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URINARY CYCLIC AMP IN VITAMIN D DEFICIENCY RICKETS*

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The advanced stage of vitamin D deficiency rickets is characterized by increased urinary loss of phosphate and amino acids. Although the pathogenesis of this tubular disorder is not well understood, the elevated plasma level of parathyroid hormone (PTH) has been implicated[1,2]. There is evidence to indicate that the phosphaturic response to infused PTH is markedly depressed[3] in vitamin D deficient rats while the calcium mobilization response of bone is either severely impaired[4] or completely absent[5]. The lack of vitamin D metabolites may also play a role, since animal experiments have demonstrated a PTH dependent action of 25-hydroxycholecalciferol on tubular reabsorption of phosphate[6]. The cyclic adenosine 3'-5' monophosphate (cAMP) has been shown to be an intermediate in the expression of PTH action on kidney[7] and bone[8]. It has been recognized that the concentration of calcium in the circulation is an important controlling factor for PTH secretion. Secondary hyperparathyroidism, which is known to occur in vitamin D deficiency rickets, is a consequence of the direct action of the plasma calcium level. For this reason the correlation between the calcium level and this nucleotide may reflect the interrelationship between the PTH and the urinary cAMP. Our study has been undertaken to determine the urinary excretion of cAMP and the correlation between the levels of serum calcium and this nucleotide level. Further, we investigated the effects of calcium infusion on the excretion of urinary cAMP, because the elevation of the serum calcium level suppresses PTH secretion. In addition the effect of vitamin D2 therapy on the excretion of this nucleotide was investigated.

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Material and Methods

Twenty-eight randomly selected infants with vitamin D deficiency rickets, their ages ranging from 4 to 24 months, were studied, and there was a control group of 11 age-matched, optimally nourished, healthy infants. None of these infants had received vitamin D prior to the study, nor had they symptoms of steatorrhea or renal disease. The diagnosis of vitamin D deficiency rickets was established by the usual radiological and biochemical criteria.

Procedures.

Both the control cases and the patients received a standard diet containing 1000 mg calcium and 600 mg phosphate per day for two days prior to testing which was performed as follows:

Day 1: Urine was collected over three 8-hour periods for 24 hours and stored at −20 °C until analyzed.

Day 2: After obtaining venous blood at 8 a.m. for determining the amount of plasma calcium and phosphorus, 20% calcium gluconate in saline (60 ml calcium gluconate in 360 ml saline per square meter of body surface area) was initiated for a 4-hour period. Blood samples were obtained at the end of infusion and at 8 a.m. the next morning. Urine was collected over three 8-hour periods as described for the first day. All cases received liquids of about 2000 ml per square meter of body surface area to provide sufficient hydration.

Following completion of the above procedures, 5,000 U oral vitamin D per day was initiated. When the rickets had been completely cured, 24-hour urine samples were collected so as to make a study of the excretion of cAMP (in 10 of the 28 patients who could be followed up).

Determinations

Twenty-four-hour urinary cAMP was determined by means of a radioimmunoassay kit (The Radiochemical Center, Amersham, Buckinghamshire, England). Serum calcium was measured with an atomic absorption spectrophotometer[9]; serum and urinary phosphate were essayed by the method of Gomori[10]. The phosphate clearance (C_p), the percentage of renal tubular reabsorption of phosphate (TRP %) and the phosphate creatinine clearance ratio (C_p/C_{cr}) in blood and urine[11] were calculated from the above-mentioned measurements.

Results

The basal levels of C_p, TRP % and C_p/C_{cr} in patients and control cases are shown in Figure 1. The rise in C_p as well as the fall in TRP % (25.17 ± 1.87 ml/min/1.73 m^2

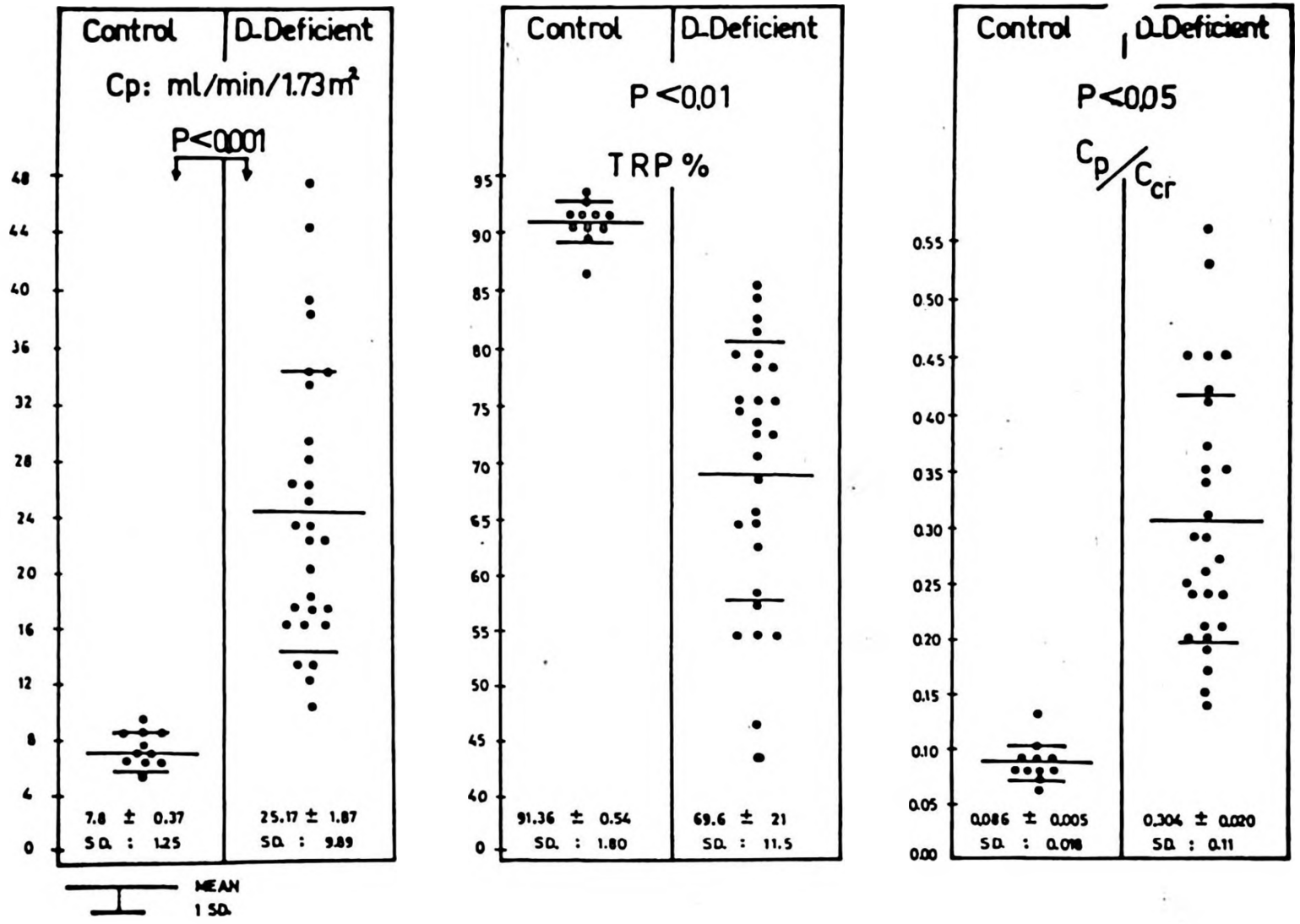

Figure 1.

Comparison of phosphate clearance, percentage of tubular reabsorption of phosphate and phosphate/creatinine clearance in vitamin D deficiency rickets patients with those of control subjects.

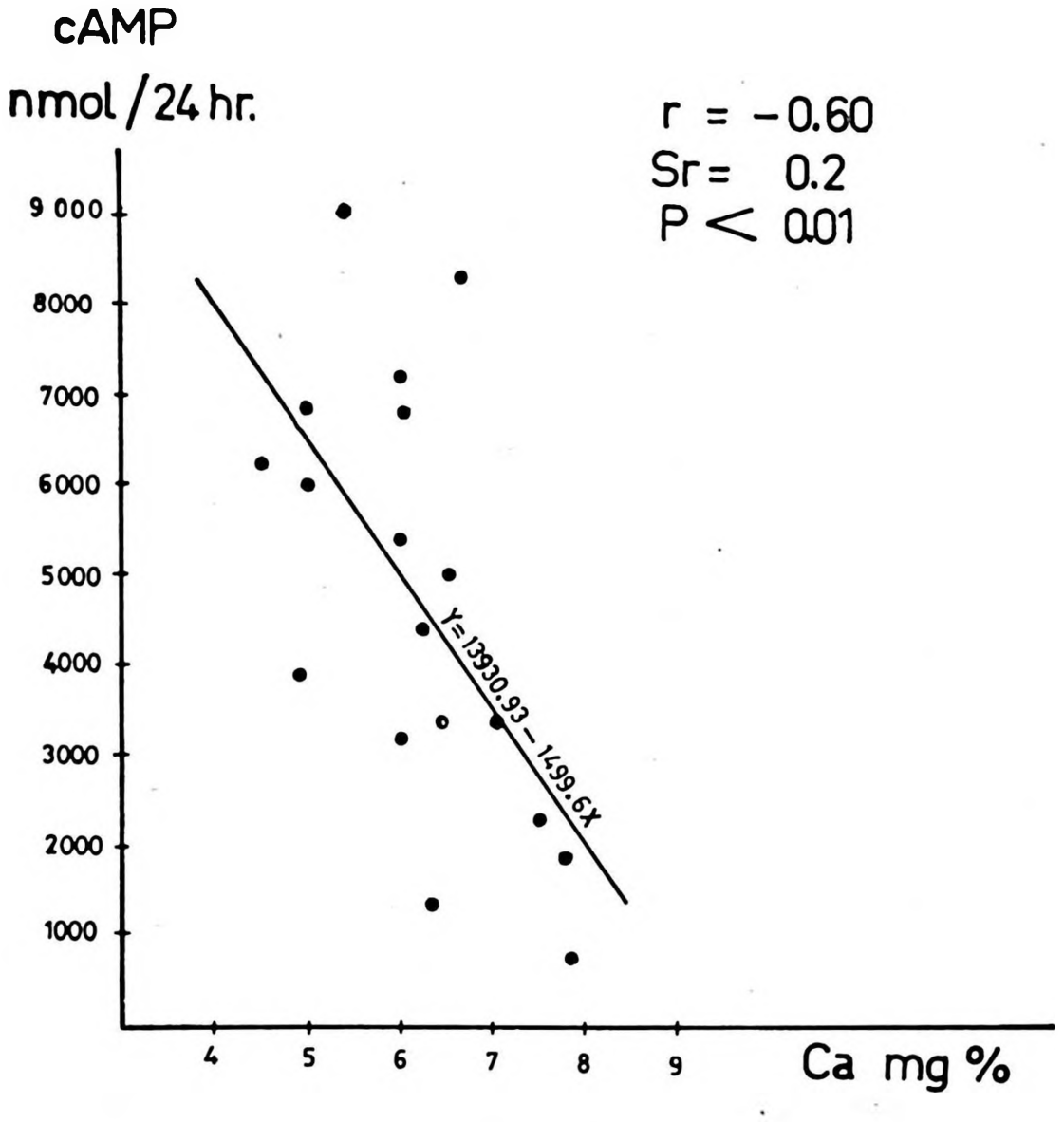

Figure 2.

Correlation between the serum calcium and urinary cAMP in patients with vitamin D deficiency rickets in whom serum calcium was below 8 mg/dl.

and 69.6 ± 21%, respectively) were strikingly different in patients with rickets as compared to controls (7.8 ± 0.37 ml/min/1.73 m^2 and 91.36 ± 0.54%, respectively). The C_p/C_{cr} ratio was considerably higher in the patients (0.304 ± 0.020) than in the control cases (0.086 ± 0.005). During the first day of the study the excretion of phosphate did not fluctuate significantly in separate 8-hour measurements. But during the second day the infusion of calcium markedly decreased the C_p in both groups (Table I).

The urinary cAMP excretion in control cases and patients is shown in Table I. The daily excretion was elevated 2.5-fold in patients with rickets. A negative correlation between the serum calcium levels and daily urinary excretion of cAMP was observed

TABLE I

Phosphate Clearance and Urinary Excretion of cAMP in Control Cases and Patients

Patients and Condition		Phosphate clearance (ml/min/1.73 m^2) Mean ± S.E.M. (SD)	3'-5' cyclic AMP in urine (nmol/24 hr) Mean ± S.E.M. (SD)
1. Basal condition			
A) Control cases	(n = 11)	7.8 ± 0.37 (1.25)	1503.8 ± 190.2 (627.9)
B) Rickets patients	(n = 28)	25.17 ± 1.87 (9.89)	4060.7 ± 420.2 (2224.0)
P value, 1A vs 1B		< 0.001	< 0.001
2. Effect of calcium infusion			
A) Control cases	(n = 11)	4.00 ± 0.23 (0.77)	819.9 ± 79.41 (263.4)
B) Rickets patients	(n = 28)	14.28 ± 1.26 (6.68)	2404.2 ± 251.9 (1333.3)
P value, 1A vs 2A		< 0.001	< 0.01
P value, 1B vs 2B		< 0.001	< 0.01
3. Effect of vitamin D			
A) Rickets patients before treatment	(n = 10)	27.9 ± 3.98 (12.59)	4072.3 ± 589.2 (1863.3)
B) Rickets patients after treatment	(n = 10)	8.4 ± 0.49 (1.57)	1407.4 ± 197.11 (623.3)
P value, 3A vs 3B		< 0.005	< 0.001
P value, 3B vs 1A		> 0.05	> 0.05

(r = −0.56). However, in 18 of the patients with rickets the initial serum calcium level was below 8 mg/dl andthe correlation coefficient was r = −0.60 (Srxy = 0.2, Figure 2). In this group the urinary excretion of cAMP (4699.5 ± 563.5 nmol/24 hr, SD = 2390.5) was significantly higher ($p < 0.05$) than that of the ten patients with a serum calcium level above 8 mg/dl (2910.4 ± 418.86 nmol/24 hr, SD = 1324.57).

The infusion of calcium decreased the urinary level of cAMP significantly both in control cases and in patients (Table I). Urinary cAMP excretion in ten patients, after complete healing of rickets, was essentially the same as that of control cases (Table I).

Discussion

In the present study the increased level of urinary cAMP in patients with vitamin D deficiency rickets is observed. It is known that about one half to two thirds of the urinary cAMP is derived from the plasma by glomerular filtration and the remainder is produced by the kidney[12,13]. Chase and Aurbach[7] demonstrated that urinary cAMP is increased following the intravenous administration of PTH and vasopressin. The amount of vasopressin required for this effect exceeded its physiological range[7], and the urinary cAMP response following parathyroid extract is at least 50 times greater than following vasopressin administration[14]. Studies on man have shown that the excretion of cAMP is diminished when sufficient fluids are given to inhibit the secretion of vasopressin[15]. Since all subjects in the present study were well hydrated, there was no question of cAMP levels being affected by vasopressin levels. The amount of cAMP produced by the action of vasopressin in the kidney is not suppressed by calcium infusion whereas the opposite was observed in the present study. Another hormone that has an effect on the excretion of urinary cAMP is calcitonin[16]. The rate of calcitonin secretion is a direct function of the plasma calcium concentration above 9 mg/dl. In the present study a negative correlation between the serum calcium and urinary cAMP levels is observed. It therefore seems unlikely that a calcitonin effect was responsible for the pattern of urinary cAMP described.

C_p is increased and TRP % is decreased in vitamin D deficiency rickets. These findings suggest the presence of secondary hyperparathyroidism, which is known to occur in this disease. Therefore it seems probable that the excessive cAMP in the urine of patients with rickets was mainly formed in the renal tubules in response to high circulating levels of PTH. In an effort to suppress PTH secretion, calcium was infused over a period of 4 hours. In fact a rapid decrease in the excretion of cAMP as well as a reduction in phosphaturia could be demonstrated. Following the treatment of rickets, the urinary cAMP values decreased to within a normal range, probably due to correction of secondary hyperparathyroidism. Although previous

studies in rats had indicated that vitamin D deficiency diminishes the activation of renal adenylate cyclase by PTH[17], Sovic and coworkers[18] have observed increased levels of urinary cAMP in two infants with severe rickets just as we have. The data presented suggest that in man the result of vitamin D deficiency can be overcome by secondary hyperparathyroidism as a result of which an increased renal tubular formation of cAMP occurs.

Summary

In this study 28 children with vitamin D deficiency rickets and 11 control cases were studied. The rise in C_p as well as the fall in TRP % were strikingly different in the rickets patients as compared to the control cases. The C_p/C_{cr} ratio was considerably higher in the rickets patients than in the controls. The daily excretion of urinary cAMP was elevated 2.5-fold in patients with rickets and further, a negative correlation between the serum calcium levels and daily urinary excretion of cAMP was observed. The infusion of calcium decreased the urinary level of cAMP significantly both in control cases and in patients. The effect of vitamin D treatment was detected, the mean value in these cases being essentially the same as that of the controls. These data suggest that in man the result of vitamin D deficiency can be overcome by secondary hyperparathyroidism as a result of which an increased renal tubular formation of cAMP occurs.

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