Kidney International, Vol. 29 (1986), pp. 563–571

Tubulopathy in nephrolithiasis: Consequence rather than cause

PHILIPPE JAEGER, LUC PORTMANN, JEAN-MARIE GINALSKI, ANNE-FRANÇOISE JACQUET, EVELYNE TEMLER, and PETER BURCKHARDT

Departments of Internal Medicine and of Radiology, University Hospital, Lausanne, Switzerland

Tubulopathy in nephrolithiasis: Consequence rather than cause. To address whether a renal tubular dysfunction is encountered in a particular patient subgroup with urolithiasis, the following parameters of tubular function were measured in urine taken in the morning from 214 stone formers after fasting: pH, excretion of lysozyme and γ-glutamyl transferase (γ-GT); fractional excretion (FE) of glucose, insulin, Mg, K, and HCO_3 after an alkali loading; and the renal threshold for phosphate (TmP/GFR). The following diagnoses were made in the patient group: primary hyperparathyroidism ($N = 8$), medullary sponge kidneys ($N = 21$), hyperuricemia ($N = 10$), cystinuria ($N = 2$), struvite stone disease ($N = 6$), idiopathic hypercalciuria of the absorptive ($N = 25$), dietary ($N = 69$) or renal ($N = 7$) type, and normocalciuric idiopathic urolithiasis ($N = 66$). In 31% of the patients TmP/GFR was below 0.80 mmole/liter and in 13% of the patients, FE HCO_3 after alkali loading was above normal. Urinary excretion of lysozyme and that of γ-GT both were elevated in 17% of the patients. FE glucose, FE insulin, FE Mg, and FE K were elevated in 8, 9, 3, and 7% of the patients, respectively. This study demonstrates that a significant number of stone formers present with signs of renal tubular dysfunction, primarily involving the proximal tubule since apparent leaks of phosphate and of bicarbonate were most frequently encountered. The defects were not specific for a given etiologic group of patients; on the other hand, occurrence was related to the presence of large stones in the pyelocaliceal system at the time data were gathered. Taken together these data suggest that the tubulopathy in nephrolithiasis is the *consequence* rather than the *cause* of the stone.

Previously, many investigators have regarded nephrolithiasis as the consequence of a renal leak of a lithogenic substance: Albright et al [1] postulated that a leak of calcium could be the first step of a cascade of events leading to renal stone formation and coined the term "idiopathic hypercalciuria"; Bordier et al [2] extended this concept postulating that a primary leak of phosphate could also lead to hypercalciuria and stone disease. Both concepts were supported by numerous investigators [3–9] and became widely accepted, although more recently many studies have challenged them [10–14]. In addition, evidence of various other tubular defects has been obtained in stone formers, leading to enzymurias [15–16], hyperinsulinuria [17], and renal tubular acidosis [18–21]. These observations support the view that overall proximal tubular function might be defective in nephrolithiasis [22].

The question of whether tubular defects could be the consequence of the disease, rather than the cause, is an issue unaddressed so far. Therefore, this study was undertaken to determine whether "leaky" tubules are a specific sign of a well-defined subgroup of idiopathic stone formers, such as renal hypercalciuria. The results support the view that tubular defects are common in many patients with nephrolithiasis, as a *consequence* of stone formation in the pyelocaliceal system.

Received for publication November 12, 1984,
and in revised form August 19, 1985

Methods

Classification of renal stone formers

This study involves 167 men (aged 21 to 76) and 47 women (aged 18 to 87) who passed one or more renal stones prior to enrollment in this study. The following ambulatory protocol was used to classify the cause of nephrolithiasis. Three 24-hr urine samples were collected when patients were on a free-choice diet, a calcium-enriched diet ($+ 3$ g $Ca \cdot day^{-1}$ for 3 days), and a low-calcium diet (< 400 mg $\cdot day^{-1}$) [23]. Aliquots of urine were analyzed for the concentration of Na, Ca, urate, and creatinine.

After the first urine collection (on the free-choice diet), a blood sample taken after the patients fasted was analyzed for total Ca, P, Mg, K, Cl, HCO_3, total protein, glucose, insulin, and, in some patients parathyroid hormone. A morning urine sample was obtained also after the patients fasted. Urine pH was measured and the sediment was examined microscopically. A urine culture was performed in patients with urogenital signs or symptoms and/or evidence by x-ray of stone formation in the pyelocaliceal system.

An intravenous pyelogram was performed in all patients and reviewed by a radiologist who was unaware of the clinical diagnosis for evidence of medullary sponge kidney. The diagnosis of medullary sponge kidney was based on the presence of pyramidal brush and/or pyramidal cysts in the absence of signs of obstruction [24–27]. The diagnosis was accepted if one single papilla was involved, provided the lesion was well-defined and there was no evidence of other caliceal lesions such as diverticula or pyelogenic cysts. The sole persistence of a papillary blush for 10 or more min after rapid injection of contrast medium (60 ml of 76% Urografin) was regarded as a minor form of medullary sponge kidney [28].

The following categories of stone formers were identified: primary hyperparathyroidism in eight patients (3 men/5 women), medullary sponge kidney (16 men/5 women), hyperuricemia with or without concurrent hyperuricosuria (10 men/0 women), cystinuria (1 man/1 woman), and struvite stone disease (1 man/5 women). The remaining 167 patients were

classified as idiopathic stone formers (ISF) and further categorized, based on urinary calcium excretion, as normocalciuric (53 men/13 women) or idiopathic hypercalciuric (IHC) (83 men/18 women). Among the 101 hypercalciuric patients, there were 25 (16/9) with absorptive hypercalciuria, 69 (62/7) with dietary hypercalciuria, and 7 (5/2) with renal hypercalciuria. The diagnosis of absorptive IHC required excessive calcium excretion on the high calcium diet, and normal calcium excretion on the low calcium diet. Dietary IHC had high calcium excretion rates either on free-choice diet only, or on the low calcium diet in the presence of excessive sodium and/or purine intake, and/or obesity with concurrent hyperinsulinemia. The term renal IHC was reserved for patients with hypercalciuria on the low calcium diet that could not be explained by any of the three previously mentioned factors.

Assessment of tubular function

Proximal and distal tubular function was estimated in stone formers by comparing the renal excretion of electrolytes and nonelectrolyte substances with normal values derived from healthy volunteers studied under the same conditions in our clinic. The following tests were performed: From urine and blood samples obtained under fasting conditions after a free-choice diet, the fractional excretion (FE) of insulin, glucose, K and Mg[1] were measured, as well as the urinary concentration of lysozyme and γGT divided by that of creatinine. FE x was derived from the ratio urinary concentration of x over the plasma concentration of x factored by the ratio plasma creatinine level over urinary creatinine concentration. The theoretical renal threshold for phosphate (TmP/GFR) was estimated from the nomogram of Walton and Bijvoet [30].

Urinary acidification was estimated from the plasma Cl and HCO_3 levels, and from the pH value of urine obtained in the morning from fasting subjects after a free-choice diet and/or the low-calcium diet. A NH_4Cl-loading test was performed on patients whose urine pH exceeded 5.85 on two occasions. Of 58 eligible subjects in this series, 37 agreed to have the analysis performed. Following the administration of NH_4Cl for 3 days (3–6 g day^{-1}, provided as 500-mg gelatine capsules), urinary acidification was determined from the pH value of fasting morning urine. In addition, FE HCO_3 was measured after 3 days of oral alkali loading administered as a mixture of Ca-gluconate and lactate (15.7 g · day^{-1}), and Ca-carbonate (2.4 g · day^{-1}). The combination of Ca-HCO_3 was chosen since HCO_3 alone would be expected to lower plasma ionized calcium, due to alkalinization, and stimulate PTH release; the latter would have led to increased excretion of bicarbonate. From a theoretical standpoint, this would have been problematic. Indeed, one group of idiopathic stone formers has been reported to have secondary hyperparathyroidism and the response of these patients to the hypocalcemic stimulus would be particularly marked, with apparent renal leak of bicarbonate. On the other hand, the oral administration of Ca-HCO_3 caused a Ca-induced depression of PTH secretion which was similar in control subjects and in each category of stone formers. This has been estimated from plasma PTH levels and nephrogenous cyclic AMP measurements (Jaeger, Bill, and Burckhardt, unpublished observations).

[1]Because a sizeable fraction of plasma Mg is protein-bound, calculation of the true FE Mg requires measurement of ultrafilterable Mg levels. However, in analogy with what has been demonstrated for Ca [29], ultrafilterable Mg can be considered to make up a reasonably constant fraction of total Mg; therefore, for the sake of the comparisons made herein, only this "apparent" FE Mg has been measured.

Determination of normal parameters of mineral metabolism and tubular function

Normal values used in this study are based on the data obtained in a population of 61 male (aged 24 to 66) and 21 female (aged 20 to 59) healthy volunteers. Upper and lower normal limits have been defined as the mean values ± 2 SD and are indicated in the legends to the figures, except for urinary excretion of calcium ($U_{Ca}V$), plasma level of Cl, and venous blood bicarbonate concentration for which they read as follows: upper normal limit of $U_{Ca}V$ at 280 mg/24 hr for men and 230 mg/24 hr for women on a free-choice diet, at 500 mg/24 hr for men and 400 mg/24 hr for women on the Ca-enriched diet, and at 250 mg/24 hr for men and 200 mg/24 hr for women on the low Ca diet; upper normal limit of plasma Cl concentration at 110 mmoles/liter and lower normal limit of venous blood HCO_3 at 22 mmoles/liter.

Chemical determinations

Lysozyme was measured in urine by turbidimetry according to a modification of the method of Litwack [31]. γ-Glutamyltransferase (γ-GT) was measured in urine according to the recommendations of the International Federation of Clinical Chemistry [32] and data expressed at 37°. In plasma and urine, glucose was measured by an enzymatic technique (hexokinase and glucose-6-phosphate dehydrogenase), and insulin according to Herbert et al [33] as modified by Ching et al [17]. Chloride and phosphate were measured by photometry with thiocyanate and mercuric chloride for the former, and molybdate without deproteinization and reading at 366 nm for the latter. Calcium and magnesium were measured by atomic absorption, sodium and potassium, by flame photometry, urate according to Kageyama, and creatinine according to Jaffé using an autoanalyser with dialysis. Blood acid-base status and urine PCO_2 were measured with an analyser (Radiometer ABL3), and urine pH with a pH-meter (Radiometer PHM 62).

Statistical analysis

Significance of differences between means has been tested applying Student's t test for unpaired data. Significance of the differences of incidence rates has been tested applying a χ^2 test with Yates correction.

Results

The individual values of fasting urinary excretion of lysozyme and γ-GT are depicted in Figures 1 and 2, respectively. For each parameter, values above normal were encountered in 17% of the whole population of patients. The incidence of the lysozyme "leak" was different among the various subgroups of stone formers: 9.5% in the normocalciuric patients, 66% in those with renal hypercalciuria ($P = 0.001$), and 100% in patients with struvite calculi ($P < 0.001$). Regarding the excretion of γ-GT, no such differences could be observed.

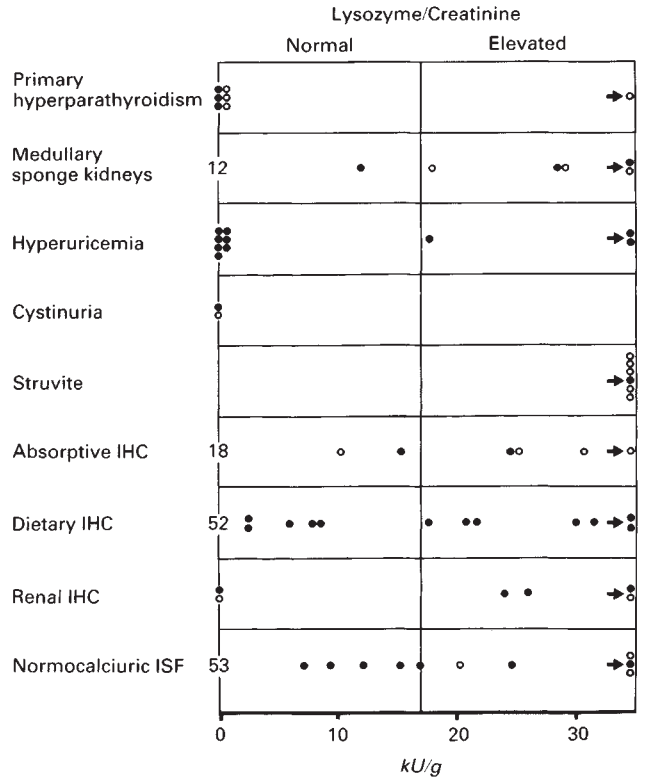

Fig. 1. *Individual values of fasting urinary excretion of lysozyme in male* (●) *and female* (○) *stone formers of the various subgroups.* The *vertical line* at 17 kU/g creatinine represents the upper normal limit for this parameter based on measurements performed in 33 normal subjects. The *numbers* on the left indicate the number of patients whose urinary lysozyme was undectectable. Abbreviations are: IHC, idiopathic hypercalciuria; ISF, idiopathic stone formers.

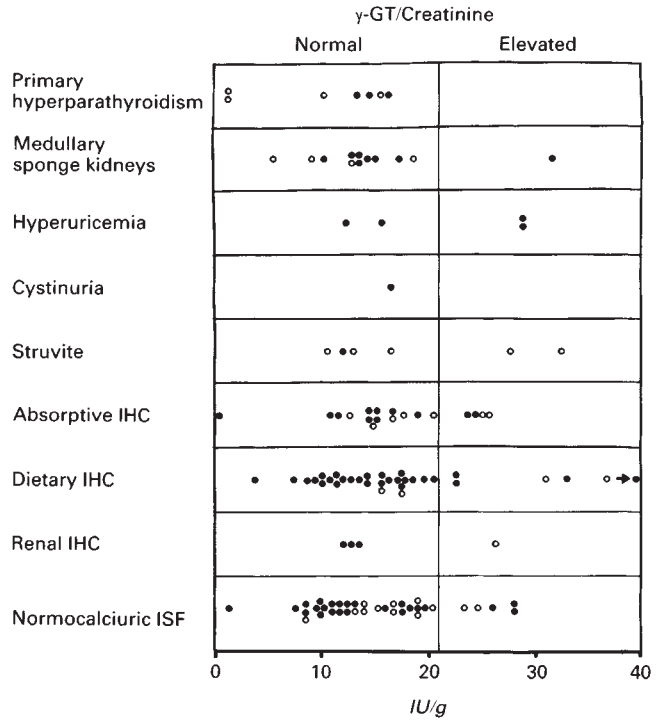

Fig. 2. *Individual values of fasting urinary excretion of γ-GT in male* (●) *and female* (○) *stone formers.* The *vertical line* at 21 IU/g creatinine indicates the upper normal limit for this parameter based on measurements performed in 33 normal subjects. Abbreviations are in Figure 1.

The individual values of fasting fractional excretion of glucose and insulin are depicted in Figures 3 and 4, respectively. For each of these parameters, values above normal were relatively infrequent, (approximately 8% of the total population), without predilection for a particular subtype of patients.

Individual values of TmP/GFR are depicted in Figure 5. Except for patients with primary hyperparathyroidism, about one third of the patients had a low renal threshold for phosphate, regardless of the cause of stone formation. The incidence of low TmP/GFR was twice as high in patients with primary hyperparathyroidism (63%) than in patients of the other groups.

In an effort to study function in more distal parts of the nephron of these patients, the fractional excretion of Mg and K were measured and the results are shown in Figures 6 and 7, respectively. Although an abnormality in these parameters was rare in the total population of stone formers, about 3 and 7%, respectively, the incidence was significantly higher in patients with struvite calculi: $P < 0.005$ and $P = 0.01$ for FE Mg and FE K, respectively.

To measure urinary acidification function of these patients when they fasted, urine pH was measured on 2 different mornings (that is, after free-choice and low calcium diets). Whereas in normal subjects urine pH was always below 5.85 (at least on one occasion), it was above this value in 28% of the patients tested. Figure 8 shows that a high fasting urine pH was

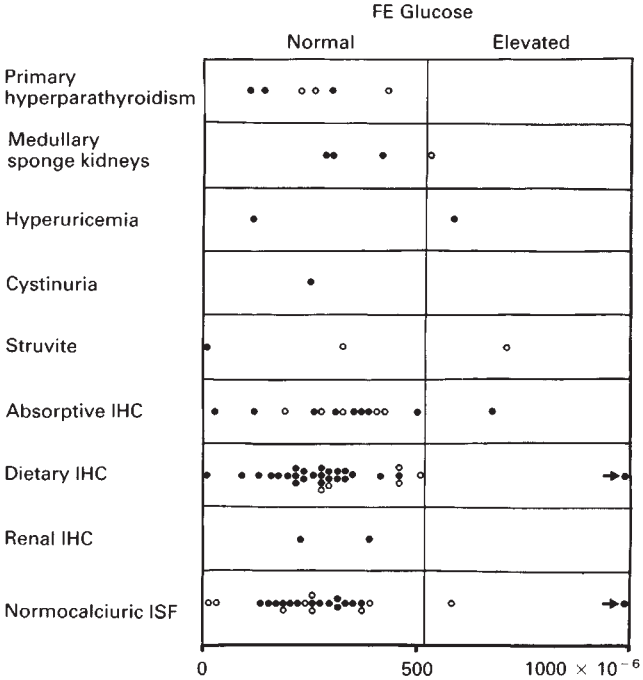

Fig. 3. *Fractional excretion* (FE) *of glucose in fasting urine in male* (●) *and female* (○) *stone formers.* The *vertical line* at 525.10^{-6} indicates the upper normal limit for this parameter based on measurements performed in 30 normal subjects. Abbreviations are in Figure 1.

more common in patients with struvite calculi (100% of the patients, $P < 0.0005$), and with primary hyperparathyroidism

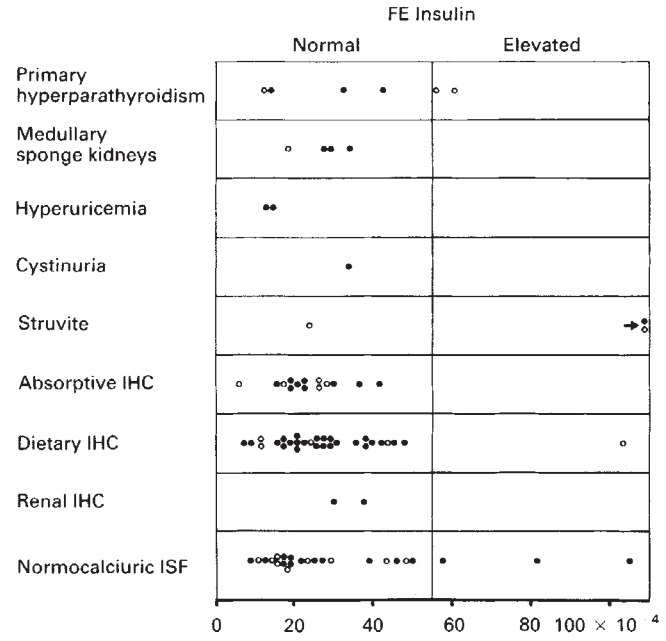

Fig. 4. *Fractional excretion* (FE) *of insulin in fasting urine in male* (●) *and female* (○) *stone formers.* The *vertical line* at 55.10^{-4} represents the upper normal limit for this parameter based on measurements performed in 30 normal subjects. Abbreviations are in Figure 1.

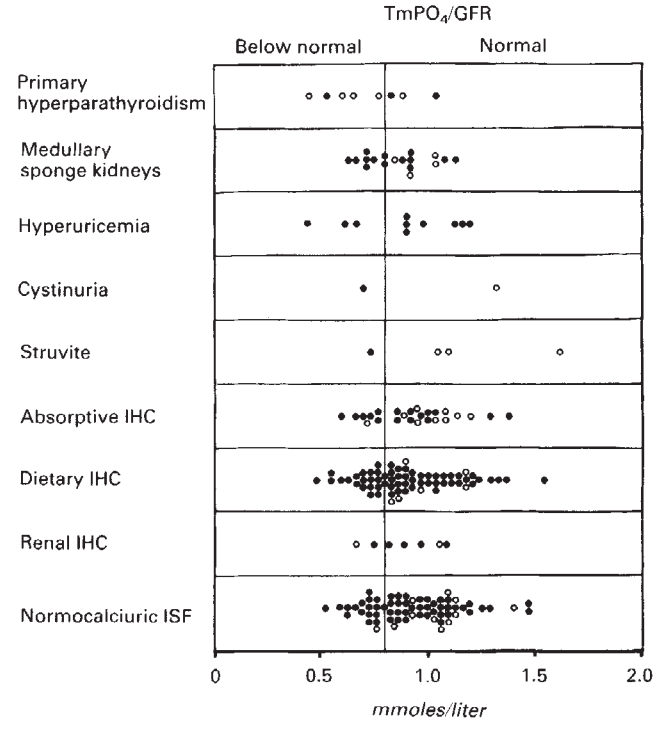

Fig. 5. *Individual values of theoretical renal threshold for phosphate (TmP/GFR) in male* (●) *and female* (○) *stone formers.* The *vertical line* at 0.80 mmole/liter indicates the lower normal limit for this parameter based on measurements performed in 46 normal subjects. Abbreviations are in Figure 1.

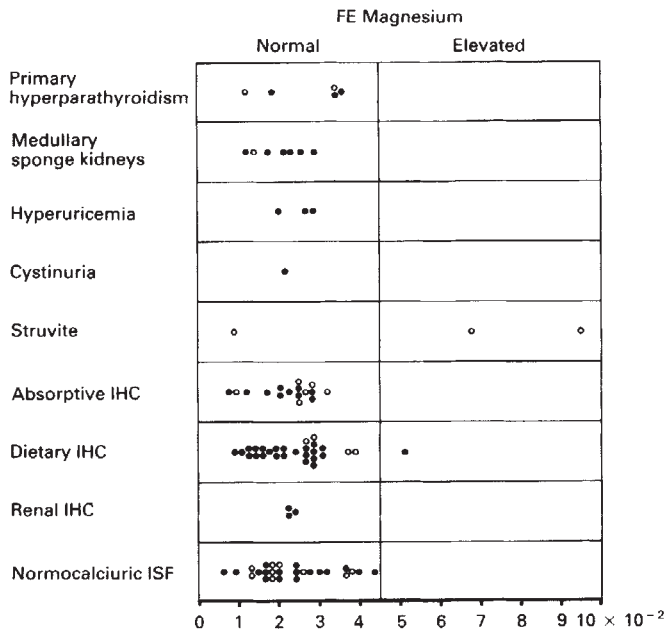

Fig. 6. *Fractional excretion* (FE) *of magnesium in fasting urine in male* (●) *and female* (○) *stone formers.* The *vertical line* at $4.5 \cdot 10^{-2}$ indicates the upper normal limit for this parameter based on measurements performed in 33 normal subjects. Abbreviations are in Figure 1.

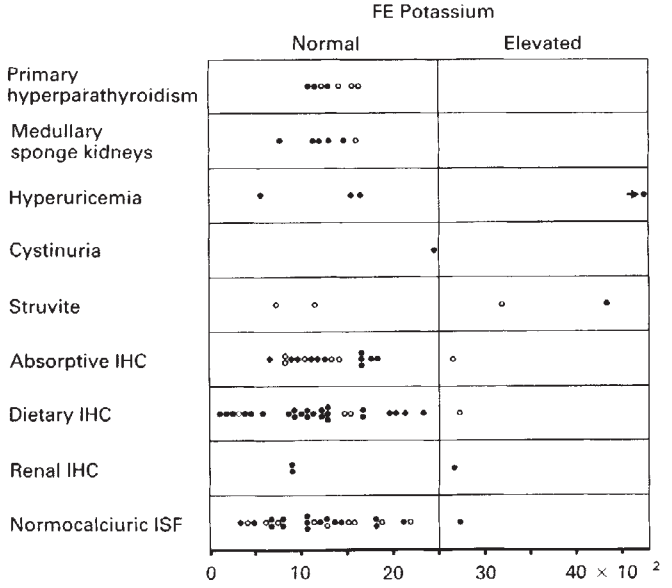

Fig. 7. *Fractional excretion* (FE) *of potassium in fasting urine in male* (●) *and female* (○) *stone formers.* The *vertical line* at $25 \cdot 10^{-2}$ indicates the upper normal limit for this parameter based on measurements performed in 32 normal subjects. Abbreviations are: IHC, idiopathic hypercalciuria; ISF, idiopathic stone formers.

(86% of the patients tested, $P = 0.003$) than in the rest of the population. On the other hand, no hyperuricemia patient had an elevated urine pH, an observation reported by others [34–36]. A high fasting urine pH was also uncommon in the normocalciuric patients, compared with the rest of the population ($P = 0.001$).

To further evaluate the cause of the high urine pH encountered in approximately one third of the stone formers, a NH_4Cl-loading test was performed in most of the latter patients; the results are depicted in Figure 9. In all the patients tested but

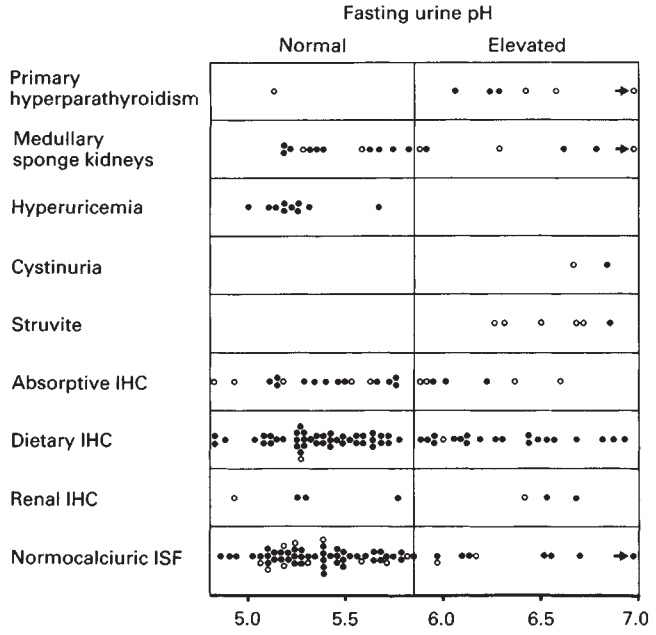

Fig. 8. *Individual values of fasting urine pH (lowest value of two measurements) in male* (●) *and female* (○) *stone formers.* The *vertical line* at 5.85 indicates the upper normal limit for this parameter based on measurements performed in 33 normal subjects. See Figure 7 for abbreviations.

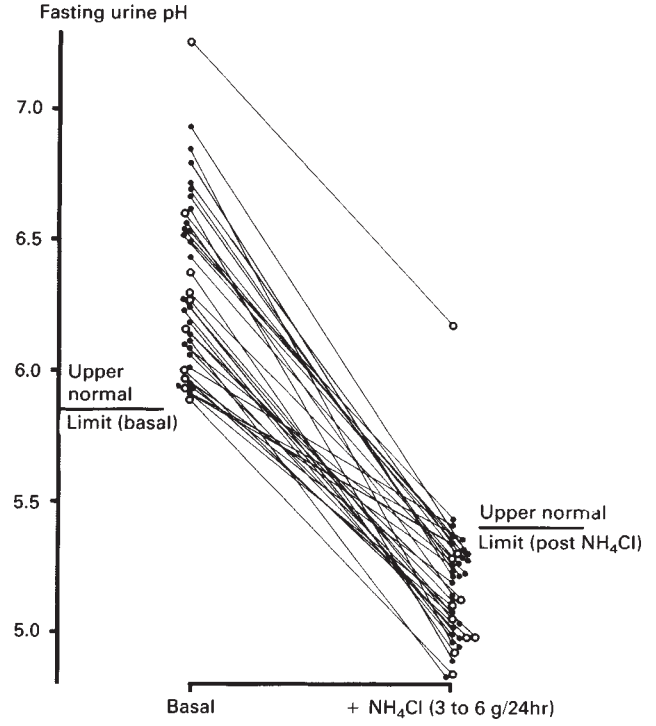

Fig. 9. *Individual values of fasting urine pH before and after 3 days on NH_4Cl in 27 male* (●) *and 10 female* (○) *stone formers.* The upper normal limit is based on measurements performed in 14 normal subjects.

one, fasting urine pH decreased to or below 5.4 after 3 days of NH_4Cl loading demonstrating normal distal acidification. One

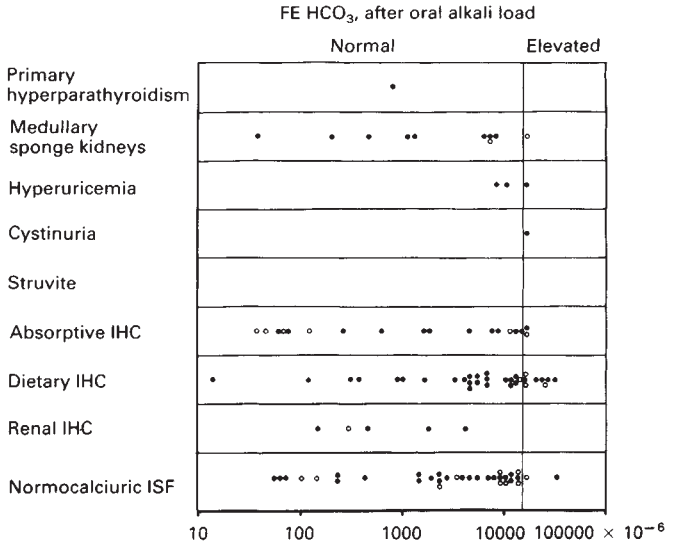

Fig. 10. *Fractional excretion* (FE) *of bicarbonate measured in fasting urine after 3 days of oral alkali loading in male* (●) *and female* (○) *stone formers.* The *vertical line* at $15000 \cdot 10^{-6}$ indicates the upper normal limit for this parameter based on measurements performed in 28 normal subjects. See Figure 7 for abbreviations.

patient, however, was refractory to both the chronic, as well as an acute NH_4Cl-loading test (0.1 g/kg over 30 min), despite development of a marked metabolic acidosis (arterial HCO_3 level at 15.8 mmoles/liter). She had medullary sponge kidneys, severe bilateral nephrocalcinosis, and overt metabolic acidosis. She was considered to have complete distal renal tubular acidosis (RTA). To evaluate proximal tubular acidification function, 102 of these patients were chronically loaded with a known amount of alkali, and fractional excretion of bicarbonate was measured. Data are depicted in Figure 10 and demonstrate that 13 patients had an apparent bicarbonate leak when submitted to this test, leading to the diagnosis of proximal RTA. All patients had a normal venous blood level of bicarbonate (> 22 mmoles/liter) and chloride (< 110 mmoles/liter). They were considered therefore to have the incomplete form of proximal RTA.

Finally, as an approach to potential causes of this renal tubular dysfunction in stone formers, the characteristics of patients with multiple tubular defects (that is, at least three; Table 1) were compared with those of patients in whom all the aforementioned parameters had been measured and found normal (Table 2). Fifteen patients exhibited multiple tubular defects and 17 exhibited no defect at all. As shown in the tables, both patient groups were similar in terms of age, sex, total number of stones ever formed, and number of stones newly formed over the last 2 years prior to the work-up, as well as nephrotomies in the past. The incidence of patients with a family history of nephrolithiasis was similar in both groups. In addition, both groups contained patients belonging to most of the various categories of stone formers. The only significant difference between the two patient groups was the number of stones present in the pyelocaliceal systems at the time of the work-up, which was significantly larger in the group with than in the group without tubular defects ($P < 0.05$). In particular the incidence of large (that is, > 2 cm diameter) pyelic stones was

Table 1. Characteristics of the 15 patients with multiple tubular defects

Patient Numbers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Compared with Table 2	
Sex	M	F	F	F	F	F	F	F	M	M	F	M	M	M	F	6 M/15	NS
Age, *years*	76	49	40	46	52	72	50	56	43	35	50	28	61	43	34	49.0 ± 3.4[a]	NS
Acid-base	x	x	x	x		x	x	x	x	x	x	x		x	x		
TmP/GFR	x						x	x	x			x	x	x	x		
Lysozyme	x	x	x	x	x	x	x			x		x	x	x	x		
γ-GT		x			x	x			x		x		x				
FE glucose			x	x	x					x			x				
FE insulin				x			x	x									
FE Mg				x		x											
FE K	x		x								x		x				
Diagnosis	NC	NC	NC	NC	NC	PHP	PHP	PHP	IHC-A	IHC-A	IHC-D	IHC-D	HU	HU	ICH-R		
Family history	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+	3+/15	NS
Nb of stones formed, *total*	3	3	2	2	1	2	5	2	6	>40	4	2	5	5	6	5.87 ± 2.47[a]	NS
Nb of stones formed, *last 2 years*	1	3	1	2	1	1	1	2	1	3	1	1	1	2	1	1.47 ± 0.19[a]	NS
Pyelocaliceal stone in place at time of analyses	1	2 (b)	1	2 (b)	0	1	1	2 (u)	2 (b)	0	0	1	0	0	1	0.93 ± 0.21[a]	$P < 0.05$
Large (i.e., >2 cm ø) calculi in place at time of analyses	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	5+/15	$P = 0.03$
Occurrence of previous nephrotomies	–	–	+	–	–	–	+	–	+	–	–	–	–	+	–	4+/15	NS

Abbreviations: Acid-base parameters, blood HCO_3 or plasma Cl concentrations, fasting urine pH, or fractional excretion of HCO_3 in fasting urine after alkali loading; X, indicates that the value of the parameters was abnormal; NC, normocalciuric idiopathic stone former; PHP, primary hyperparathyroidism; IHC, idiopathic hypercalciuria of the absorptive (A), dietary (D) or renal (R) type; HU, hyperuricemia; MSK, medullary sponge kidneys; (u), unilateral; (b), bilateral.

[a], mean ± SEM

significantly higher in the group with tubular defects ($P = 0.015$).

Discussion

This study confirms previous observations that renal tubular defects can be encountered in patients with nephrolithiasis [1–9, 15–22]; it shows, however, that this tubulopathy occurs with a rather even frequency in stone formers of any etiology or subgroup.

An apparent renal leak of phosphate is by far the most frequent of these findings. Thirty-one percent of all the patients studied had a low renal threshold for phosphate, and this defect was equally frequent among hypercalciuric and normocalciuric stone formers. The latter observation agrees well with Broadus et al [14] in whose recent study 36% of the nonhypercalciuric patients had a low TmP/GFR. It weakens the concept of phosphate leak as a cause of absorptive hypercalciuria via stimulation of $1,25(OH)_2D$ synthesis [2, 8]. However, it should be noted that a low TmP/GFR does not necessarily reflect an intrinsic renal tubular defect. It can be the consequence of hypersecretion of hormones such as in primary hyperparathyroidism; it might also be the consequence of dietary indiscretion such as a high glucose intake which has been shown to depress tubular phosphate reabsorption both in animals [37] and humans [38].

That many stone formers have a high urine pH has already been appreciated [39]. The high urine pH in patients with staghorn calculi reflects in part high ammonia generation via urea-splitting organisms. In patients with primary hyperparathyroidism, it probably reflects a PTH-induced renal leak of bicarbonate although the existence of this effect in a chronic condition remains to be proven. It may also be found in patients with a high dietary intake of alkali (such as heavy club soda drinkers or vegetarians). In hypercalciuric stone formers, however, it has classically been ascribed to a defect of the tubular acidification mechanisms involving the terminal part of the nephron, (distal RTA). In the present series, only one patient had distal RTA, whereas 13% of the stone formers tested had a high fractional excretion of bicarbonate after alkali loading, that is, proximal RTA. This observation is surprising, because it generally has been accepted that patients with proximal tubular RTA have neither hypercalciuria nor stone disease [40–42].

Table 2. Characteristics of the 17 patients without tubular defect

Patient Numbers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Sex	M	M	M	M	M	M	M	M	M	F	F	F	F	F	F	M	M	11 M/17
Age, *years*	47	50	51	46	28	36	49	38	35	60	53	31	27	28	45	44	41	41.7 ±2.4[a]
Acid-base																		
TmP/GFR																		
Lysozyme																		
γ-GT																		
FE glucose																		
FE insulin																		
FE Mg																		
FE K																		
Diagnosis	HCI-D	HCI-D	HCI-D	HCI-D	HCI-D	HCI-D	HCI-A	HCI-A	HCI-A	HCI-A	HCI-A	NC	NC	NC	NC	HCI-R	MSK	
Family history	+	+	−	−	+	−	−	+	+	−	−	−	−	+	−	+	+	8+/17
Nb of stones formed, *total*	2	10	3	4	2	1	2	2	8	5	6	1	9	6	14	17	1	5.18[a] ±1.14
Nb of stones formed, *last 2 years*	1	4	2	1	1	1	1	1	2	5	3	1	3	6	1	2	1	2.12[a] ±0.38
Pyelocaliceal stone in place at time of analyses	0	1	0	0	0	0	0	0	0	3 (u)	0	0	0	0	1	0	0	0.29[a] ±0.19
Large (i.e., >2 cm ø) calculi in place at time of analyses	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	0+/17
Occurrence of previous nephrotomies	−	−	+	−	−	−	+	−	−	+	+	−	−	−	−	−	−	4+/17

For abbreviations, see Table 1.

Indeed, delivery to the distal tubule of either bicarbonate or citrate is increased in these patients, the former stimulating distal calcium reabsorption [43] and the latter lowering the risk of precipitation of calcium salts in tubular fluid [44]. Reasons for this apparent discrepancy have not been reached, although a similar observation has recently been made by others [20]. One explanation could be that the defect was present in its incomplete form, that is, without overt metabolic acidosis; indeed, in incomplete proximal RTA bicarbonate leakage persists because the blood bicarbonate level remains above renal threshold; this, in turn, leads to high urine pH, and by that to an increased risk of precipitation of calcium phosphate.

The observation of renal leaks of lysozyme, γ-glutamyl transferase, glucose, and insulin deserves a particular mention. Whereas the apparent phosphate and bicarbonate leakages can always be ascribed, at least in part, to dietary indiscretions, the latter set of data, obtained from patients in the fasting condition, cannot. This leads to the conclusion that in stone formers of any subgroup, various transport systems of the proximal tubule are often defective. The fact that measurable amounts of lysozyme, insulin, and glucose appear in final urine reflects damage to the early portion of the proximal tubule, that is, the pars convoluta, since all of them are freely filtered but entirely reabsorbed along this part of the nephron [45–48]. On the other hand, the presence of γ-GT in urine probably reflects a damage to a more terminal portion of the proximal tubule; indeed, γ-GT is a high molecular weight unfiltered hydrolase contained in the brushborder membrane of the proximal tubule, with particularly high density in the pars recta [49–50].

Concerning lysozyme excretion, it could have been argued that the high urinary levels seen in patients with struvite stones could have been liberated from leukocytes in the urine rather than arising from tubular epithelium. In fact, this question has been answered by a study which showed that lysozyme is undetectable in patients with infection of the lower urinary tract despite the presence of severe leukocyturia [51]. The different appearance of these markers of proximal tubular dysfunction could be partially explained by differences in sensitivity of the various transport systems to the renal "nox." However, it should also be emphasized that in this study, all the analyses were performed on samples taken while patients fasted, that is, when plasma levels are very close to the renal threshold concentrations. For example, deriving the fractional excretions of glucose and insulin from 24-hr urine collections instead of from morning urine samples from fasting patients would lead to unmask a larger number of patients with glucose or insulin

leaks. For insulinuria, for instance, this would probably have yielded an incidence rate closer to that reported by Ching et al [17]. To see whether portions of the nephron beyond the pars recta participate in the tubulopathy of the stone formers, fasting fractional excretion of Mg and K have also been measured as function markers of the thick ascending limb of the loop and the terminal nephron, respectively. Contrary to what has been stated recently [22, 52], magnesium leaks appear to be exceptional in stone formers, an observation which is easier to reconcile with the classic views on lithogenesis. The leak of potassium is less exceptional; it has been encountered in various subgroups of stone formers with a frequency similar to that of glucose and insulin. Its meaning is uncertain, but it could reflect a rather rare damage of the terminal nephron of some patients with nephrolithiasis.

Taken together, these data show that renal tubular damages are frequently encountered in stone formers, irrespective of the subgroup to which the patient belongs. These lesions lead primarily to a dysfunction of the proximal tubule of which several transport systems are involved.

With respect to the cause of the tubulopathy, two hypotheses were considered: *(1)* Either the transport defects are the primary event and the stones secondary, or *(2)* the stones come first and the tubular defects ensue. Because the leaky tubules were found in stone disease of any cause but not in one specific subgroup such as the idiopathic nephrolithiasis, and because patients with multiple tubular defects were precisely those with numerous and large stones within the pyelocaliceal system at the time of the analyses, it is concluded that the tubulopathy encountered in nephrolithiasis is most probably the *consequence* rather than the *cause* of the stone.

Acknowledgments

This work has been supported in part by the Swiss National Science Foundation, grant No 3.923.0.83. This work was presented in part at the Annual Meeting of the Swiss Society of Internal Medicine, Lugano 1984. The authors thank Prof. J. P. Hayslett (Yale University School of Medicine) for critical review of the manuscript.

Reprint requests to Dr. P. Jaeger, Department of Internal Medicine, University Hospital (CHUV), CH-1011 Lausanne, Switzerland

References

1. Albright F, Henneman P, Benedict PH, Forbes AP: Idiopathic hypercalciuria. A preliminary report. *Proc Roy Soc Med* 46:1077–1081, 1953
2. Bordier P, Ryckewart A, Gueris J, Rasmussen H: On the pathogenesis of so-called idiopathic hypercalciuria. *Am J Med* 63:398–409, 1977
3. Muldowney FP, Freaney R, Ryan JG: The pathogenesis of idiopathic hypercalciuria: evidence for renal tubular calcium leak. *Q J Med New Series XLIX* 193:87–94, 1980
4. Coe FL, Canterbury JM, Firpo JJ, Reiss E: Evidence for secondary hyperparathyroidism in idiopathic hypercalciuria. *J Clin Invest* 52:134–142, 1973
5. Pak CYC, Kaplan RA, Bone H, Townsend J, Waters O: A simple test for the diagnosis of absorptive, resorptive and renal hypercalciurias. *N Engl J Med* 292:497–500, 1975
6. Aubert J, Ulmann A, Lacour B, Bader C, Funck-Brentano JL: Mise en évidence d'un trouble de la conservation rénale du phosphore dépendant du glucose chez des lithiasiques normo-ou hypercalciuriques. *Néphrologie* 1:159–162, 1980
7. Barilla DE, Zerwekh JE, Pak CYC: A critical evaluation of the role of phosphate in the pathogenesis of absorptive hypercalciuria. *Miner Electrolyte Metabol* 2:302–309, 1979
8. Shen FH, Baylink DJ, Nielsen RL, Sherrad DJ, Ivey JL, Haussler MR: Increased serum 1,25-dihydroxyvitamin D in idiopathic hypercalciuria. *J Lab Clin Med* 90:955–962, 1977
9. Wikström B, Backman U, Danielson BG, Fellström B, Hellsing K, Johansson G, Ljunghall S: Phosphate metabolism in renal stone formers. I. Indices of phosphate handling in calcium stone patients and healthy subjects. *Scand J Urol Nephrol* 61(suppl):I1–I18, 1981
10. Peacock M, Nordin BEC: Tubular reabsorption of calcium in normal and hypercalciuric subjects. *J Clin Pathol* 21:353–358, 1968
11. Muldowney FP, Treaney R, Moloney MF: Importance of dietary sodium in the hypercalciuria syndrome. *Kidney Int* 22:292–296, 1982
12. Yendt ER: Medullary sponge kidney and nephrolithiasis. *N Engl J Med* 306:1106–1107, 1982
13. Tschöpe W, Ritz E, Schmidt-Gayle H: Is there a renal phosphorus leak in recurrent renal stone formers with absorptive hypercalciuria? *Eur J Clin Invest* 10:381–386, 1980
14. Broadus AE, Insogna KL, Lang R, Mallette LE, Oren DA, Gertner JM, Kliger AS, Ellison AF: A consideration of the hormonal basis and phosphate-leak hypothesis of absorptive hypercalciuria. *J Clin Endocrinol Metab* 58:161–169, 1984
15. Baggio B, Gambaro G, Ossi E, Favaro S, Borsatti A: Increased urinary excretion of renal enzymes in idiopathic calcium oxalate nephrolithiasis. *J Urol* 129:1161–1162, 1983
16. Kregzde J, Lambert LL, Davidson WD: Lyzozymuria in renal calculosis following spinal cord injury. *Urol Int* 24:310–317, 1969
17. Ching KN, Karam JH, Choy FB, Kolb FO, Grodsky GM, Forsham PH: Hyperinsulinuria in patients with renal calculi. *Clin Chim Acta* 40:383–389, 1972
18. D'Angelo A, Pagano F, Ossi E, Lupo A, Valvo E, Messa P, Tessitore N, Maschio G: Renal tubular defects in recurring bilateral nephrolithiasis. *Clin Nephrol* 6:352–360, 1976
19. Cintron-Nadal E, Lespier LE, Roman-Miranda A, Martinez-Maldonado M: Renal acidifying ability in subjects with recurrent stone formation. *J Urol* 118:704–706, 1977
20. Backman U, Danielson BG, Johansson G, Ljunghall S, Wickström B: Incidence and clinical importance of renal tubular defects in recurrent renal stone formers. *Nephron* 25:96–101, 1980
21. Mateos F, Garcia Puig J, Gaspar G, Martinez ME, Ramos T, Martinez Pineiro JA: Renal tubular acidosis in recurrent renal stone formers. *Eur Urol* 10:55–59, 1984
22. Sutton RAL, Walker VR: Responses to hydrochlorothiazide and acetazolamide in patients with calcium stones. *N Engl J Med* 302:709–713, 1980
23. Jaeger P, Portmann L, Jacquet AF, Burckhardt P: Influence of the calcium content of the diet on the incidence of mild hyperoxaluria in idiopathic renal stone formers. *Am J Nephrol* 5:40–44, 1985
24. Lindvall N: Roentgenologic diagnosis of medullary sponge kidney. *Acta Radiol* 51:193–206, 1959
25. Palubinskas AJ: Renal pyramidal structure opacification in excretory urography and its relation to medullary sponge kidney. *Radiology* 81:963–970, 1963
26. Lang EK: Roentgenologic assessment of medullary cysts. *Sem Roentg* 9:145–154, 1975
27. Witten DM, Myers GH Jr, Utz DC: Renal cysts, in *Emmett's Clinical Urography* Vol. 3, Philadelphia, London, Toronto, W.B. Saunders, 1977, pp 1369–1466
28. Pinet A: Images d'addition des calices. *Rev d'Enseig Rad* 1:77–92, 1980
29. Transbøl I, Hornum I, Hahnemann S, Hasner E, Øhlenschlaeger H, Diemer H, Lockweed K: Tubular reabsorption of calcium in the differential diagnosis of hypercalcaemia. *Acta Med Scand* 188:505–522, 1970
30. Walton RJ, Bijvoet OLM: Nomogram for the derivation of renal threshold phosphate concentration. *Lancet* 2:309–310, 1975
31. Litwack G: Photometric determination of lysozyme activity. *Proc Soc Exp Biol Med* 89:401–403, 1955
32. Shaw LM, Strømme JH, London JL, Theodorsen L: IFCC methods for the measurement of catalytic concentration of enzymes. Part 4: IFCC method for γ-glutamyltransferase. *J Clin*

Chem Clin Biochem 21:633–646, 1983
33. HERBERT V, LAV KS, GOTTLIEB CW, BLEICHER SJ: Coated-charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–1384, 1965
34. YU TF, GUTMAN AB: Uric acid nephrolithiasis in gout: predisposing factors. *Ann Int Med* 67:1133–1148, 1967
35. FALLS WF JR: Comparison of urinary acidification and ammonium excretion in normal and gouty subjects. *Metabolism* 21:433–445, 1972
36. HOLMES EW JR: Uric acid nephrolithiasis, in *Nephrolithiasis*, edited by COE FL, BRENNER BM, STEIN JH, New York, Edinburgh and London, Churchill Livingstone, Chap 9, 1980 pp. 188–207
37. AUBERT J, TEMPLIER B, LACOUR B, CLAVEL J, ULMANN A: Modification in the renal phosphate handling after oral administration of glucose to conscious rats. *Miner Electrolyte Metabol* 10:98–102, 1984
38. ULMANN A, AUBERT J, BOURDEAU A, CHEYNEL CL, BADER C: Effects of weight and glucose ingestion on urinary calcium and phosphate excretion: implications for calcium urolithiasis. *J Clin Endocrinol Metab* 54:1063–1068, 1982
39. ROBERTSON WG, PEACOCK M, HEYBURN PJ, MARSHALL DH, CLARK PB: Risk factors in calcium stone disease of the urinary tract. *Br J Urol* 50:449–454, 1978
40. BRENES LG, BRENES JN, HERNANDEZ MM: Familial proximal renal tubular acidosis: a distinct entity. *Am J Med* 63:244–252, 1977
41. LEMAN J JR, WILZ DR, BRENES LG: Acid, calcium and phosphorus balances in proximal renal tubular acidosis *(abstract)*. *Kidney Int* 10:561, 1976
42. BRENNER RJ, SPRING DB, SEBASTIAN A, MCSHERRY EM, GENANT HK, PALUBINSKAS AJ, MORRIS RC JR: Incidence of radiographically evident bone disease, nephrocalcinosis and nephrolithiasis in various types of renal tubular acidosis. *N Engl J Med* 307:217–221, 1982
43. SUTTON RAL, WONG NLM, DIRKS JH: Effects of metabolic acidosis and alkalosis on sodium and calcium transport in the dog kidney. *Kidney Int* 15:520–533, 1979
44. MEYER JL, SMITH LH: Growth of calcium oxalate crystals. II. Inhibition by natural urinary crystal growth inhibitors. *Invest Urol* 13:36–39, 1975
45. BARRATT TM, CRAWFORD R: Lysozyme excretion as a measure of renal tubular dysfunction in children. *Clin Sci* 39:457–465, 1970
46. HARRISON, JF, LUNT GS, SCOTT P, BLAINEY JD: Urinary lysozyme, ribonuclease and low molecular-weight protein in renal disease. *Lancet* i:371–375, 1968
47. CHAMBERLAIN MJ, STIMMLER L: The renal handling of insulin. *J Clin Invest* 46:911–919, 1967
48. SPITZ IM, RUBENSTEIN AH, BERSOHN I, WRIGHT AD, LOWY C: Urine insulin renal disease. *J Lab Clin Med* 75:998–1005, 1970
49. HEINLE H, WENDEL A, SCHMIDT U: The activities of the key enzymes of the γ-glutamyl cycle in microdissected segments of the rat nephron. *FEBS Lett* 73:220–224, 1977
50. GUDER WG, ROSS BD: Enzyme distribution along the nephron. *Kidney Int* 26:101–111, 1984
51. WAUTERS JP, FAVRE H: L'intérêt de la mesure du lysozyme urinaire dans le diagnostic des néphropathies. *Schweiz Med Wschr* 100:1903–1907, 1970
52. BATAILLE P, PRUNA A, GREGOIRE I, FINET M, LEDEME N, GALY CL, DE FREMONT JF, FOURNIER A: Evidence for magnesium depletion in idiopathic hypercalciuria *(abstract)*. *Urol Res* 12:64, 1984