# Pseudohypoparathyroidism: Defective Excretion of 3′,5′-AMP in Response to Parathyroid Hormone

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ABSTRACT Urinary excretion of cyclic adenosine 3′,5′-monophosphate (3′,5′-AMP) was tested in normal subjects and patients with pseudohypoparathyroidism, idiopathic hypoparathyroidism, surgical hypoparathyroidism, and pseudopseudohypoparathyroidism under basal conditions and after a 15 min infusion of purified parathyroid hormone. Basal excretion of the nucleotide was less than normal in the patients with hypocalcemic disorders and greater than normal in pseudopseudohypoparathyroidism. Parathyroid hormone caused a marked increase in excretion of 3′,5′-AMP in all subjects except those with pseudohypoparathyroidism; nine patients with this disorder did not respond to the hormone and four showed a markedly deficient response. Radioimmunoassay showed that parathyroid hormone circulated in increased amounts in plasma from patients with pseudohypoparathyroidism and became undetectable when serum calcium was increased above 12 mg/100 ml. Suppression of parathyroid hormone secretion by induction of hypercalcemia did not alter the deficient response to exogenous hormone. The results indicate that: (*a*) parathyroid hormone circulates in abnormally high concentrations in pseudohypoparathyroidism and secretion of the hormone responds normally to physiological control by calcium; (*b*) testing urinary excretion of 3′,5′-AMP in response to infusion of purified parathyroid hormone appears to be an accurate and sensitive index for establishing the diagnosis of pseudohypoparathyroidism; and (*c*) the metabolic defect of the disorder can be accounted for by a lack of or defective form of parathyroid hormone–sensitive adenyl cyclase in bone and kidney.

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## INTRODUCTION

Albright, Burnett, Smith, and Parson (1) proposed that the pathophysiology of pseudohypoparathyroidism could be attributed to refractoriness of the receptor tissues to the action of parathyroid hormone. This hypothesis was based on the observation that patients with this disorder did not show the characteristic phosphaturic response to parathyroid extract. The recent findings that the mechanism of action of parathyroid hormone is mediated through activation of adenyl cyclase in kidney (2–4) and bone (5) suggested further investigation into the nature of the biochemical defect in pseudohypoparathyroidism. Parathyroid hormone affects specifically the membrane-bound enzyme adenyl cyclase in the renal cortex (3) and in bone (5) causing a marked increase in the concentration of adenosine 3′,5′-monophosphate (3′,5′-AMP) in these tissues (6, 7[1]). Cyclic 3′,5′-AMP itself or dibutyryl 3′,5′-AMP effects hypercalcemia, hypophosphatemia, and hyperphosphaturia in vivo (8, 9) and also mimics many of the actions of parathyroid hormone in vitro (10, 11).

One consequence of the hormone-induced rise in the concentration of 3′,5′-AMP in renal cortex is a striking increase in the rate of excretion of the nucleotide in the urine (12). In the current study we extended these observations to man and found a similar response to parathyroid hormone in normal subjects and in patients with idiopathic hypoparathyroidism, pseudopseudohypoparathyroidism, and hypoparathyroidism caused by surgical removal of the parathyroid glands (surgical hypoparathyroidism). In contrast, parathyroid hormone did not cause increased urinary excretion of phosphate or 3′,5′-AMP in patients with pseudohypoparathyroidism. This observation leads to the conclusion that the metabolic defect accounting for refractoriness to parathyroid

[1] Aurbach, G. D., and L. R. Chase. Cyclic 3′,5′-adenylic acid in bone and the mechanism of action of parathyroid hormone. *Fed. Proc.* In press.

hormone in pseudohypoparathyroidism is a lack of or defective form of adenyl cyclase in renal and skeletal tissue.

## METHODS

*Patients.* The diagnosis of postsurgical, idiopathic, pseudo- or pseudopseudohypoparathyroidism was made on the basis of physical findings, roentgenographic study, determination of serum calcium, and response to parathyroid extract (13). Patients with pseudohypoparathyroidism (Table I) showed at least one unique characteristic of the disorder (physical appearance, skeletal anomalies, or subcutaneous calcification), hypocalcemia at the time of study or in the past, and a defective phosphaturic response to parathyroid hormone. The diagnosis of pseudopseudohypoparathyroidism was established by the characteristic physical and roentgenographic findings of pseudohypoparathyroidism without hypocalcemia in the past or at the time of examination; the phosphaturic response to parathyroid hormone was normal. Patients with idiopathic hypoparathyroidism showed hypocalcemia, usually controlled with vitamin D therapy, but none of the unique physical characteristics of pseudohypoparathyroidism; they responded normally to parathyroid hormone. Patients with surgical hypoparathyroidism had developed hypocalcemia as a direct consequence of surgery for primary thyroid or parathyroid disorders.

*Study protocol.* Patients were given a routine hospital diet containing approximately 800 mg of calcium and 1200 mg of phosphorus. Medications, including supplemental calcium and vitamin D, if required, were withheld only during the period 8 hr before and during each test period. Food was withheld from midnight before each test period until completion of a test. Tests were carried out on 3 successive days (test days I, II, and III) and on each day 250 ml of water were given by mouth hourly from 6:00 a.m. until noon. On the 1st day, urine was collected at hourly intervals from 8:00 a.m. until noon; sometimes an infusion of "vehicle" in 50 ml of 0.9% sodium chloride containing 0.5% human serum albumin was given intravenously from 9:00 to 9:15 a.m. On the 2nd day, 300 USP units of purified parathyroid hormone in 50 ml of 0.9% sodium chloride and 0.5% human serum albumin were infused from 9:00 to 9:15 a.m. Urine was collected from 8:00–9:00 a.m., 9:00–9:30, 9:30–10:00, 10:00–11:00, and 11:00 to noon. On the 3rd day, calcium (10–20 mg/kg) as calcium gluconate in 250 ml of 0.9% sodium chloride was infused from 9:00 a.m. to 1:00 p.m. An infusion of parathyroid hormone as described for day II was given between 12:30 and 12:45 p.m. Urine was collected hourly from 8:00 a.m. until noon and from noon to 12:30, 12:30 to 1:00, and 1:00 to 1:30 p.m. Urine volumes were measured and aliquots frozen immediately and stored at −20°C. Plasma, collected in heparin-coated syringes at 8:55 a.m. and 12:25 p.m., was separated, frozen in acetone–solid $CO_2$, and stored at −20°C.

*Assay methods.* Urine was fractionated for 3′,5′-AMP as follows: to one ml of urine were added 0.03 μc adenosine 3′,5′-monophosphate-$^3$H, 0.2 ml of 8% zinc sulfate, and 0.2 ml of saturated barium hydroxide. The solution was mixed thoroughly and centrifuged at 1000 *g* for 10 min. The supernatant fluid was applied to a 0.5 × 3 cm column of Dowex AG 50W-X8, 100–200 mesh in the hydrogen form, and the 3′,5′-AMP eluted from the column with water in the effluent fraction from 3.5 to 5.5 ml. 0.2 ml of this fraction was added to 10 ml of scintillation solution (14) and tested for recovery of tritiated 3′,5′-AMP. The remaining effluent fluid was lyophilized, dissolved in 100–200 μl of 0.05 M Tris-HCl buffer, pH 8.0 containing 1.8 mM $MgCl_2$ and 0.1% bovine serum albumin and assayed for 3′,5′-AMP by converting the cyclic nucleotide enzymatically to adenosine triphosphate and detecting the latter by means of an ATP-$^{32}P_i$ exchange reaction (15). A sample from one large batch of pooled urine was tested as an internal control in each assay. Urine samples from subjects with pseudohypoparathyroidism were assayed in parallel with samples from subjects showing a normal response to parathyroid hormone. Occasionally, parathyroid hormone was taken to other institutions to test only a patient with pseudohypoparathyroidism. In those instances an additional vial of hormone was included to be returned to the National Institutes of Health for assay by injection into a normal subject.

Urinary inorganic phosphate and creatinine were assayed by the methods of Fiske and SubbaRow (16) and Folin and Wu (17), respectively, as modified for the Technicon AutoAnalyzer. Serum calcium was assayed by atomic absorption spectrometry (18). Radioimmunoassay for parathyroid hormone was carried out according to Berson, Yalow, Aurbach, and Potts (19) with the modification that separation of free from antibody-bound hormone was accomplished with dextran-coated, activated charcoal as described by Herbert, Lau, Gottlieb, and Bleicher for insulin (20).

Statistical analyses used were standard procedures (21) with application of Student's *t* test for degree of significance.

*Materials.* Purified bovine parathyroid hormone (22) in 2% cysteine-HCl, pH 4.5 was passed through a sterile Millipore filter, diluted with sterile gelatin, and stored in sealed vials at 4°C. Each vial contained 0.25 mg of parathyroid hormone in 15% gelatin–0.11% cysteine–HCl. The hormone used in this study was processed at the same time from a single preparation of purified material. Bioassays (23) immediately after preparation and after 2 yr of storage gave similar results, 300 USP U/vial, showing that no significant loss of potency occurred during this period. The "vehicle," 0.11% cysteine–HCl, pH 4.5 in 15% gelatin, was also stored in aliquots of 0.5 ml at 4°C. Glucagon for injection, USP was from Eli Lilly & Company. Tritiated 3′,5′-AMP (2250 mc/mmole) was purchased from Schwarz Bioresearch; other chemicals were reagent grade from standard suppliers.

## RESULTS

*Urinary excretion of 3′,5′-AMP under basal conditions.* Results for normal subjects and patients are expressed in nanomoles of 3′,5′-AMP excreted per minute (Table II) or nanomoles of 3′,5′-AMP excreted per milligram of creatinine (Table III) for each collection period on day I. In some studies urinary creatinine was not determined. Excretion of 3′,5′-AMP (nmoles/min or nmoles/mg of creatinine) was not significantly different ($P > 0.1$) from one period to another during the test. Thus, data for the four collection periods were combined for each group of patients and are expressed as excretion from 8:00 a.m. until noon. The rate of excretion of 3′,5′-AMP in this period was significantly less than normal for patients with hypocalcemic disorders (idiopathic, pseudo-, and surgical hypoparathyroidism) and significantly greater than normal for patients with pseudopseudohypoparathyroidism. Excretion

TABLE I
*Subjects with*

| Subject | Age | Sex | History | | | | Physical examination | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Seizures | Carpopedal spasm | Hypocalcemia | Delayed dentition | Obesity | Round face | Short stature | Cataracts | Mental retardation |
| | *yr* | | | | | | | | | | |
| B. A. | 16 | M | − | − | + | + | − | + | + | + | + |
| S. B. | 16 | F | − | − | + | + | + | + | + | − | + |
| S. C. | 19 | F | + | + | + | + | + | + | + | − | + |
| J. E. | 43 | M | − | − | + | + | + | + | − | + | + |
| B. H. | 17 | F | + | + | + | + | + | + | + | + | + |
| E. H. | 29 | F | + | + | + | + | − | + | − | + | − |
| A. J. | 46 | M | + | − | + | + | − | + | − | + | + |
| N. M. | 19 | M | + | + | + | − | − | − | − | − | + |
| R. M. | 30 | M | + | + | + | + | + | + | + | + | + |
| J. S. | 37 | F | − | + | + | + | + | + | + | − | + |
| B. T. | 18 | M | + | − | + | + | + | + | − | + | + |
| L. T. | 19 | M | + | + | + | + | − | − | − | + | + |
| K. U. | 28 | M | − | + | + | − | + | + | + | − | + |

Ca, calcium; P, phosphorus.

of 3′,5′-AMP relative to urinary creatinine was significantly different from normal only in the group of patients with pseudopseudohypoparathyroidism. Further studies with samples obtained from four normal volunteers at 4-hr intervals for 2 days showed no diurnal pattern in excretion of 3′,5′-AMP. Thus, one could predict that extrapolation of the rate of excretion obtained between 8:00 a.m. and noon would give a valid ap-

TABLE II
*Urinary Excretion of Cyclic 3′, 5′-AMP under Basal Conditions**

| Subjects | Number | Cyclic 3′, 5′-AMP‡ | | | | | |
|---|---|---|---|---|---|---|---|
| | | 8:00–9:00 | 9:00–10:00 | 10:00–11:00 | 11:00–12:00 | 8:00–12:00 | 9:00–12:00 |
| | | *nmoles/min* | | | | | *Total μmoles* |
| Normal | 11 | 3.9 ±0.5 | 3.4 ±0.4 | 3.0 ±0.6 | 3.6 ±0.5 | 3.5 ±0.2 | 0.62 ±0.07 |
| Pseudohypoparathyroidism | 13 | 2.5 ±0.5 | 3.0 ±0.8 | 2.6 ±0.6 | 2.6 ±0.6 | 2.7 ±0.3§ | 0.47 ±0.11§ |
| Idiopathic hypoparathyroidism | 5 | 2.0 ±0.3 | 2.2 ±0.6 | 2.2 ±0.4 | 2.1 ±0.4 | 2.1 ±0.2‖ | 0.38 ±0.0‖ |
| Pseudopseudohypoparathyroidism | 4 | 4.6 ±1.1 | 4.1 ±0.7 | 4.7 ±1.0 | 5.6 ±1.0 | 4.8 ±0.5‖ | 0.86 ±0.14‖ |
| Surgical hypoparathyroidism | 6 | 2.4 ±0.6 | 2.2 ±0.5 | 2.4 ±0.6 | 2.4 ±0.6 | 2.3 ±0.3‖ | 0.42 ±0.10‖ |

* Day I of test protocol.
‡ Mean ±SE.
§ Significantly different from normal subjects $P < 0.05$.
‖ Significantly different from normal subjects $P < 0.01$.

| Roentgenographic and laboratory studies | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Subcutaneous calcification | Exostoses | Basal ganglia calcification | Brachydactyly | Serum Ca | Serum P | Therapy, calcium and (or) vitamin D | Associated findings | Reference |
| + | + | + | + | 7.1 | 5.9 | − | | 47 |
| + | + | − | + | 7.3 | 7.9 | − | Amenorrhea, mother with pseudopseudohypoparathyroidism | 49 |
| + | − | + | + | 10.2 | 4.7 | + | Irregular menses | |
| + | − | + | + | 8.8 | 5.9 | − | | 43 |
| + | + | − | + | 7.7 | 5.3 | + | | 47 |
| − | − | − | − | 9.3 | 3.8 | + | | |
| + | + | + | − | 7.7 | 4.0 | + | Thyrotropin deficiency | 48 |
| − | − | − | − | 9.4 | 5.7 | + | | |
| + | − | + | + | 9.5 | 3.5 | + | Parathyroidectomy | |
| + | + | − | + | 7.5 | 4.6 | + | Irregular menses | |
| − | − | − | − | 10.6 | 3.6 | + | Sibs, mother with pseudopseudohypoparathyroidism | |
| − | − | − | − | 10.3 | 3.4 | + | | |
| + | − | + | + | 9.1 | 3.7 | + | Thyrotropin deficiency, mother with pseudopseudohypoparathyroidism | 47 |

proximation for total daily excretion of the nucleotide. The extrapolated result, 1.1–8.6 μmoles (95% confidence limits), for normal subjects in fact agrees well with a previous report (24) for total daily excretion.

*Effect of parathyroid hormone on urinary excretion of 3′,5′-AMP in normal subjects.* Infusion of 300 U of purified parathyroid hormone into 12 normal volunteers caused a marked and rapid increase in urinary excretion of 3′,5′-AMP (Fig. 1 A, Table IV). The rate of excretion reached a maximum within 30 (eight sub-

TABLE III

*Urinary Excretion of Cyclic 3′, 5′-AMP under Basal Conditions**

| Subjects | Number | Cyclic 3′, 5′-AMP‡ 8:00–9:00 | 9:00–10:00 | 10:00–11:00 | 11:00–12:00 | 8:00–12:00 |
|---|---|---|---|---|---|---|
| | | *nmoles/mg of creatinine* | | | | |
| Normal | 10 | 3.9 ±0.7 | 4.1 ±0.5 | 3.1 ±0.6 | 4.5 ±0.9 | 3.9 ±0.3 |
| Pseudohypoparathyroidism | 12 | 3.3 ±0.5 | 3.1 ±0.5 | 3.3 ±0.5 | 3.0 ±0.8 | 3.2 ±0.3 |
| Idiopathic hypoparathyroidism | 4 | 3.4 ±1.1 | 2.9 ±0.7 | 4.0 ±0.7 | 3.8 ±0.4 | 3.5 ±0.4 |
| Pseudopseudohypoparathyroidism | 4 | 5.9 ±1.8 | 6.2 ±1.3 | 5.2 ±1.5 | 7.9 ±1.3 | 6.3 ±0.7§ |
| Surgical hypoparathyroidism | 4 | 3.1 ±0.3 | 2.8 ±0.4 | 3.4 ±0.4 | 2.9 ±0.1 | 3.0 ±0.2 |

* Day I of test protocol.

‡ Mean ±SE.

§ Significantly different from normal subjects $P < 0.01$.

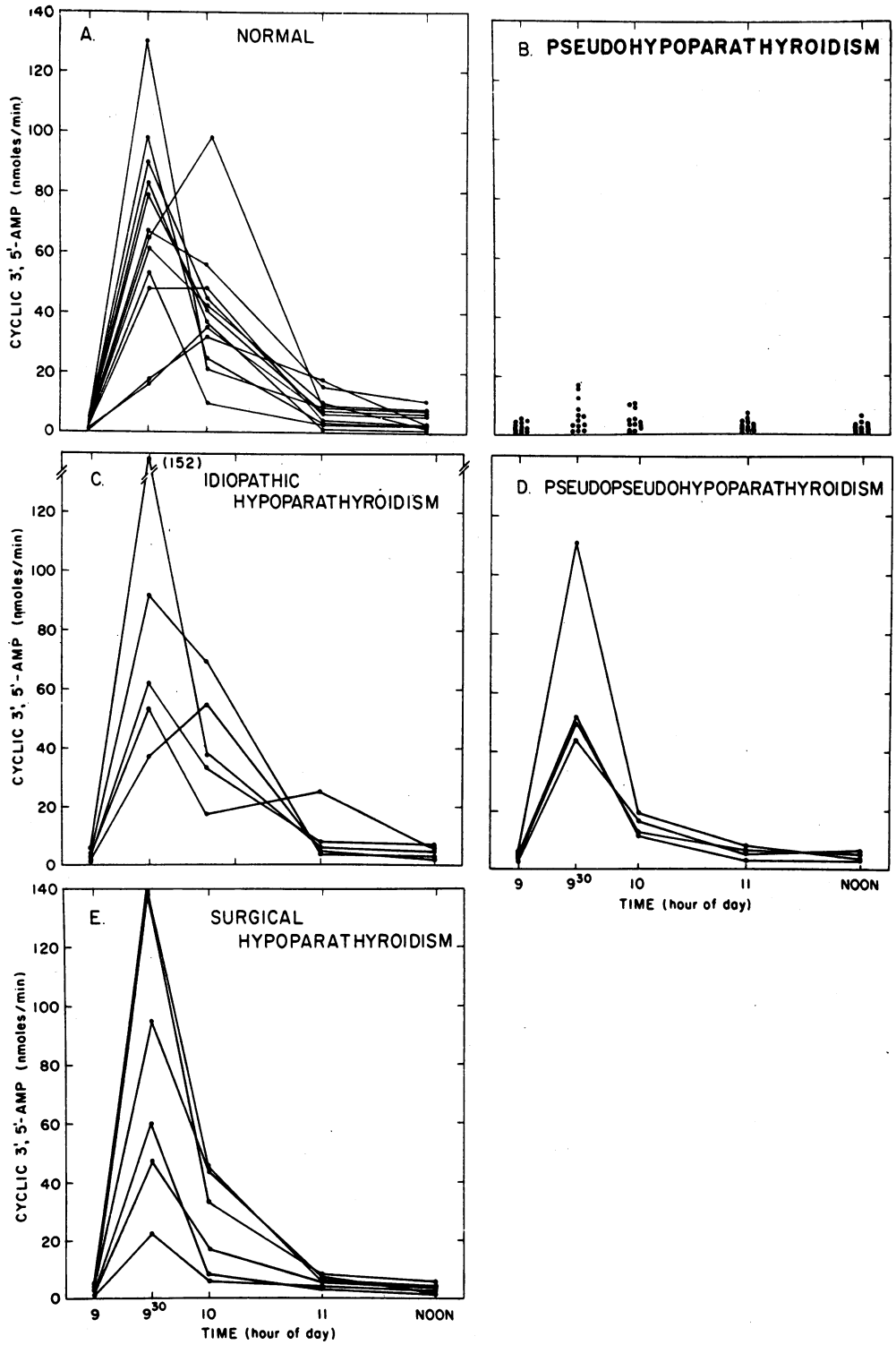


FIGURE 1 Effect of parathyroid hormone on the urinary excretion of cyclic 3′,5′-AMP. 300 U of parathyroid hormone were infused from 9:00 to 9:15 a.m. and urine collected as described in the text for day II of the test protocol. Results represent the rate of excretion of cyclic 3′,5′-AMP for each interval and are plotted to coincide with the end of the period. Each continuous line represents the pattern of excretion for one subject. Individual patterns of excretion are not shown in Fig. 1 B where each point represents the result for one subject.

TABLE IV

*Effect of Parathyroid Hormone on Urinary Excretion of Cyclic 3', 5'-AMP**

| Subjects | Number | Cyclic 3', 5'-AMP‡ | | | | | |
|---|---|---|---|---|---|---|---|
| | | 8:00–9:00 | 9:00–9:30 | 9:30–10:00 | 10:00–11:00 | 11:00–12:00 | 9:00–12:00 |
| | | *nmoles/min* | | | | | *Total μmoles* |
| Normal | 12 | 3.3 ±0.7 | 67.9 ±9.5 | 40.4 ±6.4 | 7.3 ±1.4 | 4.8 ±0.8 | 3.90 ±0.35 |
| Pseudo-hypoparathyroidism | 13 | 2.7 ±0.5 | 6.4 ±1.6 | 4.5 ±0.9 | 3.0 ±0.6 | 2.1 ±0.4 | 0.63 ±0.12 |
| Idiopathic hypoparathyroidism | 5 | 2.8 ±0.6 | 79.4 ±20.4 | 42.2 ±9.0 | 9.6 ±4.0 | 3.5 ±0.6 | 4.43 ±0.54 |
| Pseudopseudo-hypoparathyroidism | 4 | 4.2 ±0.6 | 64.2 ±15.7 | 14.6 ±1.5 | 5.4 ±0.8 | 4.8 ±1.0 | 2.98 ±0.49 |
| Surgical hypoparathyroidism | 6 | 3.5 ±0.9 | 85.8 ±21.3 | 25.0 ±7.3 | 4.5 ±1.0 | 3.2 ±0.5 | 3.78 ±0.91 |

* Day II of test protocol. 300 U of parathyroid hormone were infused from 9:00 to 9:15 a.m.
‡ Mean ±SE.

jects) to 60 (four subjects) min and declined thereafter, approaching the baseline by 3 hr. The total excretion of 3',5'-AMP for this 3-hr period was 3.90 ±0.35 μmoles (mean ±SE) (Table IV) compared with 0.62 ±0.07 μmoles (Table II) for the comparable 3-hr period on the control day. Infusion of "vehicle" without parathyroid hormone caused no change in excretion of 3',5'-AMP during this period. Infusion of 50 U of parathyroid hormone into one normal volunteer caused a 5-fold increase in urinary 3',5'-AMP within 30 min compared with a 20-fold increase in response to 300 U of the hormone.

*Effect of parathyroid hormone on urinary excretion of 3',5'-AMP in patients with disorders related to the parathyroid glands.* Results for patients with idiopathic, surgical, pseudo-, and pseudopseudohypoparathyroidism are illustrated in Fig. 1 B–E which represents the rate of excretion of 3',5'-AMP for each collection period. Results expressed in nanomoles of 3',5'-AMP excreted per minute and nanomoles of 3',5'-AMP excreted per milligram of creatinine are listed in Tables IV and V. All patients except those with pseudohypoparathyroidism and one with surgical hypoparathyroidism showed responses to parathyroid hormone that were similar, both qualitatively and quantitatively, to those of normal subjects. 9 of the 13 patients with pseudohypoparathyroidism showed no response to parathyroid hormone and four (N. M., B. T., L. T., and J. S., see Table I) showed clearly deficient minimal responses. The patient with surgical hypoparathyroidism

TABLE V

*Effect of Parathyroid Hormone on Urinary Excretion of Cyclic 3', 5'-AMP**

| Subjects | Number | Cyclic 3', 5'-AMP‡ | | | | |
|---|---|---|---|---|---|---|
| | | 8:00–9:00 | 9:00–9:30 | 9:30–10:00 | 10:00–11:00 | 11:00–12:00 |
| | | *nmoles/mg of creatinine* | | | | |
| Normal | 11 | 2.9 ±0.6 | 45.9 ±10.2 | 38.7 ±7.5 | 7.5 ±1.8 | 4.8 ±1.0 |
| Pseudo-hypoparathyroidism | 13 | 3.5 ±0.5 | 6.1 ±1.2 | 4.8 ±1.0 | 3.2 ±0.5 | 2.7 ±0.3 |
| Idiopathic hypoparathyroidism | 4 | 3.0 ±0.4 | 171.2 ±59.7 | 105.3 ±37.8 | 15.5 ±7.8 | 6.3 ±1.8 |
| Pseudopseudo-hypoparathyroidism | 4 | 4.2 ±1.3 | 73.8 ±19.9 | 20.2 ±2.6 | 7.6 ±1.9 | 6.9 ±1.6 |
| Surgical hypoparathyroidism | 6 | 3.5 ±1.0 | 106.7 ±27.2 | 35.9 ±10.4 | 9.0 ±2.6 | 5.0 ±1.0 |

* Day II of test protocol. 300 U of parathyroid hormone were infused from 9:00–9:15 a.m.
‡ Mean ±SE.

who responded minimally to parathyroid hormone evidenced hypercalcemic nephropathy (serum calcium 18.1 mg/100 ml and blood urea nitrogen 35 mg/100 ml), nephrolithiasis, and chronic pyelonephritis before surgical removal of an 11 gm parathyroid adenoma. Postoperatively, hypocalcemia developed and impaired renal function persisted (blood urea nitrogen 46 mg/100 ml and creatinine clearance 25 ml/min); thus, it is possible that severe renal disease in some way inhibited formation or excretion of 3′,5′-AMP. Infusion of vehicle alone into several patients in each group caused no change in urinary excretion of 3′,5′-AMP.

Response to parathyroid hormone was also analyzed on the basis of maximal ratio of nucleotide to creatinine (Fig. 2) and total excretion of 3′,5′-AMP (Fig. 3 and Table IV) after injection of hormone. The total excretion for the comparable 3 hr period on test day I is shown in Table II. In terms of these parameters, patients with pseudohypoparathyroidism again showed a markedly deficient response to parathyroid hormone. Results for these patients did not overlap with those of normal subjects or patients with other forms of hypoparathyroidism with the single exception of the patient with surgical hypoparathyroidism described above. Although the total excretion of 3′,5′-AMP was deficient for this patient, the maximal response to parathyroid hormone was still greater than for any of the patients with pseudohypoparathyroidism.

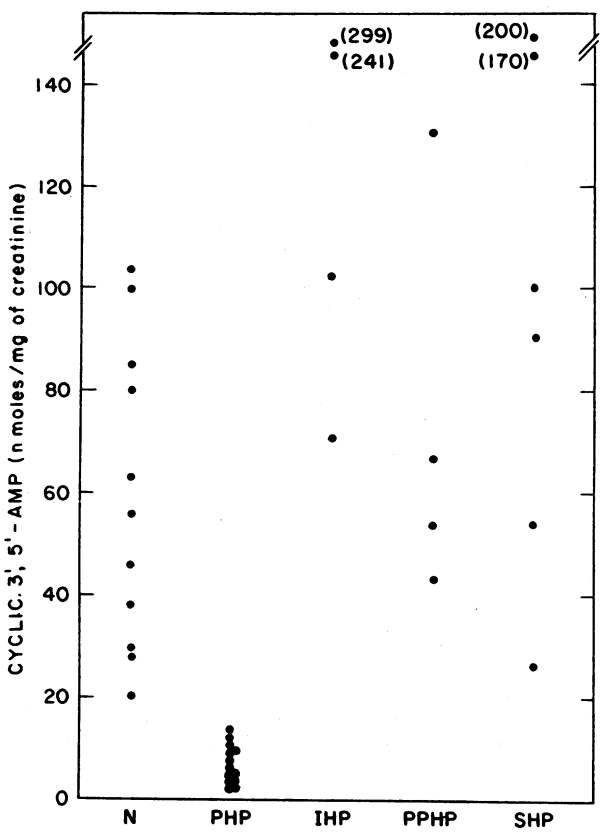


FIGURE 2 Effect of parathyroid hormone on the urinary excretion of cyclic 3′,5′-AMP. 300 U of parathyroid hormone were infused from 9:00 to 9:15 a.m. and urine collected as described in the text for day II of the test protocol. Each point represents the maximal response (highest ratio of nucleotide to creatinine observed during any period) to parathyroid hormone in nmoles of cyclic 3′,5′-AMP per mg of creatinine for one subject. N, normal; PHP, pseudohypoparathyroidism; IHP, idiopathic hypoparathyroidism; PPHP, pseudopseudohypoparathyroidism; SHP, surgical hypoparathyroidism.

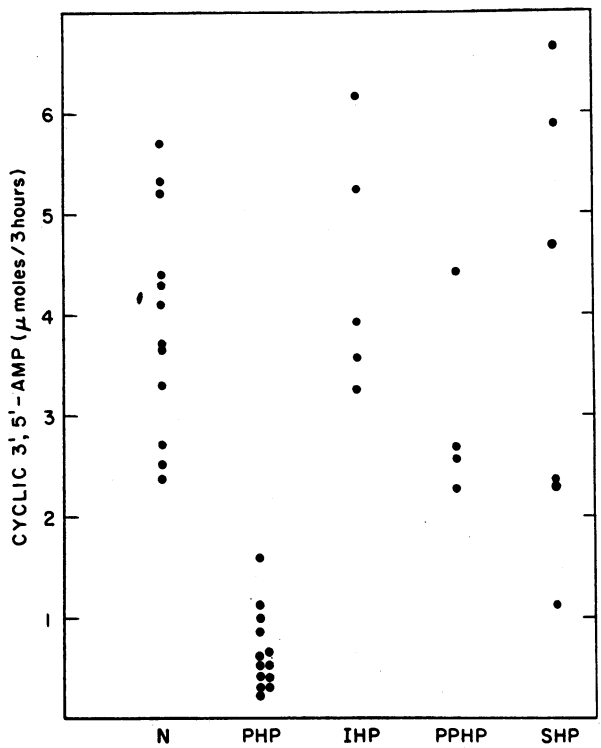


FIGURE 3 Effect of parathyroid hormone on the urinary excretion of cyclic 3′,5′-AMP. 300 U of parathyroid hormone were infused from 9:00 to 9:15 a.m. and urine collected as described in the text for day II. Each point represents μmoles of cyclic 3′,5′-AMP excreted from 9:00 a.m. to noon for one subject. N, normal; PHP, pseudohypoparathyroidism; IHP, idiopathic hypoparathyroidism; PPHP, pseudopseudohypoparathyroidism; SHP, surgical hypoparathyroidism.

Four additional patients (not represented in the figures or tables), thought to have pseudohypoparathyroidism but not evaluated personally by the authors, were infused with purified parathyroid hormone at other institutions through the cooperation of their physicians. Three did not respond but the fourth, a 40 yr old male with mental retardation, short stature, lenticular opacities, shortened fourth and fifth metacarpal bones, and

TABLE VI

*Effect of Calcium Infusion on the Concentration of Parathyroid Hormone in Plasma from Patients with Pseudohypoparathyroidism**

| Patient | Calcium 8:55 a.m. | Calcium 12:25 p.m. | PTH‡ 8:55 a.m. | PTH‡ 12:25 p.m. |
|---|---|---|---|---|
| | *mg/100 ml* | | *mμg/ml* | |
| A. J. | 8.2 | 13.4 | 1.8 | ND |
| B. A. | 7.6 | 12.1 | 3.0 | ND |
| J. E. | 8.8 | 17.0 | 3.1 | ND |

PTH, parathyroid hormone; ND, not detectable.

* Calcium (10–20 mg/kg) as calcium gluconate was infused from 9 a.m. to 1 p.m.

‡ Equivalents of purified bovine parathyroid hormone. Normal <0.5 mμg/ml. Not detectable <0.2 mμg/ml.

past history of hypocalcemia (calcium at the time of the study was 8.6–9.8 mg/100 ml) showed an increase from 3.25 to 54.0 nmoles of 3′,5′-AMP/min within 30 min after injection of hormone; total excretion rose from 0.5 to 2.77 μmoles in 3 hr. Phosphate excretion increased 230% in response to the hormone.

*Effect of calcium infusion on the urinary response to parathyroid hormone in pseudohypoparathyroidism.* Tests with ruminant species (25, 26) and human subjects with secondary hyperparathyroidism (27) showed that secretion of parathyroid hormone became completely suppressed as the concentration of calcium in plasma rose toward 12 mg/100 ml. It was important to determine whether secretion of parathyroid hormone was under physiological control by calcium in subjects with pseudohypoparathyroidism and, if so, whether the pattern of excretion of 3′,5′-AMP after exogenous hormone would revert to normal under conditions where secretion of endogenous hormone was suppressed. 10 of the patients with this disorder were given calcium intravenously, sufficient to raise the concentration in serum to 12 mg/100 ml or greater. Results of radioimmunoassay[2] for parathyroid hormone in plasma from three of these patients before and after 3.5 hr of calcium infusion are listed in Table VI. Parathyroid hormone in plasma was abnormally high before infusion but was undetectable at the time of hypercalcemia. The 10 patients with pseudohypoparathyroidism given parathyroid hormone during the period of hypercalcemia evidenced the same defective response in excretion of 3′,5′-AMP as found on the 2nd test day, whereas infusion of parathyroid hormone into four normal subjects under the same conditions caused a marked increase (Fig. 4) similar to the response observed on day II.

*Family studies.* Pseudohypoparathyroidism, apparently inherited as a sex-linked, dominant trait (28), and pseudopseudohypoparathyroidism may appear within a single family in the same or succeeding generations. S. B. and K. U., with pseudohypoparathyroidism (see Table I), were the progeny of mothers with pseudopseudohypoparathyroidism. Parathyroid hormone caused a marked increase in urinary 3′,5′-AMP in each mother, but not in the affected children. Tests were made on several members of another family which included three brothers with pseudohypoparathyroidism. Two of the affected brothers (D. T. and B. T., Table I) were tested with parathyroid hormone and did not respond. The father, evidencing calcification of the choroid plexus but not of the basal ganglia, and a son, with an epileptic disorder and probable cerebral palsy but without hypocalcemia or other signs of pseudohypoparathyroidism, responded normally. The mother, with short stature and marked subcutaneous calcification but no current or past history of hypocalcemia, also responded normally. It is probable that she represents a case of pseudopseudohypoparathyroidism. The third sibling with pseu-

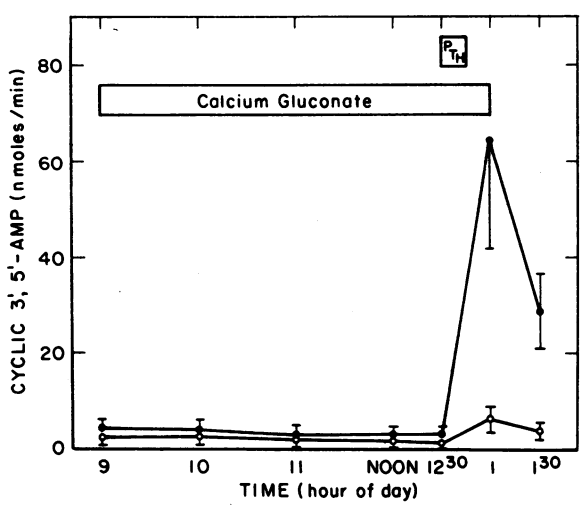


FIGURE 4 Effect of calcium on urinary excretion of cyclic 3′,5′-AMP in response to parathyroid hormone in normal subjects and patients with pseudohypoparathyroidism. Sufficient calcium (10–20 mg/kg) was infused as calcium gluconate from 9:00 a.m. to 1:00 p.m. to raise serum calcium above 12 mg/100 ml. 300 U of parathyroid hormone were infused from 12:30 to 12:45 p.m. and urine collected as described in the text for day III. The mean response ±1 SD (vertical bar) is plotted at the end of each collection period and represents nmoles of cyclic 3′,5′-AMP excreted per minute during the period. ●——●, normal subjects (n = 4); ○——○, pseudohypoparathyroidism (n = 10); PTH, parathyroid hormone.

[2] We wish to acknowledge the collaboration of Doctors R. Reitz and John T. Potts, Jr., in this aspect of the study. A detailed presentation of these radioimmunoassay studies will be the subject of a subsequent communication.

TABLE VII

*Effect of Glucagon and Parathyroid Hormone on Urinary Excretion of Cyclic 3', 5'-AMP*

| Subject | Glucagon | | Cyclic 3', 5'-AMP | | |
|---|---|---|---|---|---|
| | Route | Dose | Day I* | Day II‡ | Glucagon§ |
| | | *mg* | | *μmoles/3 hr* | |
| Control | i.v. | 0.5 | 1.06 | NT | 2.83 |
| Control | i.v. | 0.5 | 0.58 | NT | 2.58 |
| Pseudopseudohypoparathyroidism | i.m. | 5 | 1.24 | 2.58 | 4.52 |
| Pseudohypoparathyroidism (J. S.) | i.m. | 5 | 0.83 | 0.97 | 2.00 |
| | i.v. | 0.5 | | | 2.61 |
| Pseudohypoparathyroidism (A. J.) | i.m. | 5 | 0.38 | 0.62 | 1.35 |
| | i.v. | 0.5 | | | 7.64 |

i.v., intravenous; i.m., intramuscular; NT, not tested.

* Day I of test protocol—no infusion.

‡ Day II of test protocol—infusion of parathyroid hormone, 300 U.

§ Glucagon was administered at 9 a.m. Urine was collected as on day II of the test protocol.

dohypoparathyroidism and a normal son by a previous marriage of the father were not available for study.

*Effect of glucagon on urinary excretion of 3',5'-AMP.* It has been reported recently (29) that glucagon also causes increased urinary excretion of 3',5'-AMP. This response presumably reflects activation of adenyl cyclase in the liver with consequent increase in 3',5'-AMP in plasma (29), whereas the response to parathyroid hormone is manifested through direct activation of the enzyme in renal cortex (3). Injection or infusion of glucagon was used to test the response of the enzyme in an extrarenal tissue. The protocol was as described for day II except that glucagon was administered instead of parathyroid hormone. Two control subjects without abnormalities of calcium metabolism, one patient with pseudopseudohypoparathyroidism, and two patients with pseudohypoparathyroidism showed a marked increase in urinary excretion of 3',5'-AMP (Table VII).

*Phosphaturic response to parathyroid hormone.* The classical Ellsworth-Howard test (13), based on the phosphaturic response to crude parathyroid extract, frequently gives an equivocal result, often attributed to

TABLE VIII

*Effect of Parathyroid Hormone on Urinary Excretion of Phosphate and Cyclic 3', 5'-AMP in Normal Subjects**

| Subject | Phosphate/creatinine ratio | | | | Cyclic 3', 5'-AMP | | | |
|---|---|---|---|---|---|---|---|---|
| | Day I | | Day II | | Day I | | Day II | |
| | Min. | Max. | Min. | Max. | Min. | Max. | Min. | Max. |
| | | | | | *nmoles/mg of creatinine* | | | |
| 1 | 0.37 | 0.55 | 0.38 | 0.70 | 3.7 | 4.3 | 3.0 | 80.8 |
| 2 | 0.45 | 0.80 | 0.80 | 1.30 | 1.5 | 4.8 | 2.0 | 84.6 |
| 3 | 0.41 | 0.52 | 0.23 | 0.52 | 2.3 | 8.4 | 1.9 | 56.4 |
| 4 | 0.15 | 0.71 | 0.56 | 0.87 | 1.1 | 2.5 | 1.8 | 28.6 |
| 5 | 0.19 | 0.30 | 0.21 | 0.42 | 0.9 | 4.8 | 1.0 | 45.6 |
| 6 | 0.33 | 0.42 | 0.31 | 0.67 | 4.9 | 7.8 | 4.8 | 62.8 |
| 7 | 0.59 | 0.80 | 0.37 | 0.72 | 2.5 | 3.5 | 2.8 | 38.0 |
| 8 | 0.41 | 1.1 | 0.45 | 0.85 | 1.5 | 2.3 | 2.8 | 19.5 |
| 9 | 0.19 | 0.62 | 0.19 | 1.60 | 2.7 | 8.8 | 4.1 | 99.9 |
| 10 | — | — | 0.16 | 0.23 | — | — | 2.2 | 32.0 |
| 11 | 0.39 | 0.56 | 0.47 | 0.75 | 1.9 | 4.0 | 2.2 | 104.7 |

Min., minimum; Max., maximum.

* Data represent the range for four collection periods on day I (no infusion or infusion of vehicle) and five collection periods on day II (infusion of parathyroid hormone, 300 U, from 9:00 to 9:15 a.m.).

inactive parathyroid extract. Moreover, experience indicates that, at best, the phosphaturic response to parathyroid extract is inconstant in normal subjects (30–32). The current data (Table VIII) support this conclusion. The phosphaturic response to purified parathyroid hormone in normal subjects was variable and frequently small in magnitude, whereas the response manifested in excretion of 3′,5′-AMP was constant and marked. It was also observed that most subjects showed basal rates of excretion that were more constant from day to day for 3′.5′-AMP than for phosphate.

## DISCUSSION

Our previous studies showed that parathyroid hormone caused a marked increase in urinary excretion of 3′,5′-AMP (12) as a consequence of direct stimulation of renal adenyl cyclase (2, 3). The finding that 3′,5′-AMP became virtually undetectable in urine from rats after hypercalcemia was induced suggested that excretion of this nucleotide was primarily a function of the rate of secretion of parathyroid hormone. In contrast to these studies, the current findings indicate that only a fraction of total 3′,5′-AMP excreted by human subjects is controlled by parathyroid hormone. Inhibiting secretion of endogenous parathyroid hormone by infusing calcium into human subjects caused only a slight fall in urinary excretion of 3′,5′-AMP, and patients with idiopathic or surgical hypoparathyroidism continued to excrete 3′,5′-AMP at a rate only slightly less than normal. In fact, further study is necessary to be sure that the latter difference is real, since it was statistically significant only for results expressed as nanomoles of 3′,5′-AMP excreted per minute. It is likely then that other factors significantly influence the rate of excretion of 3′,5′-AMP in man. One report (33) showed that urinary 3′,5′-AMP in man increased in response to vasopressin, an effect expected in view of the action of this hormone on adenyl cyclase in the renal medulla (3). However, for reasons unknown at present, this effect of vasopressin in vivo is small, and in several experiments of our own (Chase, Melson, and Aurbach, unpublished observations) no significant increase in urinary excretion of the nucleotide was detected after injection of vasopressin into normal subjects or patients with diabetes insipidus. Glucagon in pharmacological amounts causes increased urinary 3′,5′-AMP in man presumably through direct activation of adenyl cyclase in the liver (29), but it is not known if endogenous glucagon controls the rate of excretion of the nucleotide under normal physiological conditions. Further studies will be necessary to evaluate the possibility that this hormone or others that activate adenyl cyclase in extrarenal tissues are important in regulating this process. In any event, the urinary excretion of 3′.5′-AMP has proved to be a sensitive and reliable index of responsiveness to parathyroid hormone. Normal subjects show a marked response uniformly much greater than baseline (see Table VIII), whereas the phosphaturic response to the hormone is often small and indistinguishable from diurnal variations (30–32). Thus, one may anticipate that measurement of 3′,5′-AMP will be established as a definitive diagnostic test for pseudohypoparathyroidism.

Subsequent to the thesis of resistance to parathyroid hormone by Albright et al. (1), other proposals have been offered to explain the pathophysiology of pseudohypoparathyroidism. Thyrocalcitonin has been found in high concentration in the thyroid of several subjects with pseudohypoparathyroidism (34–36), but this finding is a manifestation of hypocalcemia (37, 38), not hypersecretion of the hormone. Removal of the thyroid glands from two patients with pseudohypoparathyroidism caused only a transient rise in serum calcium in one patient (36) and no detectable change in the other (39). Further, studies with rats show that thyrocalcitonin does not inhibit the activation of renal adenyl cyclase by parathyroid hormone, nor does it influence urinary excretion of 3′,5′-AMP (2, 5, 12). Thus, thyrocalcitonin does not appear to be an etiological factor in pseudohypoparathyroidism. Previous studies (40), as well as our own, showed that immunologically reactive parathyroid hormone circulates in high concentration in pseudohypoparathyroidism. Our results also show that secretion of parathyroid hormone in these patients is under normal physiological control since induced hypercalcemia caused a sharp decrease in circulating hormone from abnormally high concentrations to amounts undetectable by radioimmunoassay. One might postulate that the hormone secreted in this disorder is biologically ineffective and acts as an inhibitor by blocking sites for parathyroid hormone at tissue receptors. The study of Tashjian, Frantz, and Lee (35) showing that extracts from the parathyroid glands of a patient with pseudohypoparathyroidism caused a hypercalcemic response in parathyroidectomized test animals makes this thesis untenable. In the current study, induction of hypercalcemia with consequent inhibition of secretion of immunologically reactive parathyroid hormone did not correct the defective renal response to exogenous hormone. Moreover, this defect in excretion of 3′,5′-AMP was also apparent in tests on one patient with pseudohypoparathyroidism (R. M.) who had undergone total parathyroidectomy 3 yr before study. Thus, the results given here, as well as previous studies (35, 40), indicate that biologically and immunologically active hormone is synthesized and secreted at increased rates by the parathyroid glands of patients with pseudohypoparathyroidism. The possibility that neutralizing antibody might circulate in these patients was not supported by direct

tests for antibody to parathyroid hormone in the plasma of affected subjects (40, 41).

The above considerations, then, reinforce the deduction of Albright and his associates that the defect in the disorder is localized to the receptor tissues. The recent experiments indicating that the mechanism of action of parathyroid hormone is mediated through activation of adenyl cyclase and the results reported here showing defective excretion of 3′,5′-AMP after injection of hormone, suggest that the metabolic abnormality in the syndrome can be accounted for by a defect in the membrane-bound enzyme adenyl cyclase. Alternatively one could postulate a defect in the receptor site at the plasma membrane of the cell. Since there is as yet no proof that the receptor site for the hormone and adenyl cyclase are functionally separable, the argument is not yet of compelling importance. Other interpretations might include increased destruction of 3′,5′-AMP perhaps through the phosphodiesterase mechanism. This possibility is unlikely particularly since there has never been described a genetic disorder attributed to excessive enzyme activity. It will be important to relate the postulated defect of adenyl cyclase to the mode of inheritance of the disorder, currently best explained as a sex-linked, dominant trait (28). The finding that subjects with pseudopseudohypoparathyroidism excrete more 3′,5′-AMP than normal subjects under basal conditions may be of significance in this regard.

Earlier clinical observations (42, 43) showed that repeated intramuscular injections of parathyroid extract caused a slight rise in concentration of calcium in the blood of some patients with pseudohypoparathyroidism, suggesting that in these patients the metabolic defect was incomplete. The current finding that exogenous parathyroid hormone caused a deficient but detectable increase in urinary 3′,5′-AMP in 4 of the 13 patients with pseudohypoparathyroidism is consistent with this hypothesis. A single patient with pseudohypoparathyroidism responded normally to parathyroid hormone. Unfortunately, we did not have the opportunity to evaluate this patient personally to verify the diagnosis. It is possible that the diagnosis was inaccurate, that the patient had regained responsiveness to parathyroid hormone,[8] or that he represents a variant of the disorder different from that usually found in pseudohypoparathyroidism.

Results of previous studies (5) indicate that adenyl cyclase from particular tissues responds only to specific hormones. It is likely then that patients with pseudohypoparathyroidism are deficient only in the particular form of adenyl cyclase sensitive to parathyroid hormone; this is supported by the finding that two patients with pseudohypoparathyroidism responded normally to glucagon. To substantiate this hypothesis it will be important to obtain samples of bone, kidney, and other tissues to test directly for hormonal activation of adenyl cyclase in vitro. One would predict that adenyl cyclase in renal cortex and skeletal tissue from patients with pseudohypoparathyroidism will be insensitive to parathyroid hormone but the enzyme in renal medulla, liver, and skeletal muscle will respond to vasopressin, glucagon, and epinephrine, respectively.

Although the current studies were limited to tests reflecting the effect of parathyroid hormone on renal tissue, the facts that skeletal and renal tissue respond abnormally to the hormone in pseudohypoparathyroidism and the action of the hormone on both tissues is mediated through a common primary mechanism, lead to the conclusion that the metabolic defect in pseudohypoparathyroidism can be accounted for by a partial or total lack of parathyroid hormone-sensitive adenyl cyclase in both renal and skeletal tissue.

[8] Several cases (43–46) have shown apparent spontaneous correction of hypocalcemia; however, normocalcemia was not well documented in these cases and the phosphaturic response to parathyroid extract was not tested under these conditions.

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