	43.5
2	2.7	97	6.7	34.5

Conclusions: (1) Dietary restriction of sodium is a useful and safe means of reducing urinary cystine; (2) drugs other than sodium bicarbonate should be used to alkalinize the urine in patients with recurrent cystine stones.

F4. Nonoperative Treatment of Extreme Bilateral Nephroureterolithiasis

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Innovative techniques like extracorporeal shock-wave lithotripsy (ESWL), percutaneous nephrostolithotomy (PCNL), and ureteroscopy (URS) have fundamentally changed the treatment of renal and ureteral stones. As an example, the case of a 73-year-old man with extreme bilateral nephroureterolithiasis is demonstrated. He was admitted to the hospital on September 11, 1985, with bilateral hydronephrosis, acute renal failure, and incipient urosepsis. Apart from multiple large renal pelvis and caliceal stones on both sides, the right ureter contained about 8 stones above the iliac crest with a diameter up to 1.8 cm. On the left side, 6 lumbar stones up to 2 cm in diameter were diagnosed. The treatment sequence (under epidural anesthesia) was a follows: Bilateral percutaneous nephrostomy on September 12; Retrograde manipulation of ureteral stones and left percutaneous nephrostolithotomy on September 1; First ESWL treatment on the left side on September 23; Right ureteroscopy with ultrasound lithotripsy combined with retrograde stone manipulation, followed by ESWL on the right side on September 26; Second left percutaneous nephrolithotomy on October 10; URS II, PCNL I, and ESWL II on the right side on October 16; ESWL III R and ESWL II L on October 21; Removal of percutaneous nephrostomy tubes on October 22. On discharge (October 23), the collecting system revealed minimal fragments on both sides in the calices. Intravenous pyelography revealed normal excretion and drainage. Renal function was normal again.

F5. Obstructing Urethral Stones Treated by Extracorporeal Piezoelectric Lithotripsy

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We report the first case of urethral stone treated by piezoelectric shock-wave lithotripsy. A 65-year-old male was admitted with a urethral stone obstructing the proximal pars pendulans urethra and causing dysuria. The stone could be palpated easily and showed a diameter of about 1 cm on X-ray. It could be treated successfully and without any anesthesia by extracorporeal piezoelectric shock-wave lithotripsy. Endourethral stone therapy often causes mucosal lesions. Other shock-wave systems may harm the testes by using X-rays for focusing. Even if a concomitant urethral stricture – as in this case – must be treated, primary stone removal facilitates the urethromia interna. Thus, EPL might be the treatment of choice for urethral stones.

F6. Factitious Struvite Stones

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The patient to be reported is a 30-year-old female hospital employee who had a 3-year history of repeated hospital admissions with renal colic, hematuria and apparent passage of 2–3 mm stones, analyzed as struvite. She had received numerous courses of intramuscular narcotics. Apart from tiny calcifications in the right kidney, present at the time of the first attack, all subsequent X-ray and ultrasound examinations of the kidneys were negative. Metabolic investigations for the causes of the stones were negative and urine cultures were sterile. Because of the discrepancy between the apparent symptoms and the negative laboratory tests, the possibility of factitious disease was suspected. A search of the patient's belongings yielded evidence that the "hematuria" was being feigned, together with a plentiful supply of apparent renal calculi, analyzed as struvite plus calcium carbonate. Confronted with the evidence, the patient appeared somewhat relieved and indicated the stones were of feline origin, collected from local veterinarians on the pretext of conducting research on cat stones. The motives for this subterfuge proved to be complex, but the patient has remained free of recurrence.