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Pathophysiology of incomplete renal tubular acidosis in recurrent renal stone formers: evidence of disturbed calcium, bone and citrate metabolism

P. J. Osther[1], J. Bollerslev[2], A. B. Hansen[3], K. Engel[3], P. Kildeberg[4]

Departments of [1]Urology, [2]Medical Endocrinology, [3]Clinical Chemistry, and [4]Pediatrics, Odense University Hospital, Odense, Denmark

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Summary. Urinary acidification, bone metabolism and urinary excretion of calcium and citrate were evaluated in 10 recurrent stone formers with incomplete renal tubular acidosis (iRTA), 10 recurrent stone formers with normal urinary acidification (NUA) and 10 normal controls (NC). Patients with iRTA had lower plasma standard bicarbonate after fasting ($P < 0.01$) and lower urinary excretion of titratable acid ($P < 0.05$) and citrate ($P < 0.01$) compared with NUA patients and NC, and higher urinary excretion of ammonia ($P < 0.05$) compared with NC ($P < 0.05$). Hypercalciuria was found in 6 of 10 patients with iRTA compared with 3 of 10 with NUA, and 0 of 10 NC. The citrate/calcium ratio in urine was significantly reduced in iRTA compared with the value in NUA ($P < 0.01$), and in NUA compared with NC ($P < 0.05$). Biochemical markers of bone formation (serum osteocalcin) and bone resorption (urinary hydroxyproline) were significantly increased in iRTA compared with NUA and NC ($P < 0.01$), indicating increased bone turnover in stone formers with iRTA. Stone formers with iRTA thus presented with disturbed calcium, bone and citrate metabolism – the same metabolic abnormalities which characterize classic type 1 RTA. Mild non-carbonic acidosis during fasting may be a pathophysilogical factor of both nephrolithiasis and disturbed bone metabolism in stone formers with iRTA.

Key words: Bone turnover – Calcium – Citrate – Renal stones – Renal tubular acidosis

Renal tubular acidosis (RTA) is a common designation of disturbances of the transtubular transport of hydrogen ions in which glomerular function is normal or close to normal. Traditionally, RTA is divided into "proximal" (type 2) and "distal" (type 1) types. Nephrolithiasis more commonly occurs in type 1 RTA (RTA-1), in which the maximal pH gradient across the epithelium of the collecting ducts is reduced [3, 4, 20, 22]. Even in conditions of acid loading the pH of the urine pH(U), will usually remain above 6 and the excretion of ammonium ions will be correspondingly limited.

The risks of RTA-1 in its fully developed form are well known: hyperchloraemic hypokalaemic acidosis associated with nephrocalcinosis and/or recurrent calcium nephrolithiasis and clinical bone disease [22]. Classic RTA-1 is a rare condition in patients with nephrolithiasis (< 1%) [14, 17]. Incomplete urinary acidification defects in which the characteristic tubular defect in hydrogen ion secretion occurs in the absence of metabolic (non-carbonic) acidosis [incomplete RTA (iRTA)] have, however, been reported with relatively high frequencies in recurrent calcium stone formers [1, 8, 14, 25, 27].

Non-carbonic acidosis has been considered to be the main pathogenetic factor of both the lithogenic constitution of the urine (hypercalciuria and hypocitraturia) and the metabolic bone disease (osteomalacia) in RTA-1, causing dissolution of bone salts, impaired tubular reabsorption of calcium and increased renal citrate consumption [17].

Hypercalciuria and hypocitraturia have also been proposed as the main lithogenic factors in iRTA [4]. The pathophysiological background for this is not fully understood. The fact that no cases of osteomalacia have been reported in stone formers with iRTA has indicated that mechanisms other than acidosis are involved in the lithopathogenesis of iRTA [4, 5]. In previous studies concerning the pathogenesis of stone formation in iRTA, bone metabolism has been rather neglected, however, and the existence of subclinical bone lesions cannot be excluded.

The aim of the present study was to examine the relation between urinary acidification, urinary excretion of calcium and citrate, and bone metabolism in recurrent calcium stone formers with iRTA.

Materials and methods

The study comprised three groups of participants: 10 recurrent renal stone formers with *iRTA* assessed by a standard short ammonium

Correspondence to: P. J. Osther, Institute of Clinical Research, Odense University Hospital, Kloevervaenget 10,3, DK-5000 Odense C

Table 1. Details of patients

	iRTA ($n=10$)	NUA ($n=10$)	NC ($n=10$)
Median age (years) (range)	35.5 (21–69)	50.5 (30–55)	41 (27–54)
Female/male ratio	5/5	5/5	5/5
Stone analyses: CaP/CaOxP	3/7	1/9	-

iRTA, Incomplete renal tubular acidosis; *NUA,* normal urinary acidification; *NC,* normal controls; *CaP,* calcium phosphate; *CaOxP,* calcium oxalate or mixture of calcium oxalate and CaP

chloride loading test; 10 recurrent renal stone formers with normal urinary acidification (*NUA*) assessed by a standard short ammonium chloride loading test; 10 healthy persons (normal controls, *NC*) who showed no evidence of disease. The participants did not receive any form of medical treatment. Recurrent stone disease was defined as two or more stones during the 5 years prior to the study.

Details of the three groups are presented in Table 1. Intravenous pyelography disclosed no signs of anatomical abnormalities or other urological disorders. None of the patients was immobilized or had a history of previous or actual gastrointestinal disorder. All stone patients (iRTA and NUA) had normal acid-base status in the normal (non-acid-loaded, non-fasting) condition. The procedure of the short ammonium chloride loading test was as described by Wrong and Davies [28]. The diagnostic criteria of iRTA were (1) inability to lower pH(U) below 5.4 during ammonium chloride loading (2 mmol/kg body mass) and (2) normal plasma standard bicarbonate [$c\mathrm{HCO_3^-}(\mathrm{aB_s})$] in the normal condition. The minimum pH(U) during ammonium chloride loading was 6.39 (5.53–6.67) in iRTA and 4.92 (4.82–5.28) in NUA. There were no dietary restrictions. Patients with positive urine cultures were not examined.

Renal function was evaluated by serum creatinine, and in the stone patients also by diethylenetriamine pentaacetic acid (DTPA) clearance. Acid-base metabolism was, apart from the short ammonium chloride loading test, evaluated by $c\mathrm{HCO_3^-}(\mathrm{aB_s})$ after 8 h of fasting, the 24-h urinary excretion of total ammonia [$\dot{n}\mathrm{tNH_3(U)}$] and titratable acid, [$\dot{n}$TA(U)]. The 24-h urinary excretion of citrate, [$\dot{n}$Ci(U)], was also measured.

Bone formation was evaluated by fasting serum osteocalcin (S-OC) and total alkaline phosphatase (S-AP). Bone resorption was evaluated by 24-h urinary excretion of hydroxyproline [$\dot{n}$HP(U)]. Calcium and phosphate metabolism were evaluated by serum ionized calcium ($\mathrm{S\text{-}Ca^{2+}}$) serum parathyroid hormone, [S-PTH(64-85), serum phosphate (S-P) and the 24-h urinary excretion of total calcium, [$\dot{n}$tCa(U)] and of total phosphorus [$\dot{n}$tP(U)]. Hypercalciuria was defined as $\dot{n}\mathrm{tCa(U)} > 0.1$ mmol/kg body mass in 24 h.

S-OC was determined by radioimmunoassay (Medicinsk Laboratorium, Copenhagen). $\dot{n}$HP(U) was measured by the method described by Pødenphant et al. [16]. $\mathrm{S\text{-}Ca^{2+}}$ was measured by a calcium-ion-selective electrode (ICA 1, Radiometer, Copenhagen) employing a built-in correction for pH. S-PTH was determined by radioimmunoassay. Titration according to Jørgensen [9] was used for the measurement of $\dot{n}$TA(U) and $\dot{n}\mathrm{tNH_3(U)}$. The titration was carried out to an end-point at $\mathrm{pH(U)} = 7.40$ and $Pco_2 = 0$, which means that cTA(U) equals the concentration of non-carbonic acid in urine [cNCA(U) [10]. pH(U) was measured immediately after sampling using a pH meter (Radiometer, Copenhagen). The urinary concentration of phosphorus [ctP(U)] was measured according to Baginski et al. [2], and the urinary concentration of citrate [cCi(U)] was determined by a commercially available method using citrate lyase prepared from *Aerobacter aerogenes* [26]. All other analyses were performed using standard laboratory methods. Data on S-OC, S-PTH and S-P were not available in the control group (NC).

Quantities are presented either as complete symbols, e.g. $c\mathrm{Na^+(S)}$, or in abbreviated form, e.g. $\mathrm{S\text{-}Na^+}$.

Statistical methods

All measurements are given as the median, with 95% confidence intervals (CI) of the median in parentheses. Differences were compared using the Mann-Whitney rank sum test. A probability value of $P < 0.05$ was considered significant.

Table 2. Acid-base data

	iRTA ($n=10$)		NUA ($n=10$)		NC ($n=10$)		P
	Median	95% CI	Median	95% CI	Median	95% CI	
Blood							
Fasting $c\mathrm{HCO_3^-}(\mathrm{aB_s})$ (mmol/l)	19	18/21	23	20/25	-	-	<0.01
Urine							
$\dot{n}$TA(U) (mmol/24 h)	-1.6	-37.2/12.4	14.2	1.3/23.6	12.4	4.8/18.3	<0.05[a] <0.05[b] n.s.[c]
$\dot{n}\mathrm{tNH_3(U)}$ (mmol/24 h)	47.6	27.5/72.5	36.8	27.3/56.6	29.4	18.3/41.3	n.s.[a] <0.05[b] n.s.[c]
$\dot{n}$Ci(U) (mmol/24 h)	0.9	0.2/2.7	2.3	1.5/3.6	2.6	2.0/2.9	<0.05[a] <0.05[b] n.s.[c]

[a] iRTA vs NUA; [b] iRTA vs NC; [c] NUA vs NC

95% CI, 95% confidence interval; $c\mathrm{HCO_3^-}(\mathrm{aB_s})$, plasma standard bicarbonate; $\dot{n}$*TA(U),* 24-h urinary excretion of titratable acid; $\dot{n}\mathrm{tNH_3(U)}$, 24-h urinary excretion of total ammonia; $\dot{n}$*Ci(U),* 24-h urinary excretion of citrate; *n.s.,* not significant

Table 3. Renal function

	iRTA ($n=10$)	NUA ($n=10$)	NC ($n=10$)
Serum creatinine (μmol/l) (range)	84.5 (73–159)	85.0 (73–103)	74.0 (58–88)
DTPA clearance (ml/1.73 m per minute) (range)	85 (53–122)	88 (76–101)	–

DTPA, Diethylenetriamine pentaacetic acid

Results

Acid-base data

The stone patients with iRTA had a significantly lower $cHCO_3^-(aB_s)$ after 8 h of fasting than the patients with NUA: 19 (18–21) mmol/l and 23 (20–25 mmol/l respectively ($P<0.01$) (Table 2).

$\dot{n}$TA(U) was significantly reduced in iRTA compared with the values in NUA and NC ($P<0.05$). No difference in $\dot{n}$TA(U) between the latter two groups was observed (Table 2). $\dot{n}tNH_3(U)$ was significantly increased in iRTA compared with NC ($P<0.05$); when compared with NUA the difference was insignificant (Table 2). $\dot{n}$Ci(U) was significantly decreased in iRTA compared with NUA and NC ($P<0.05$).

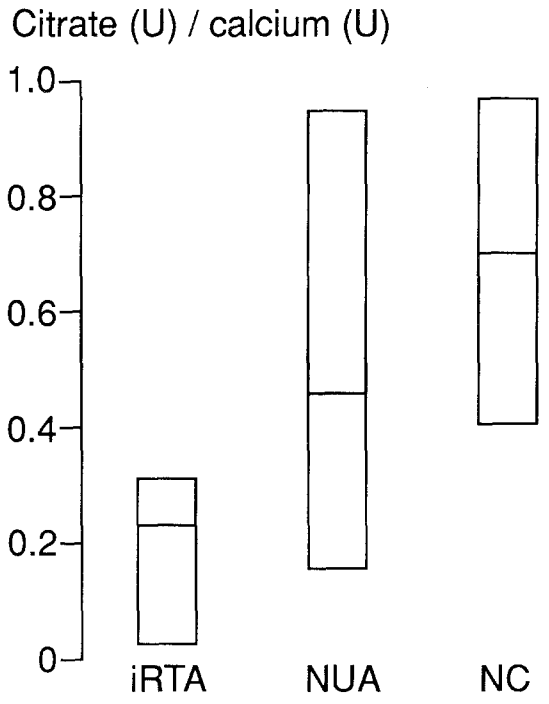

Fig. 1. Molar citrate/calcium ratio in urine. *Horizontal bars* represent medians; *framed areas* 95% confidence intervals of the medians *iRTA,* incomplete renal tubular acidosis; *NUA,* normal urinary acidification; *NC,* normal controls

Table 4. Calcium and bone metabolic data

	iRTA ($n=10$)		NUA ($n=10$)		NC ($n=10$)		P
	Median	95% CI	Median	95% CI	Median	95% CI	
Blood							
S-Ca^{2+} (mmol/l)	1.27	1.23/1.30	1.28	1.22/1.31	1.22	1.20/1.33	n.s.[a] n.s.[b] n.s.[c]
S-P (mmol/l)	1.08	0.99/1.20	1.00	0.70/1.23	–	–	n.s.
S-AP (U/l)	153	89/216	115	89/185	–	–	n.s.
S-PTH (pmol/l)	44	23/64	40	29/46	–	–	n.s.
S-OC (μg/l)	7.3	6.2/16.2	6.1	3.8/7.4	–	–	<0.05
Urine							
$\dot{n}$tCa(U) (mmol/24 h)	8.1	3.3/11.5	5.5	3.4/8.0	3.4	2.9/4.7	n.s.[a] <0.01[b] <0.05[c]
$\dot{n}$tP(U) (mmol/24 h)	23.9	10.8/40.7	18.6	13.2/31.0	16.0	9.0/21.5	n.s.[a] n.s.[b] n.s.[c]
$\dot{n}$HP(U) (μmol/24 h)	340	217/1365	204	127/357	185	140/227	<0.05[a] <0.01[b] n.s.[c]

[a] iRTA vs NUA; [b] iRTA vs NC; [c] NUA vs NC

S-Ca^{2+}, Serum ionized calcium; *S-P,* serum phosphate; *S-AP,* serum total alkaline phosphatase; *S-PTH,* serum parathyroid hormone; *S-OC,* serum osteocalcin; *$\dot{n}$tCa(U),* 24-h urinary excretion of calcium; *$\dot{n}$tP(U),* 24-h urinary excretion of total phosphorus; *$\dot{n}$HP(U),* 24-h urinary excretion of hydroxyproline

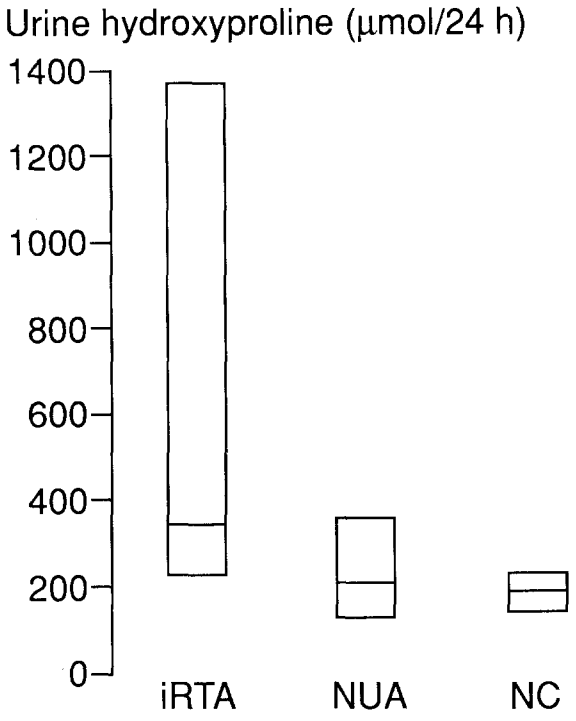

Fig. 2. The 24-h urinary excretion of hydroxyproline (µmol/24 h). *Horizontal bars* represent medians; *framed areas* 95% confidence intervals of the medians

Renal function

There were no differences in serum creatinine between the three groups, and no difference in DTPA clearance between the iRTA and NUA groups (Table 3).

Calcium-bone metabolic data

$\dot{n}$Ca(U) was higher in iRTA ($P<0.01$) and NUA ($P<0.05$) than in NC. Hypercalciuria was found in 6 of 10 (26–88%) of the iRTA group and in 3 of 10 (12–74%) of the NUA group. None of the NC group had hypercalciuria (Table 4).

The molar citrate/calcium ratio [Ci(U)/Ca(U)] was reduced in iRTA compared with NUA ($P<0.01$), and reduced in NUA compared with NC ($P<0.05$) (Fig. 1). There were no differences in $\dot{n}$tP(U) between the three groups (Table 4).

S-OC was elevated in iRTA compared with NUA ($P<0.05$). $\dot{n}$HP(U) was also elevated in iRTA relative to both NUA ($P<0.01$) and NC ($P<0.01$), indicating increased bone turnover in iRTA (Table 4, Fig. 2).

There were no differences in S-Ca^{2+}, S-P and S-PTH(65-84) between the three groups.

Discussion

The syndrome of iRTA was originally defined in three patients with generalized nephrocalcinosis who were unable to excrete a highly acid urine even after ammonium chloride loading [28]. The absence of hyperchloraemic acidosis was explained by the ability of the patients to excrete large amounts of ammonia [23, 28]. Later this definition was broadened to include other non-acidotic conditions with a tubular defect in the ability to lower urine pH, such as amphotericin nephropathy, hypergammaglobulinaemia, medullary sponge kidney, idiopathic hypercalciuria and calcium nephrolithiasis [1, 6, 7, 13, 14]. Our patients with recurrent calcium nephrolithiasis and urinary acidification defect seem to fit well within the frame of this broader classification of iRTA.

It is considered that RTA-1 enhances stone formation by three different risk factors: hypercalciuria, an alkaline urine and a low urinary citrate [4, 23]. However, the relative imporance of these is debatable. Hypocitraturia seems to be the most common risk factor for stone formation in RTA-1 as well as in iRTA [4, 5]. In the present study hypocitraturia ($\dot{n}Ci(U)_{24h} < 1.8$ mmol) was found in 8 of 10 calcium stone formers with iRTA, which is in accordance with other studies [7, 12]. The presence of hypocitraturia is thus very characteristic of iRTA but not an obligatory finding.

The reported prevalence of hypercalciuria in iRTA has varied from 23% to 100% [5, 21, 29]. We found hypercalciuria in 6 of 10 patients with iRTA, compared with 3 of 10 stone patients with NUA.

Since both hypocitraturia and hypercalciuria are characteristic findings in iRTA, it is not surprising that the molar citrate/calcium ratio in urine usually is very low (<0.35) in iRTA (Fig. 1). This index might prove to be a valuable diagnostic tool in the screening and follow-up of stone patients, since a low value not only predicts a high risk for calcium stone formation but also seems to indicate the possible presence of an underlying metabolic abnormality [15].

Osteomalacia is a well-recognized entity of classic RTA-1 [5, 7, 11]. The most widely accepted cause of bone disease in RTA-1 is non-carbonic acidosis, on the basis that clinical osteomalacia in RTA-1 has been shown to heal with base therapy alone [11, 19]. No cases of osteomalacia have been reported in patients with iRTA. This fact has led to the assumption that mechanisms other than acidosis are of importance in the pathophysiology of stone formation in iRTA (as well as in classic RTA-1) [4, 5]. The bone metabolism of stone formers with iRTA has, however, never been evaluated by any means other than radiography, which is not a sensitive technique, the existence of subclinical bone lesions cannot therefore be excluded. Using biochemical markers of bone formation and bone resorption, we found that stone formers with iRTA had evidence of increased bone turnover compared with stone patients with NUA and normal controls (Fig. 2, Table 4). One of the most conspicious findings in our patient data was the rather low values of plasma standard bicarbonate after fasting in stone formers with IRTA (Table 2). Thus, a possible explanation of disturbed bone metabolism in iRTA could be mild non-carbonic acidosis during fasting, which represents a relative acid load in most individuals. Because of the defect in tubular hydrogen ion secretion, stone formers with iRTA might intermittently (during fasting or after intake of food with concentrations of non-metabolizable base lower than normal) exist in a state of positive balance of non-

metabolizable acid, resulting in a slow attrition of skeletal stores of base, decreased tubular reabsorption of calcium and increased renal citrate consumption. This imbalance is periodically restored when the ingestion of food (normally rich in non-metabolizable base) during the day normalizes the extracellular acid-base status.

As can be seen in Table 3, one of the patients with iRTA had slightly decreased renal function as evaluated by serum creatinine level and DTPA clearance. However, no significant correlations between renal function tests and the biochemical markers of bone turnover in our group of patients with iRTA could be demonstrated. The role of renal insufficiency in the bone metabolism of iRTA patients cannot, however, be completely disregarded, and needs to be further evaluated. Furthermore, it should be mentioned that biochemical markers of bone formation and resorption such as serum osteocalcin and urinary hydroxyproline are indirect measures of bone metabolism, and future studies on bone metabolism in these patients might benefit from the use of more direct and sensitive techniques.

Our results nevertheless indicate that non-carbonic acidosis plays a role in the pathophysiology of renal stone formation even in iRTA. These observations support the original hypothesis, which referred to iRTA as a mild form of classic RTA-1 [28]. This hypothesis also explains why bicarbonate and citrate therapy have been successful in preventing stone recurrence in stone formers with iRTA [1, 18].

Histomorphometric and biochemical investigations in stone patients with idiopathic hypercalciuria have shown signs of bone mineralization defects [24]. It was proposed that a primary tubular phosphate leak could lead to hypophosphataemia, causing a defect in the mineralization process. Our patients with iRTA did not present with hypophosphataemia, and this cannot explain the bone metabolic disturbances in our cases. Furthermore, the acid-base status was not evaluated in the former study, and the presence of mild urinary acidification defects in those patients cannot therefore be excluded.

Our results suggest that moderate urinary acidification defects might play a role in the development of skeletal mineralization defects, as well as kidney stones, through moderate alterations in the extracellular acid-base status. Further studies on bone metabolism in calcium nephrolithiasis should therefore take the acid-base metabolism of the patients into consideration.

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References

1. Backman U, Danielson BG, Johanson G, Ljunghall S, Wikström B (1980) Incidence and clinical importance of tubular defects in recurrent renal stone formers. Nephron 25:96
2. Baginski ES, Foà PP, Zak B (1967) Microdetermination of inorganic phosphate, phospholipids, and total phosphate in biological materials. Clin Chem 13:326
3. Battle D (1983) Renal tubular acidosis. Med Clin North Am 67:859
4. Buckalew VM Jr (1989) Nephrolithiasis in renal tubular acidosis. J Urol 141:731
5. Buckalew VM Jr, Caruana RJ (1985) The pathophysiology of distal (type 1) renal tubular acidosis. In: Gonick HC, Buckalew VM Jr. (eds) Renal tubular disorders: pathophysiology, diagnosis, and management. Dekker, New York, p 357
6. Buckalew VM Jr, McCurdy DK, Ludwig GD, Chaykin LB, Elkinton JR (1968) Incomplete renal tubular acidosis: physiologic studies in three patients with a defect in lowering urine pH. Am J Med 48:32
7. Caruana RJ, Buckalew VM Jr (1988) The syndrome of distal (type 1) renal tubular acidosis. Medicine (Baltimore) 67:84
8. Gault MH, Parfrey PS, Robertson WG (1988) Idiopathic calcium phosphate nephrolithiasis. Nephron 48:265
9. Jørgensen K (1957) Trimetric determination of the net excretion of acid/base in urine. Scand J Clin Lab Invest 9:287
10. Kildeberg P (1983) Acid-base status of biological fluids: amount of acid, kind of acid, anion-cation difference, and buffer value. Scand J Clin Lab Invest 43:103
11. McSherry E, Morris RC Jr (1978) Attainment and maintenance of normal stature with alkali therapy in infants and children with classic renal tubular acidosis. J Clin Invest 61:509
12. Nicar MJ, Skurla C, Sakhaee K, Pak CYC (1983) Low urinary citrate excretion in nephrolithiasis. Urology 21:8
13. Osther PJ, Hansen AB, Røhl HF (1988) Renal acidification defects in medullary sponge kidney. Br J Urol 61:392
14. Osther PJ, Hansen AB, Røhl HF (1989) Screening renal stone formers for distal renal tubular acidosis. Br J Urol 63:581
15. Parks JH, Coe FL (1986) A urinary calcium-citrate index for the evaluation of nephrolithiasis. Kidney Int 30:85
16. Pødenphant J, Larsen N-E, Christiansen C (1984) An easy and reliable method for determination of urinary hydroxyproline. Clin Chim Acta 142:145
17. Pohlman T, Hruska KA, Menon M (1977). Renal tubular acidosis. J Urol 132:431
18. Preminger GM, Sakhaee AK, Skurla C, Pak CYC (1985) Prevention of recurrent calcium stone formation with potassium citrate therapy in patients with distal renal tubular acidosis. J Urol 134:20
19. Richards P, Chamberlain MJ, Wrong OM (1972) Treatment of osteomalacia of renal tubular acidosis by sodium bicarbonate alone. Lancet II:994
20. Rodríguez-Soriano J, Vallo A, Castillo G, Oliveros R (1985) Pathophysiology of primary distal renal tubular acidosis. Int J Pediatr Nephrol 6:77
21. Schneeberger W, Hesse A, Vahlensieck W (1992) Recurrent nephrolithiasis in renal tubular acidosis: metabolic profiles, therapy and course. Urol Res 20:98
22. Sebastian A, Morris RC Jr (1977) Renal tubular acidosis. Clin Nephrol 7:216
23. Seldin DW, Wilson JD (1966) Renal tubular acidosis. In: Stanbury JB, Wyngaarden JB, Fredrickson DS (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, p 1230
24. Steinicke T, Mosekilde L, Christensen MS, Melsen F (1989) A histomorphometric determination of iliac bone remodeling in patients with recurrent renal stone formation and idiopathic hypercalcuria. APMIS 97:309
25. Tannen RL, Falls WF, Brackett NC (1975) Incomplete renal tubular acidosis: some clinical and physiological features. Nephron 15:111
26. Warty VS, Busch RP, Virji MA (1984) A kit for citrate in foodstuffs adapted for assay of serum and urine. Clin Chem 30:1231
27. Williams G, Chisholm GD (1976) Stone screening and follow-up are necessary? Br J Urol 47:745
28. Wrong O, Davies HEF (1959) The excretion of acid in renal disease. Q J Med 28:259
29. Wrong OM, Feest TG (1980) The natural history of distal renal tubular acidosis. Contrib Nephrol 21:137