Demonstration of a Lack of Change in Serum 1α,25-Dihydroxyvitamin D in Response to Parathyroid Extract in Pseudohypoparathyroidism

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A B S T R A C T   Studies were carried out to compare the effects of parathyroid extract (PTE) on serum and urinary calcium (Ca) and phosphorus (P), serum 25-hydroxyvitamin D (25-OHD), serum 24,25-dihydroxyvitamin D (24,25(OH)$_2$D), serum 1α,25-dihydroxyvitamin D (1α,25(OH)$_2$D), and urinary cyclic AMP in two normal subjects, two patients with hypoparathyroidism (HP) and six patients with pseudohypoparathyroidism (PHP), some of whom were on suboptimal treatment with vitamin D. Two of the patients with PHP were studied while on long-term treatment with 1α,25-(OH)$_2$D$_3$. Before PTE, serum 1α,25(OH)$_2$D was at the lower limit of normal in one patient and was abnormally low in the other five patients. None of these individuals was on treatment with 1α,25(OH)$_2$D$_3$. Serum 25-OHD and 24,25(OH)$_2$D were either increased or at the upper limit of normal in the patients given vitamin D and were normal in the other patients. PTE lowered the serum P and increased the serum 1α,25(OH)$_2$D, serum and urinary Ca, urinary P, and urinary cyclic AMP in the normal subjects and patients with HP. In individual studies, changes in serum 1α,25(OH)$_2$D and serum Ca occurred in parallel before, during, and after PTE. In contrast, PTE had very little effect in the patients with PHP. Whereas there were highly significant positive correlations between serum 1α,25(OH)$_2$D and serum Ca, and highly significant negative correlations between serum P and serum 1α,25(OH)$_2$D in each of the normal subjects and patients with HP, there were significant correlations in only one of the patients with PHP. An increase in serum Ca in response to PTE was

observed in one of the two patients with PHP who were on long-term treatment with 1α,25(OH)$_2$D$_3$. In these individuals, PTE produced only slight increases in serum 1α,25(OH)$_2$D. Serum 25-OHD and 24,25(OH)$_2$D were not changed by PTE in any of the subjects or patients. The results provide evidence that hypocalcemia in HP and PHP arises in part from low circulating 1α,25-(OH)$_2$D, and indicate that the lack of change in serum 1α,25(OH)$_2$D with PTE in patients with PHP is related to impaired renal adenylate cyclase and phosphaturic responses. These and previous results support the idea that diminished renal production of 1α,25(OH)$_2$D, because of a defect in the parathyroid hormone-responsive adenylate cyclase system, may be a contributing factor in the pathogenesis of the abnormal calcium metabolism in PHP.

INTRODUCTION

The metabolic defect that accounts for the kidney's lack of response to parathyroid hormone (PTH)[1] in pseudohypoparathyroidism (PHP) was attributed to an abnormal adenylate cyclase system in the renal tubule (1, 2). Thus, PTH and parathyroid extract (PTE) consistently increase urinary cyclic AMP and phosphate in normal subjects and in patients with hypoparathyroidism (HP), but not in patients with PHP (1–3).

Available evidence also indicates that there is a defect in the renal production of 1α,25-dihydroxyvitamin D (1α,25[OH]$_2$D) in PHP, which causes impaired in-

[1] *Abbreviations used in this paper:* HP, hypoparathyroidism; 25-OHD, 25-hydroxyvitamin D; 1α,25(OH)$_2$D, 1α,25-dihydroxyvitamin D; 1α,25(OH)$_2$D$_3$, 1α,25-dihydroxyvitamin D$_3$; 24,25(OH)$_2$D, 24,25-dihydroxyvitamin D; PHP, pseudohypoparathyroidism; PTE, parathyroid extract; PTH, parathyroid hormone.

testinal absorption of calcium, hypocalcemia, and secondary hyperparathyroidism (4-7). These abnormalities are reversed by treatment with small doses of $1\alpha,25$-dihydroxyvitamin D_3 ($1\alpha,25[OH]_2D_3$) (4, 6-8). Previous studies showed that PTE readily increases the serum calcium in normal subjects and in patients with HP, but not in those with PHP (2, 3, 5, 9, 10). This abnormal response was attributed to an associated defect in the adenylate cyclase system in the skeleton (1, 2).

The present studies were carried out to compare the effects of PTE on serum $1\alpha,25(OH)_2D$ in normal subjects, in patients with HP, and in patients with PHP. The results indicate that PTE readily increases serum $1\alpha,25(OH)_2D$ in normals and in patients with HP, but not in those with PHP.

METHODS

Two normal subjects—a man and a woman—two patients with HP, and six patients with PHP were hospitalized at the Clinical Research Center of the Indiana University Medical School, Indianapolis, Ind. They were given a constant daily diet and fluid intake, and distilled water to drink. The diets were estimated to contain 400 and 900 mg/d of calcium and phosphorus, respectively. Daily fasting blood samples were obtained for determination of serum calcium, phosphorus, 25-hydroxyvitamin D (25-OHD), 24,25-dihydroxyvitamin D ($24,25[OH]_2D$), and $1\alpha,25(OH)_2D$. 24-h urines were obtained for measurement of calcium and phosphorus. Serum and urinary calcium (11) and phosphorus (12) were analyzed by methods modified for the Auto Analyzer. Serum PTH was measured by radioimmunoassay with antiserum (chicken 9, kindly provided by Dr. Eduardo Slatopolsky, St. Louis, Mo.) which is predominantly directed against the carboxylterminal portion of the hormone and which was used at a final concentration of 1:20,000 as previously described (13). The normal range is 0.55 ng/ml or less, and serum PTH can be detected in 85% of normal subjects. Urinary cyclic AMP was determined by the method of Gilman (14) as previously described (2).

Serum 25-OHD, $24,25(OH)_2D$, and $1\alpha,25(OH)_2D$ were measured by methods previously reported (15, 16). Serum (3-4 ml) was extracted with methanol-methylene chloride (2:1, vol/vol). The extract was fractionated by chromatography on a Sephadex LH 20 column (0.9 × 15 cm) in the solvent system n-hexane:chloroform:methanol (9:1:1, vol/vol). The pooled eluates contained vitamin D, 25-OHD, and 24,25-$(OH)_2D$ and $1\alpha,25(OH)_2D$ together. The last two metabolites were separated by high pressure liquid chromatography on a μ-Porosil column (Waters Associates, Inc., Milford, Mass.) and CO: PELL PAC guard column (Whatman, Inc., Clifton, N. J.) in tandem in the solvent system n-hexane:isopropanol run in a concave gradient elution mode (97:3 to 90:10, vol/vol). 25-OHD and $24,25(OH)_2D$ were assayed by the normal rat plasma protein-binding method reported previously (15). Recovery was determined by adding 3,500 dpm of [^{3}H]25-OHD$_3$ and [^{3}H]24,25(OH)$_2$D$_3$ to serum samples and averaged 85 and 82%, respectively. The intra- and interassay variations were 12% or less. Detection limit for the two assays was 0.1 ng/ assay tube. Mean values for 25-OHD and $24,25(OH)_2D$ in normal sera were 27.7±6.4 (n = 95, ±SD) and 1.4±0.6 ng/ml (n = 85), respectively. 95% confidence values were 14.9-40.5 ng/ml for 25-OHD and 0.8-2.0 ng/ml for $24,25(OH)_2D$. Serum $1\alpha,25(OH)_2D$ was measured by chick cytosol receptor assay employing an intestinal mucosal cytosol receptor from rachitic

chicks, a Tris-HCL, KCL, thioglycerol-glycerol buffer system, and dextran-charcoal-human plasma separation of bound from free $1,25(OH)_2D$ as previously described (15, 16). Intra- and interassay variations were 5% or less. Recovery was determined by the addition to serum of 3,500 dpm[^{3}H]$1\alpha,25(OH)_2D_3$ and averaged 80%. The lower limit of detection was 2 pg. Mean normal value was 34.2±7.4 pg/ml (n = 65, ±SD). The 95% confidence values were 19.4-49.0 pg/ml.

Crystalline 25-OHD$_3$, $24,25(OH)_2D_3$, and $1\alpha,25(OH)_2D_3$ were kindly provided by Dr. M. Uskokovik, Hoffman-LaRoche, Nutley, N. J. [23,24-^{3}H]25-OHD$_3$ (110 ci/mmol) was obtained from Amersham Corp., Arlington Heights, Ill. [23,24-^{3}H]24,25(OH)$_2$D$_3$ and [23,24-^{3}H]$1\alpha,25(OH)_2D_3$ were biosynthesized in vitro from [23,24-^{3}H]25-OHD$_3$ as previously described (15).

Parathyroid extract (Eli Lilly and Co., Indianapolis, Ind.) was given by intramuscular injection every 12 h. Except where noted, the dose was 3 U/kg body wt. $1\alpha,25(OH)_2D_3$ (Hoffman-LaRoche) was given as a single morning dose.

Correlation coefficient and statistical analysis of paired and nonpaired data by t test were calculated with a Hewlett-Packard calculator, model 9815A (Hewlett-Packard Co., Palo Alto, Calif.). Correlation coefficient and the test for significance between two regressions were determined by standard methods (17).

RESULTS

The clinical findings in the normal subjects and patients are summarized in Table I. Serum PTH was undetectable in the patients with HP, and was abnormally elevated in the patients with HP who were on no treatment or received inadequate doses of vitamin D.

The effects of PTE in two normal subjects are shown in Fig. 1. PTE (days 5-8) increased serum calcium and serum $1\alpha,25(OH)_2D$, lowered serum phosphorus, and increased urinary calcium, phosphorus, and cyclic AMP. Mean serum 25-OHD and 24,25(OH) did not change (Table II).

The effects of PTE in two patients with HP are shown in Fig. 2. During an initial control period (days 1-4), serum calcium and serum $1\alpha,25(OH)_2D$ were abnormally low in patient A, who was on no treatment, and were at the lower range of normal in patient B, who was receiving 50,000 U of vitamin D every other day. PTE (days 5-8) increased serum calcium and serum $1\alpha,25(OH)_2D$, lowered serum phosphorus, and increased urinary calcium, phosphorus, and cyclic AMP. Mean serum 25-OHD and serum $24,25(OH)_2D$, which initially were within the normal range in patient A and were strikingly elevated in patient B, were not altered by PTE (Table III).

The effects of PTE in four patients with PHP are shown in Fig. 3. During an initial control period (days 1-4), serum calcium was abnormally low in each of the patients, and serum $1\alpha,25(OH)_2D$ was abnormally low in three of them. Serum phosphorus was increased in three of them. In contrast to the response in normals and patients with HP, PTE (days 5-8) produced little or no change in both serum and urinary calcium and phosphorus, serum $1\alpha,25(OH)_2D$, and urinary cyclic AMP in the patients with PHP. Initially, mean serum

Pseudohypoparathyroidism and 1α,25-Dihydroxyvitamin D   783

TABLE I

TABLE I

Clinical Findings and Treatment in Normal Subjects and Patients with Hypoparathyroidism or Pseudohypoparathyroidism

Subjects	Age	Sex	Serum			Comments and treatment
			Ca	P	PTH	
	yr		*mg/dl*	*mg/dl*	*ng/ml*	
Normal						
A	22	M	10.0	3.7	—	
B	25	F	8.8	3.8	0.20	
Hypoparathyroidism						
A	47	M	6.8	4.8	Undet.*	Idiopathic; no treatment
B	58	F	9.1	4.2	Undet.*	Post-subtotal thyroidectomy; 2 g calcium gluconate and 2.5 grains thyroid daily; 50,000 U vitamin D_2 every other day for 20 yr
Pseudohypoparathyroidism						
C	17	M	6.5	6.4	1.26	Seizures, cataracts; 100,000 U vitamin D_2 daily intermittently for 10 yr
D	21	M	5.8	5.7	2.33	Short stature, cataracts, tetany, seizures, short metacarpals; 50,000 U vitamin D_2 and 16 g calcium gluconate daily just started
E	23	F	7.1	4.6	1.07	Short stature, round face, seizures, short metacarpals; no treatment
F	11	M	6.3	8.1	1.53	Tetany; no treatment
G	15	F	8.7	4.8	—	3 μg daily $1\alpha,25(OH)_2D_3$ for 53 mo
H	57	M	10.2	3.8	0.12	Short stature, round face, cataracts; 1 μg daily $1\alpha,25(OH)_2D_3$ for 24 mo

Patients were continued on the same dose of the drugs listed. Vitamin D and $1\alpha,25(OH)_2D_3$ were given as single morning doses. * Undet., undetectable.

25-OHD and 24,25(OH)₂D were increased in patient C, who was receiving 100,000 U a day of vitamin D, and were normal in the other patients. They were not altered by PTE (Table II).

The effects of PTE in two patients with PHP who were on chronic treatment with $1\alpha,25(OH)_2D_3$ are shown in Fig. 4. During an initial control period (days 1–4), serum calcium was at the lower range of normal and serum $1\alpha,25(OH)_2D$ was abnormally low in both patients. PTE produced only slight increases in serum $1\alpha,25(OH)_2D$ in each of them. There was an increase in serum calcium in patient H, but not in patient G. Mean serum 25-OHD and serum 24,25(OH)₂D were within the normal range in both patients and were not altered by PTE (Table II).

There were highly significant negative correlations between the serum phosphorus and serum $1\alpha,25-(OH)_2D$ in the normals, in patients with HP (Fig. 5), and in the patients with PHP (Fig. 6). Values for two adults with PHP (patients D and E) fell along the same axis as that of the normals and patients with HP, whereas values for two adolescents (patients C and F) fell along a separate axis. There was a significant difference among the slopes of the regression of the adolescents with PHP, that of the normals and patients with HP

($P < 0.05$), and that of the adults with PHP ($P < 0.01$). The last two slopes of the regressions were not significantly different from each other.

There were significant positive correlations between serum $1\alpha,25(OH)_2D$ and serum calcium in the normals and patients with HP (Fig. 7), and those in the patients with PHP (Fig. 8). There was a statistically significant difference between the slopes of the regressions of the two groups ($P < 0.01$).

In individual patients, there were significant negative correlations between serum phosphorus and serum $1\alpha,25(OH)_2D$, and significant positive correlations between serum $1\alpha,25(OH)_2D$ and serum calcium in each of the normals and patients with HP (Table III). In contrast, there were significant correlations in only one of the patients with PHP.

In all the studies, there was a significant correlation between mean serum 25-OHD and mean serum 24,25(OH)₂D($r = 0.962, P < 0.01$).

DISCUSSION

Our results confirm that there are low values for serum $1\alpha,25(OH)_2D$ in HP (18) and PHP (5–7, 18). They demonstrate that, whereas serum $1\alpha,25(OH)_2D$ readily
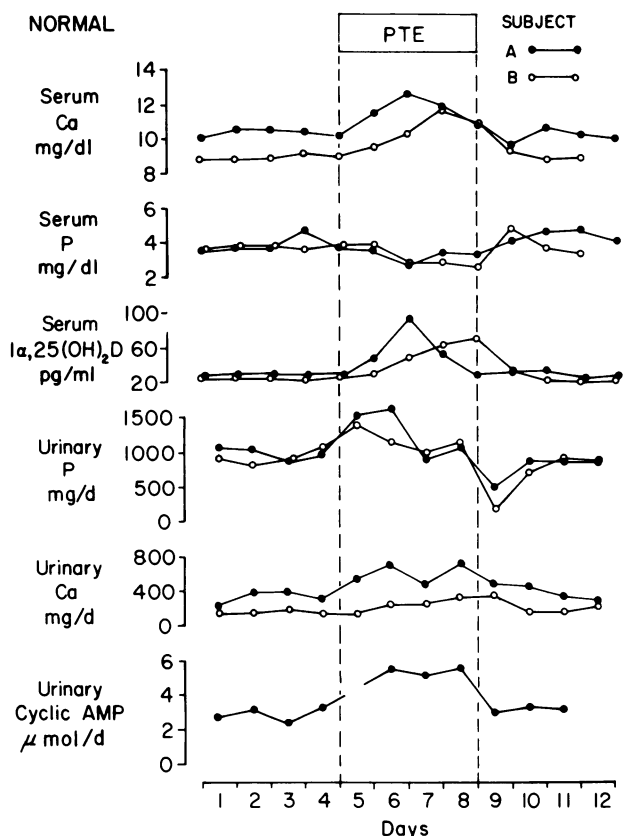
NORMAL

PTE

SUBJECT
A ●────●
B ○────○

FIGURE 1   Effects of PTE, 6 U/kg body wt per day, on serum and urinary calcium (Ca) and phosphorus (P), serum 1α,25-(OH)₂D, and urinary cyclic AMP in two normal subjects. The dose of PTE was 12 U/kg body wt per day on days 5 and 6 in subject A.

increases in response to PTE in normals and patients with HP, serum 1α,25(OH)₂D changes very little in response to PTE in patients with PHP. This attenuated response is apparently related to defective phosphaturic response and possibly to the impaired cyclic AMP response to PTH, abnormalities which are characteristic of PHP.

There were highly significant negative correlations between the serum phosphorus and serum 1α,25-(OH)₂D in each of the normals, in each of the patients with HP, and in only one of the patients with PHP. Such a relationship has been described previously in normal subjects, patients with renal stones, and patients with primary hyperparathyroidism (19). It is likely that hyperphosphatemia and impaired phosphate excretion may be responsible to a considerable degree for the abnormally low or borderline values for serum 1α,25-(OH)₂D in HP and PHP. Our results of course do not exclude the possibilities that cyclic AMP may be involved in the renal production of 1α,25(OH)₂D (20) and that impaired renal synthesis of cyclic AMP may

TABLE II
Effects of Parathyroid Extract on Serum
25-OHD and Serum 24,25(OH)₂D

Subjects		Serum 25-OHD	Serum 24,25(OH)₂D	Duration
		ng/ml	ng/ml	d
Normal				
A	C	26.0±1.3	1.1±0.1	5
	PTE	27.2±1.3	1.0±0.1	4
	C	26.8±0.8	1.2±0.2	4
B	C	23.3±0.8	0.7±0.1	5
	PTE	23.3±0.4	0.6±0.1	4
	C	23.5±1.3	1.1±0.2	4
Hypoparathyroidism				
A	C	23.0±0.4	1.1±0.1	5
	PTE	21.9±0.8	0.9±0.1	4
	C	23.9±0.6	1.1±0.1	3
B*	C	172±10	8.4±0.6	5
	PTE	169±2	7.8±0.6	4
	C	158±7	8.4±0.6	4
Pseudohypoparathyroidism				
C*	C	243±15	8.8±1.6	4
	PTE	238±7	7.2±0.8	4
	C	226±14	7.2±1.6	4
D*	C	44.4±1.8	1.4±0.4	4
	PTE	45.6±2.0	1.6±0.2	4
	C	48.4±1.8	1.6±0.4	4
E	C	26.9±1.6	1.3±0.1	5
	PTE	28.8±0.8	1.0±0.1	4
	C	26.2±2.8	1.0±0.2	2
F	C	26.2±1.4	1.3±0.1	5
	PTE	26.2±1.0	1.2±0.1	4
	C	29.9±0.7	1.4±0.1	4
G‡	C	14.7±0.2	0.7±0.1	5
	PTE	14.9±0.1	0.6±0.1	4
	C	16.6±0.3	0.7±0.1	4
H‡§	C	15.1±0.6	0.9±0.1	5
	PTE	14.2±2.0	0.7±0.2	3
	PTE	13.1±1.5	0.8±0.1	3
	C	15.3±0.6	0.6±0.1	4

Results are given as mean±SE.
PTE, 3 U/kg body wt i.m. every 12 h.
* On treatment with vitamin D₂ (see Table I).
‡ On treatment with 1α,25(OH)₂D₃ (see Table I).
§ PTE, 1.5 and 3 U/kg body wt i.m., every 12 h in sequential 3-d periods.

diminish the conversion of 25-OHD to 1α,25(OH)₂D in PHP.

There was a clear difference between the adult and adolescent patients with regard to the relationship be-

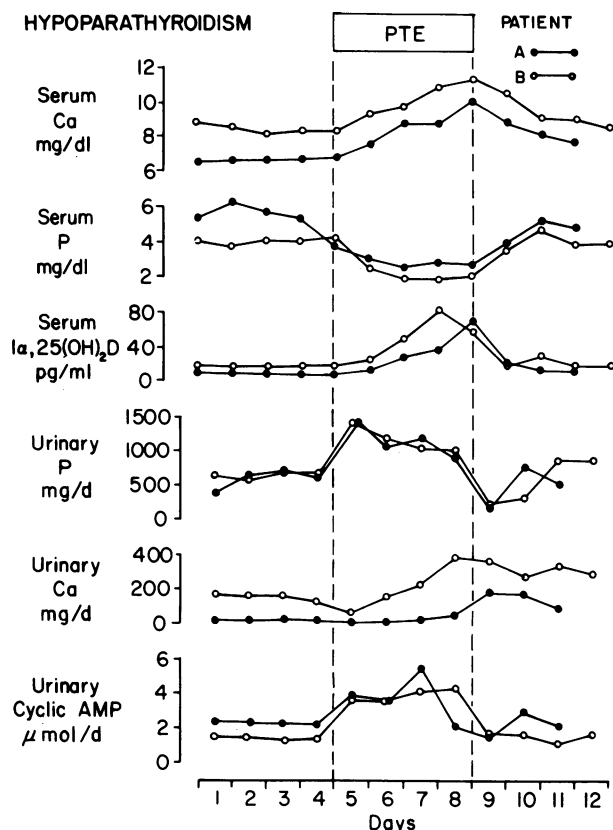FIGURE 2 Effects of PTE, 6 U/kg body wt per day, on serum and urinary calcium (Ca) and phosphorus (P), serum 1α,25-(OH)₂D, and urinary cyclic AMP in two patients with hypoparathyroidism.
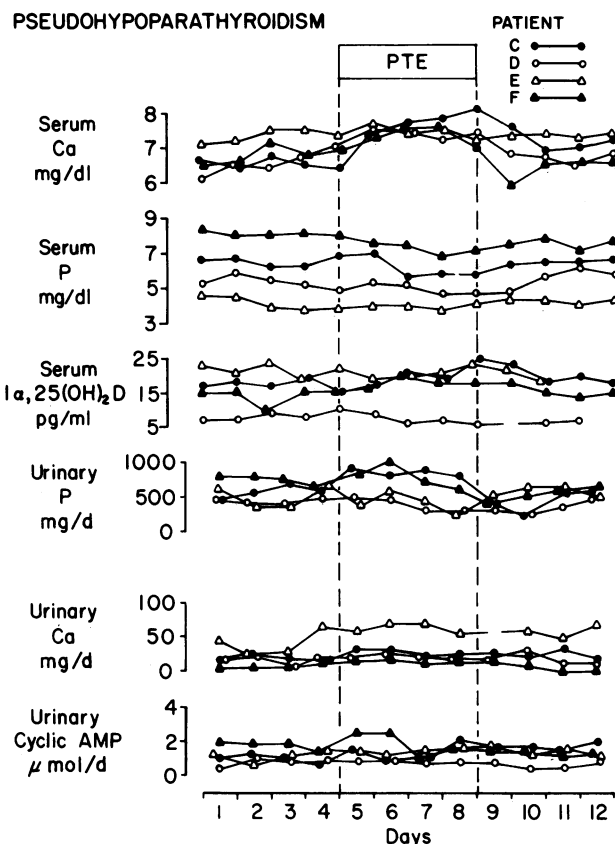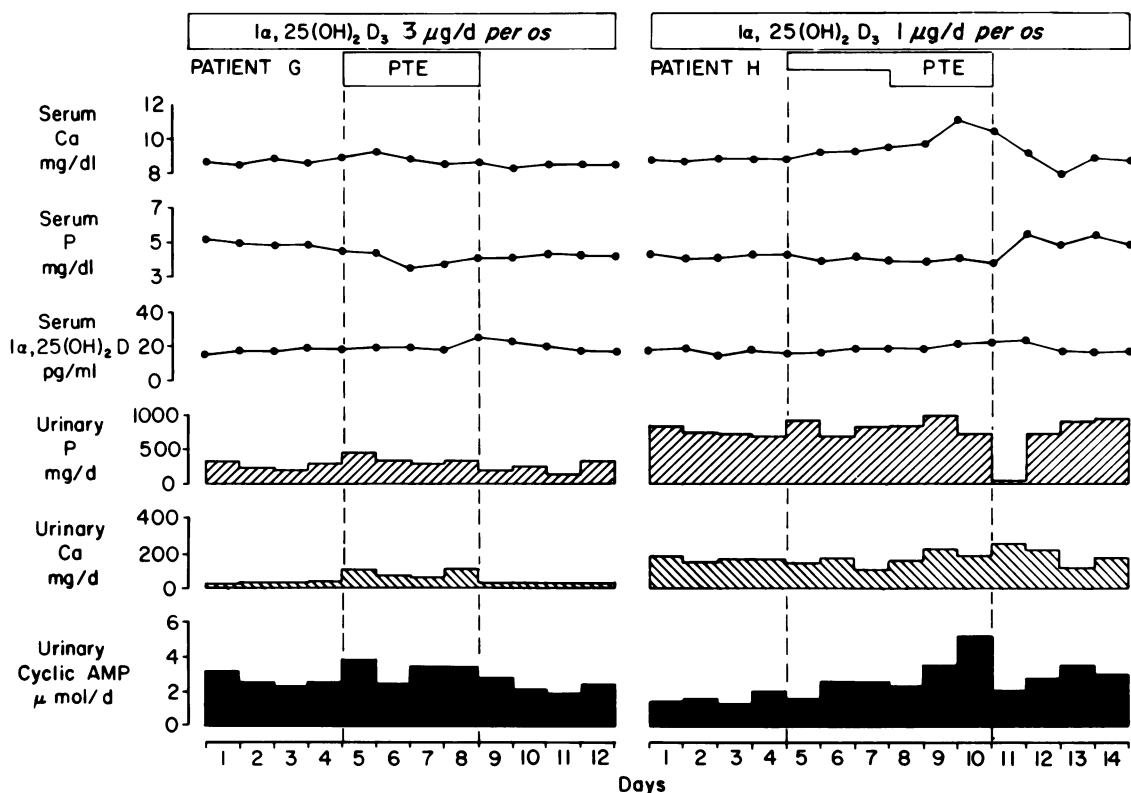

FIGURE 3 Effects of PTE, 6 U/kg body wt per day, on serum and urinary calcium (Ca) and phosphorus (P), serum 1α,25-(OH)₂D, and urinary cyclic AMP in four patients with pseudohypoparathyroidism.

TABLE III
Correlation of Serum 1α, 25(OH)₂D with Serum Calcium and Phosphorus

| Subjects | n | Serum 1α,25(OH)₂D | |
		vs. serum Ca	vs. serum P
		r	
Normal			
A	13	0.740*	0.616‡
B	12	0.916*	0.663‡
Hypoparathyroidism			
A	12	0.959*	0.806‡
B	13	0.812*	0.824*
Pseudohypopara-thyroidism			
C	13	0.755*	0.624‡
D	11	0.100	0.100
E	11	0.538	0.141
F	13	0.141	0.346

n = the number of observations.
* P < 0.01.
‡ P < 0.05.

tween the serum 1α,25(OH)₂D and serum phosphorus. It is known that serum 1α,25(OH)₂D, serum phosphorus, and serum growth hormone are normally increased during adolescence (21–24), and that growth hormone increases the phosphorus tubular maximum for reabsorption by the kidney (T_m) (22, 23). However, recent evidence indicates that growth hormone does not alter circulating 1α,25(OH)₂D (24). These results provide evidence that the increase in serum 1α,-25(OH)₂D during adolescence is not mediated by growth hormone and must result from some other mechanism.

An investigation carried out with kidney tissue from a patient with PHP showed a high K_m for ATP and decreased adenylate cyclase activity in response to PTH at subsaturating concentrations of ATP (25). The abnormality could be entirely corrected by GTP. In this patient, a defect in the nucleotide receptor or concentration of the nucleotide may have caused an abnormality in the PTH-responsive adenylate cyclase system in renal cortex. Recent preliminary investigations indicate a deficiency in the nucleotide regulatory protein

FIGURE 4   Effects of PTE, 6 U/kg body wt per day, on serum and urinary calcium (Ca) and phosphorus (P), serum $1\alpha,25(OH)_2D$, and urinary cyclic AMP in two patients with pseudohypoparathyroidism on chronic treatment with $1\alpha,25(OH)_2D_3$. The dose of PTE was 3 U/kg body wt per day on days 5–7 in patient H.

in erythrocytes of patients with PHP and skeletal abnormalities, but not in those patients with PHP and a normal skeleton (26). This raises the possibility of a generalized defect in the adenylate cyclase system, including the skeleton, which might account for hypocalcemia and the lack of calcemic response to PTE in this group of patients.

Our findings demonstrate that PTE consistently increased the serum calcium in normal subjects and patients with HP, but produced little or only modest change in serum calcium in the patients with PHP who were not being treated with $1\alpha,25(OH)_2D_3$. As noted before, this lack of response of the serum calcium to PTE is characteristic of PHP (2, 3, 5, 9, 10). A number of possible mechanisms might be responsible. Refractoriness of the skeletal system may occur because of secondary hyperparathyroidism, because of deficiency of $1\alpha,25(OH)_2D$ (4–6, 27), because of a lack of increase in circulating $1\alpha,25(OH)_2D$ as shown in the present findings, because of a defect in the skeletal adenylate cyclase system similar to that in the kidney (1, 2), or because an abnormal form of PTH is produced,

which occupies but does not activate receptors in target organs (28).

Available evidence indicates that refractoriness of bone to PTH develops after exposure to the hormone. The cyclic AMP response of rat calvaria to PTH and prostaglandin E_1 is abolished by exposure for 60 min to PTH and prostaglandin E_1, respectively (29). Similarly, isolated bone cells from mouse calvaria are down regulated by exposure to PTH (30).

There is both clinical and experimental evidence that deficiency of vitamin D or its metabolites impairs the calcemic response to PTH. In the vitamin D-deficient rat, this defect is corrected within 24 h by a single dose of vitamin D (31). Vitamin D deficiency, however, does not alter PTH-stimulated adenylate cyclase activity or accumulation of cyclic AMP in rat calvaria (31). Thus, the lack of response to PTH is not related to an abnormal adenylate cyclase system. An attenuated response to PTE also occurs in chronic renal failure. In uremic thyroparathyroidectomized dogs, this abnormal response is restored by treatment with $1\alpha,25-(OH)_2D_3$ and $24,25(OH)_2D_3$ (32). When given alone,
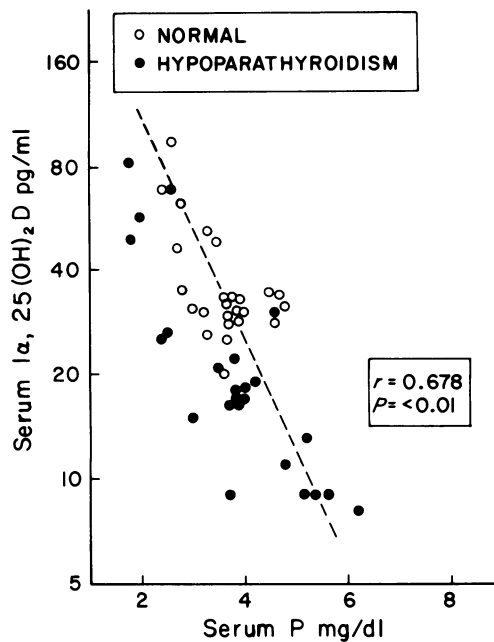
FIGURE 5 Relationship between serum phosphorus (P) and serum 1α,25(OH)₂D in two normal subjects and two patients with hypoparathyroidism. Data are from Figs. 1 and 2. Note that serum 1α,25(OH)₂D is drawn on a semi-log plot.
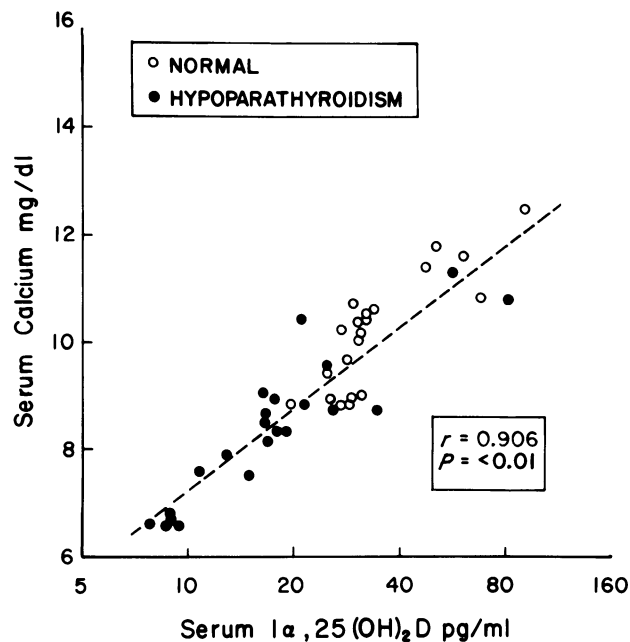

FIGURE 7 Relationship between serum 1α,25(OH)₂D and serum calcium (Ca) in two normal subjects and two patients with hypoparathyroidism. Data are from Figs. 1 and 2. Note that serum 1α,25(OH)₂D is drawn on a semi-log plot.
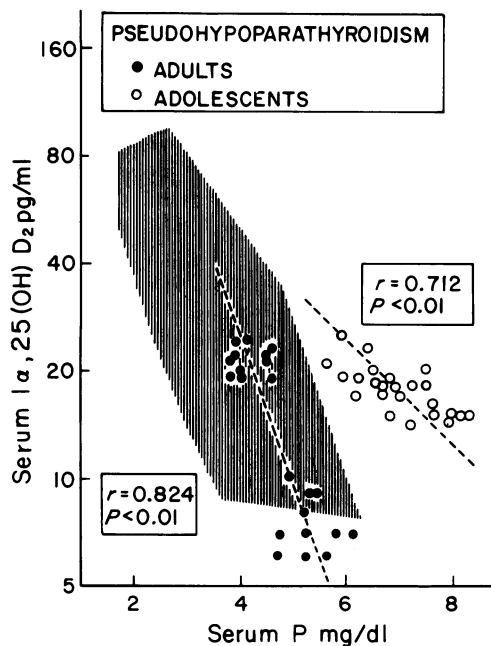

FIGURE 6 Relationship between serum phosphorus (P) and serum 1α,25(OH)₂D in two adults and two adolescents with pseudohypoparathyroidism. Data are from Fig. 3 and shaded area is from Fig. 5. Note that serum 1α,25(OH)₂D is drawn on a semi-log plot.
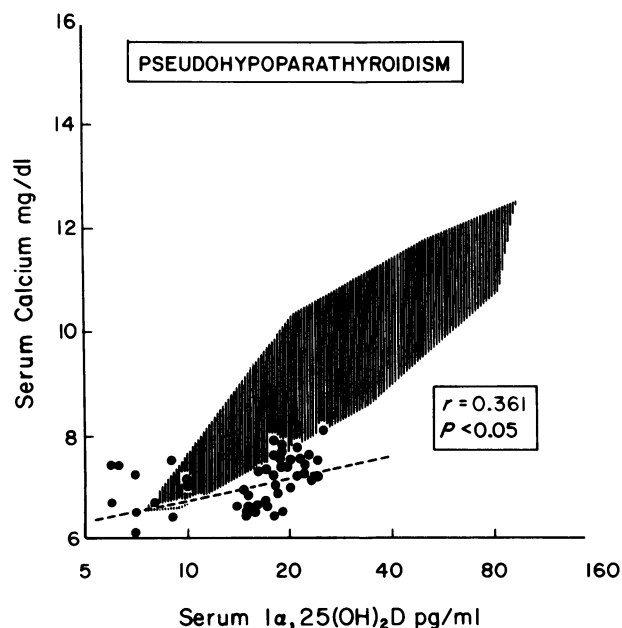

FIGURE 8 Relationship between serum 1α,25(OH)₂D and serum calcium (Ca) in four patients with pseudohypoparathyroidism. Data are from Fig. 3 and shaded area is from Fig. 7. Note that serum 1α,25(OH)₂D is drawn on a semi-log plot.

$1\alpha,25(OH)_2D_3$ is only partially effective and $24,25$-$(OH)_2D_3$ is ineffective (32). In uremia, the abnormal calcemic response is apparently related to deficiency of these metabolites of vitamin D.

Clinically, hypercalcemia does not occur in primary hyperparathyroidism when there is vitamin D deficiency until after the vitamin deficiency is corrected (33, 34). Further, isolated $1\alpha,25(OH)_2D$ deficiency was reported to cause skeletal resistance to PTH in a patient with hypocalcemia and secondary hyperparathyroidism caused by low serum $1\alpha,25(OH)_2D$ (27). The impaired calcemic response was corrected by treatment with $1\alpha,25(OH)_2D$, 1 $\mu g/d$ for 4 d. The patient initially showed a normal increase in urinary cyclic AMP and phosphorus in response to PTE and therefore did not have the classic form of PHP. It should be noted that chronic treatment with $1\alpha,25(OH)_2D_3$ improved the skeletal response to PTE in one of our patients with PHP.

It can be argued that changes in $1\alpha,25(OH)_2D$ may contribute to the calcemic effects of PTE. $1\alpha,25$-$(OH)_2D$ is normally a major determinant of fractional intestinal absorption of calcium or α (35, 36) and increases the skeletal release of calcium in a dose-dependent fashion (37). Serum $1\alpha,25(OH)_2D$, serum calcium, and α are increased in patients with primary hyperparathyroidism (35, 38) and return to normal after surgical treatment (38). On the other hand, serum 1α,-$25(OH)_2D$, serum calcium, and intestinal absorption of calcium are low in HP (16, 18) and PHP (4–7), and hypocalcemia and impaired intestinal absorption of calcium can be treated in these disorders with small doses of $1\alpha,25(OH)_2D_3$ (4, 6–8, 39, 40). In addition, our studies showed parallel changes in serum $1\alpha,25$-$(OH)_2D$ and serum calcium before, during, and after PTE in the normals and patients with HP. There were significant positive correlations between the serum calcium and serum $1\alpha,25(OH)_2D$, both collectively and in the individual studies.

PTE produced very little change in serum $1\alpha,25$-$(OH)_2D$ in our patients with PHP. The lack of change may be a contributing factor to the lack of calcemic response to PTE. In support of this hypothesis is the report that PTE produced transient increases in serum $1\alpha,25(OH)_2D$ and serum calcium in a patient with PHP that were similar to those seen in our normal subjects and patients with HP (41). As in our studies, the changes in serum $1\alpha,25(OH)_2D$ and serum calcium before, during, and after PTE occurred in parallel.

Serum $1\alpha,25(OH)_2D$ remained low and changed very little in response to PTE in both of the patients with PHP who were being treated with the metabolite. The low values during treatment with $1\alpha,25(OH)_2D_3$, which were observed previously (7), are attributed to

the short half-life of the metabolite (42), which was given as a single morning dose.

The mechanism for the lack of calcemic response to PTE during treatment with $1\alpha,25(OH)_2D_3$ in one of our patients is not clear. Variability in serum $1\alpha,25(OH)_2D$ during the day after the single morning dose or lack of increase in serum concentration of the metabolite in response to PTE could be responsible. Before treatment, a bone biopsy showed increases in osteoclasts, 2.40/ mm^2 (normal range: 0.22–0.80), trabecular osteoclastic resorptive surface, 23.9% (normal range: 2.5–4.7%) and periosteocytic lacunae, 63.9 μm^2 (normal range: 45.2–56.2 μm^2) as previously reported (6). These findings indicate excess circulating parathyroid hormone. It is difficult in the case of this patient, therefore, to attribute the abnormal calcemic response entirely to an abnormal adenylate cyclase system in the skeleton.

The possibility that a biologically inactive form of PTH (28) is regularly produced in PHP is unlikely. Several cases have been reported in which there was clinically apparent osteitis fibrosa cystica (10, 43). In one of them, an increase in urinary excretion of biologically active PTH was demonstrated (10). As already noted, changes in quantitative bone histology, increases in trabecular osteoclastic resorptive surface, and periosteocytic lacunae, which occur in PHP as a consequence of secondary hyperparathyroidism, are similar to the changes that are observed in primary hyperparathyroidism (5, 6). The same argument may be used to refute the concept for an abnormal skeletal adenylate cyclase system in PHP (1, 2). As noted already, some patients with PHP may have a deficiency in the nucleotide regulatory protein (26). This could produce a partial defect that would allow morphologic changes in response to PTH but still impair mobilization of calcium from the skelton.

In summary, we attribute the attenuated calcemic response to PTE in PHP to a combination of several factors: deficiency of $1\alpha,25(OH)_2D$, lack of change in serum $1\alpha,25(OH)_2D$, possible down regulation of the skeleton because of secondary hyperparathyroidism, and a putative defect in the adenylate cyclase system in the skeletal system (1, 2). In our view the production of an abnormal form of PTH (28) is less likely for reasons outlined. It is possible, of course, that this mechanism may be responsible in specific instances.

Finally, our findings show that PTE does not change mean serum $24,25(OH)_2D$ in the normal subjects and patients. Studies with monkey kidney cells in tissue culture demonstrated that $24,25(OH)_2D$ production from 25-OHD is inhibited by PTH and is stimulated both by calcium and by $1\alpha,25(OH)_2D$ (44). It was reported that serum $24,25(OH)_2D$ is increased in primary hyperparathyroidism (45). It appears that the effects of

PTE are predominant in our acute studies, whereas the effects of hypercalcemia and increases in serum $1\alpha,25(OH)_2D$ (16, 18, 19) are predominant in the patients with primary hyperparathyroidism. The positive correlation between serum 25-OHD and serum 24,25-$(OH)_2D$ was reported previously (46).

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