Effects of Dibutyryl Cyclic Adenosine 3′,5′-Monophosphate and Parathyroid Extract on Calcium and Phosphorus Metabolism in Hypoparathyroidism and Pseudohypoparathyroidism

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ABSTRACT It has been proposed previously that the metabolic defect in pseudohypoparathyroidism which accounts for parathyroid hormone unresponsiveness is an absence or abnormal form of the adenyl cyclase system in kidney and presumably in bone. To determine whether there is an associated defect in the response mechanism to cyclic adenosine 3′,5′-monophosphate (cyclic AMP), the effects of parathyroid extract (PTE), and dibutyryl cyclic AMP were compared in patients with either surgical hypoparathyroidism or pseudohypoparathyroidism. PTE and dibutyryl cyclic AMP both increased serum and urinary calcium, lowered the serum phosphorus, and increased urinary phosphorus in patients with hypoparathyroidism. PTE also increased urinary cyclic AMP in these patients. PTE increased serum and urinary calcium and urinary phosphorus but did not alter serum phosphorus or urinary cyclic AMP in the patients with pseudohypoparathyroidism. Dibutyryl cyclic AMP increased the serum and urinary calcium, lowered the serum phosphorus, and increased urinary phosphorus in all the patients with pseudohypoparathyroidism. The results indicate that (*a*) dibutyryl cyclic AMP can reproduce the effects of parathyroid hormone on calcium and phosphorus metabolism in man, (*b*) the response mechanism to cyclic AMP appears to be intact in pseudohypoparathyroidism, and (*c*) PTE apparently produces some of its characteristic effects on calcium and phosphorus metabolism in pseudohypoparathyroidism in the absence of an increase in urinary cyclic AMP.

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INTRODUCTION

Pseudohypoparathyroidism (PHP)[1] is a disease in which resistance of the kidneys and skeletal system to parathyroid hormone (PTH) may occur alone or in association with other congenital abnormalities (1–3). These include short stature, round face, short phalanges, metacarpal or metatarsal bones, exostoses, radius curvus, mental retardation, and expressionless face (1–3). It has been proposed that the metabolic defect is an inability of the adenyl cyclase system in the kidney to respond to parathyroid hormone (PTH) (4). It is assumed that the same defect exists in bone. Thus, whereas PTH regularly increases the urinary excretion of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) in normal subjects and in patients with hypoparathyroidism (HP), it has little or no effect in patients with PHP in this regard.

To determine whether the response mechanism to cyclic AMP is intact in PHP, the effects of parathyroid extract (PTE) and of dibutyryl cyclic AMP on calcium and phosphorus metabolism have been compared in HP and PHP.

METHODS

Two patients with surgical HP and three patients with PHP were studied. They were hospitalized either in the Clinical Research Center of Indiana University Medical Center, Robert Long Hospital, or in the Lilly Clinic, Marion County General Hospital. Patients were maintained on a

[1] *Abbreviations used in this paper:* C_P, phosphate clearance; HP, hypoparathyroidism; PHP, pseudohypoparathyroidism; PTE, parathyroid extract; PTH, parathyroid hormone.

TABLE I

Clinical Findings in Patients with Hypoparathyroidism and Pseudohypoparathyroidism

Patient	Age	Sex	Body wt.	Serum Ca	Serum P	Comments
	yr		*kg*	*mg/100 ml*		
Surgical hypoparathyroidism						
K. T.	22	F	57.3	6.4	5.5	Radical neck dissection for carcinoma of thyroid
F. B.	50	M	94.5	8.4	2.5	Radical neck dissection for carcinoma of larynx
Pseudohypoparathyroidism						
J. B.	18	M	48.2	8.6	4.7	Carpopedal spasm, cataracts, short stature, seizures
J. C.	14	M	76.5	6.4	8.1	Cataracts, seizures
F. N.	27	F	47.8	7.3	3.9	Paresthesias, carpopedal spasm, short digits, short stature, round face, mother has pseudo-pseudohypoparathyroidism

metabolic balance regimen. Each study was preceded by an equilibration period of 3 days. Patients were given a constant daily diet and fluid intake and distilled water to drink. Fasting blood samples were obtained daily and were analyzed for calcium by atomic absorption spectrometry and phosphorus (5). 24-hr urines were collected daily and analyzed for calcium, phosphorus, and cyclic AMP. Phosphate clearance (C_P) expressed as milliliters per minute was determined from 24-hr urinary phosphate and the average of the fasting serum phosphate values at the beginning and end of each collection period. For cyclic AMP, 1 ml of urine was acidified with 5% trichloroacetic acid and was extracted with water-saturated ether. The aqueous phase was lyophilized and dissolved in 0.2 M acetate buffer pH 4.0. Samples thus treated were assayed by a method which utilizes competitive binding of cyclic AMP to a kinase (6). Components for the assay were a protein kinase obtained from rabbit skeletal muscle, 40 μg; acetate buffer, 20 μmoles; ^{3}H-cyclic AMP (New England Nuclear Corp., Boston, Mass.) and either the sample for assay or cyclic AMP standard (Sigma Chemical Co., St. Louis, Mo.). Results are expressed as micromoles per day. Variance of the method was ±11%. Statistical tests of significance were carried out with Student's *t* test.

PTE, lot number 4 × R35A,[2] was administered intramuscularly every 12 hr. Dibutyryl cyclic AMP[3] was administered intravenously by continuous drip infusion in a volume of 1 liter of normal saline daily. Random samples were found by thin-layer chromatography on cellulose in the system n-butanol: acetic acid: water (2:1:1) to be essentially free of monobutyryl cyclic AMP or cyclic AMP. Water intake was diminished by the same volume during this treatment. The doses and duration of treatment with PTE and dibutyryl cyclic AMP are given in the Results.

[2] Kindly supplied by Dr. Anthony Ridolfo, Eli Lilly and Company, Indianapolis, Ind.

[3] Courtesy of Dr. G. Michal, C. F. Boehringer and Soehne, GmbH, Tutzing, West Germany.

RESULTS

The clinical findings in the five patients are summarized in Table I. All of them had been treated with vitamin D, 50,000 or 100,000 U/day before study, and vitamin D, 50,000 U/day, was continued throughout the period of observation. In two of the patients, F. B. and J. B., the initial serum calcium was borderline low and in the other three it was abnormally low. The initial serum phosphorus was normal in three of the patients, F. B., J. B., and F. N., and abnormally increased in the other two.

The effects of dibutyryl cyclic AMP and PTE in hypoparathyroidism. In patient F. B., PTE, 6, 9, or 12 U/kg per day (Fig. 1*a*, days 5 through 13), increased serum and urinary calcium, lowered the serum phosphorus, and increased urinary phosphorus and cyclic AMP. Mean C_P increased significantly during treatment with PTE as compared with the initial control period (Table II). After PTE was stopped, serum calcium had declined to control values by day 20, urinary calcium increased further, then fell, serum phosphorus increased as urinary phosphorus fell to very low levels, and urinary cyclic AMP decreased to control values. With dibutyryl cyclic AMP, 3 or 4 mg/kg per day (days 19 through 21), serum and urinary calcium increased, serum phosphorus decreased, and urinary phosphorus and cyclic AMP increased strikingly. Mean C_P also increased significantly during treatment with dibutyryl cyclic AMP (Table II). After dibutyryl cyclic AMP was stopped (days 22 through 25), the increases in serum and urinary calcium persisted, serum phosphorus increased, and urinary phosphorus and cyclic AMP decreased.

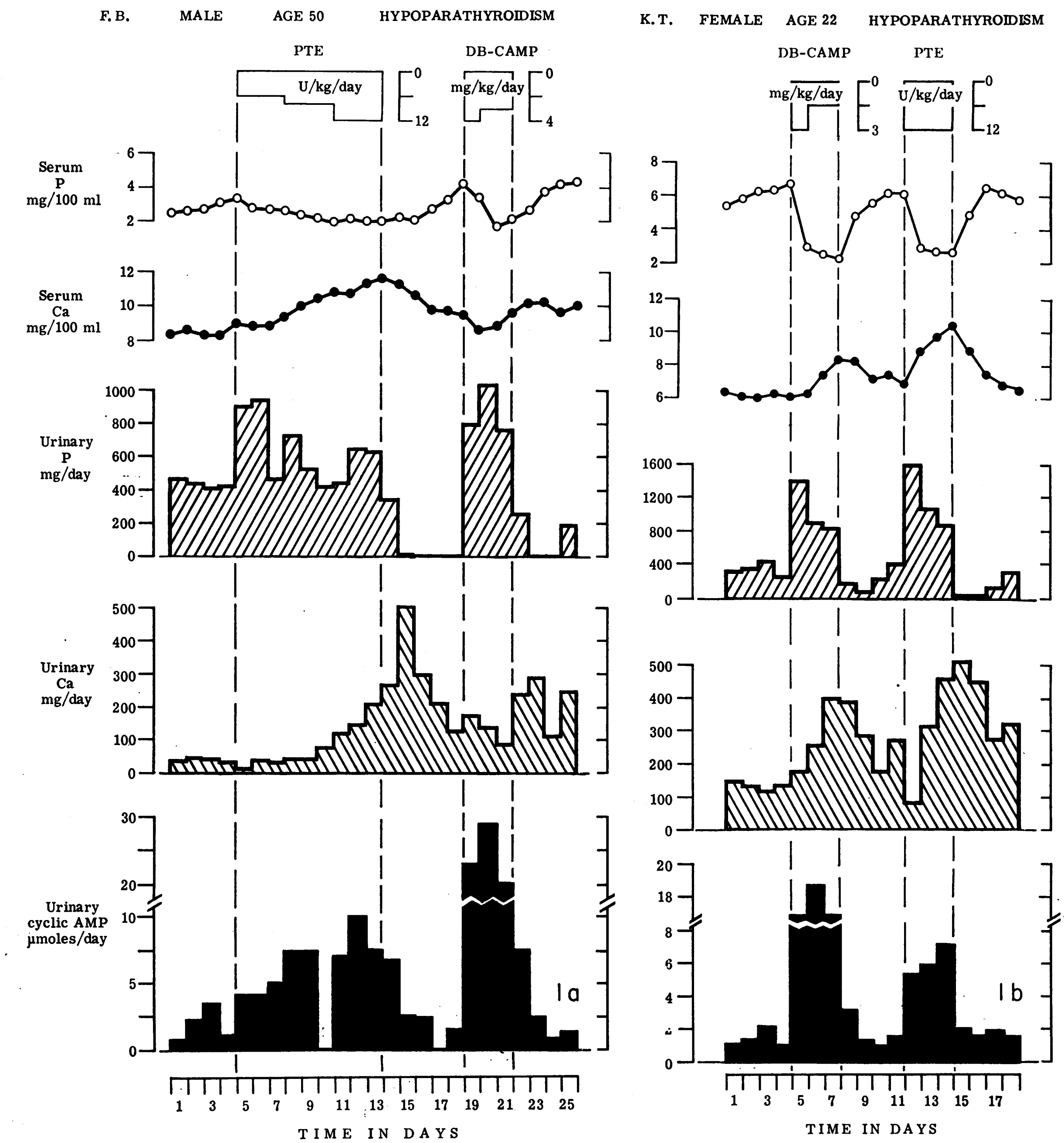


FIGURE 1 The effects of parathyroid extract (PTE) and dibutyryl cyclic adenosine 3′,5′-monophosphate (DB-CAMP) on serum and urinary calcium (Ca) and phosphorus (P) and on urinary cyclic AMP in patients F. B. (1*a*) and K. T. (1*b*) with surgical hypoparathyroidism. In F. B., urinary P was less than 5 mg/day on days 15 through 18, 23, and 24 and urinary cyclic AMP was not determined on days 10 and 17.

In patient K. T., dibutyryl cyclic AMP, 1.5 or 3 mg/kg per day (Fig. 1*b*, days 5 through 7), increased serum and urinary calcium, lowered the serum phosphorus, and increased urinary phosphorus and cyclic AMP. Mean C_P increased significantly (Table II). PTE, 12 U/kg per day (days 12 through 14), produced similar results and caused a significant increase in mean C_P. Thus in both patients with surgical HP the effects of PTE on calcium and phosphorus metabolism were reproduced by dibutyryl cyclic AMP. Whereas the changes in serum and urinary phosphorus and C_P with the two agents were similar, the increases in

Table II

The Effects of Parathyroid Extract (PTE) and Dibutyryl Cyclic Adenosine 3', 5'-Monophosphate (DB-CAMP) on Phosphate Clearance (C_P)

Patient	C_P Control	C_P PTE	P value	C_P Control	C_P DB-CAMP	P value
	ml/min			*ml/min*		
Hypoparathyroidism						
F. B.	11.0 ±0.7 (1–4)	18.5 ±1.4 (5–13)	<0.001	2.4 ±2.2 (14–18)	23.1 ±4.4 (19–21)	<0.01
K. T.	3.2 ±0.7 (8–11)	25.3 ±1.4 (12–14)	<0.001	3.9 ±0.3 (1–4)	23.7 ±1.6 (5–7)	<0.001
Pseudohypoparathyroidism						
J. C.	4.2 ±0.9 (8–11)	6.7 ±0.5 (12–14)	<0.05	4.2 ±0.7 (1–4)	12.1 ±0.9 (5–7)	<0.01
J. B.	3.9 ±1.0 (8–11)	7.1 ±1.0 (12–14)	N. S.	6.0 ±0.4 (1–4)	27.2 ±6.5 (5–7)	<0.05
F. N.	9.5 ±1.1 (8–11)	14.5 ±0.9 (12–14)	<0.02	8.9 ±0.4 (1–4)	24.3 ±0.4 (5–7)	<0.001

Numbers in parentheses represent days (see Figures).
Values represent mean ±SE.

serum calcium were not as great with the cyclic nucleotide as with PTE. Unequivocal increases in urinary cyclic AMP occurred with PTE in both patients. In F. B., urinary cyclic AMP averaged 1.9 ±0.6 (SE) μmoles/day in the control period and 6.6 ±0.7 μmoles/day during treatment ($P < 0.01$). Similarly in K. T., urinary cyclic AMP averaged 1.8 ±0.6 μmoles/day in the control period (days 9 through 11) and 6.2 ±0.5 μmoles/day during treatment ($P < 0.001$).

The effects of dibutyryl cyclic AMP and PTE in pseudohypoparathyroidism. In patient J. B., dibutyryl cyclic AMP, 3 mg/kg per day (Fig. 2*a*, days 5 through 7), changed serum calcium very little, lowered the serum phosphorus, and increased urinary calcium, phosphorus and cyclic AMP. Mean C_P increased markedly (Table II). PTE, 12 U/kg per day (days 12 through 14), increased serum and urinary calcium, increased urinary phosphorus, and did not change serum phosphorus or urinary cyclic AMP. There was a slight increase in mean C_P which was not significant.

In patient J. C., dibutyryl cyclic AMP, 3 mg/kg per day (Fig. 2*b*, days 5 through 7), increased serum and urinary calcium, lowered the serum phosphorus, and increased urinary phosphorus and cyclic AMP. Mean C_P also increased significantly (Table II). PTE, 12 U/kg per day (days 12 through 14), increased serum and urinary calcium, increased urinary phosphorus, but did not change urinary cyclic AMP. Serum phosphorus had decreased by the end of treatment (days 15 and 16) but it is not clear whether this occurred spontaneously or resulted from the PTE. There was a modest but significant increase in mean C_P.

In patient F. N., dibutyryl cyclic AMP, 3 mg/kg per day (Fig. 2*c*, days 5 through 7), increased serum and urinary calcium, lowered the serum phosphorus, and increased urinary phosphorus and cyclic AMP. Mean C_P increased significantly (Table II). PTE, 12 U/kg per day (days 12 through 14), increased serum and urinary calcium, increased urinary phosphorus, but did not change serum phosphorus or urinary cyclic AMP. Mean C_P also increased with PTE but not as much as with dibutyryl cyclic AMP.

Thus, the characteristic effects of PTE on calcium and phosphorus metabolism were readily produced by dibutyryl cyclic AMP in each of the patients with PHP and were comparable with those occurring in the patients with HP. PTE did increase serum and urinary calcium and urinary phosphorus, but did not change urinary cyclic AMP or serum phosphorus. Whereas PTE was as effective as dibutyryl cyclic AMP in increasing mean C_P in HP it was much less effective in this regard in PHP.

All patients showed increases in pulse rate when the dibutyryl cyclic AMP was infused. The drug was generally well tolerated. However, one patient, K. T., developed anorexia and nausea on the 1st day of treatment. These symptoms were eradicated when the dose was cut in half.

DISCUSSION

There are at least three lines of evidence which support the view that the effects of PTH on bone and kidney are mediated through the adenyl cyclase system.

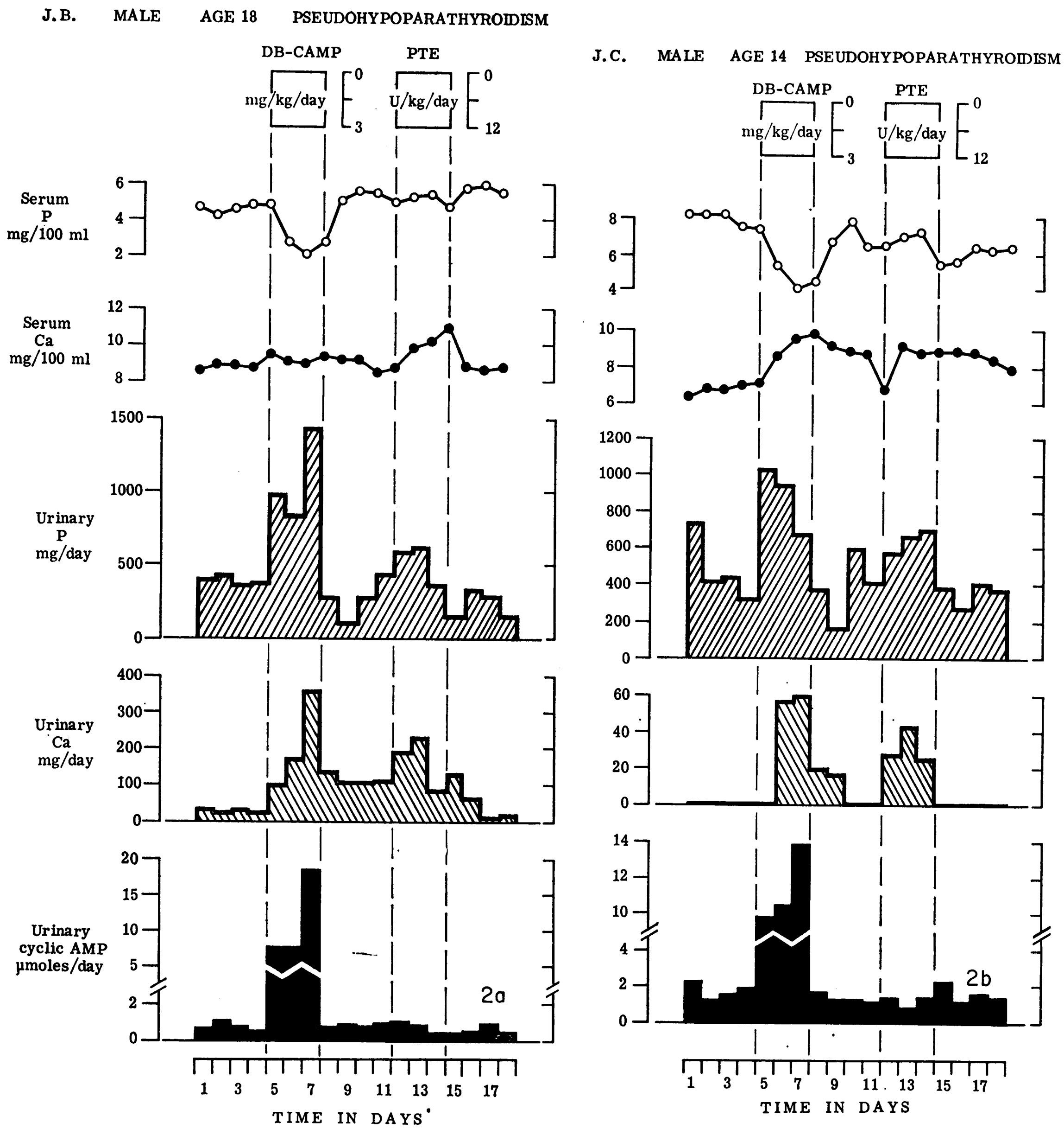
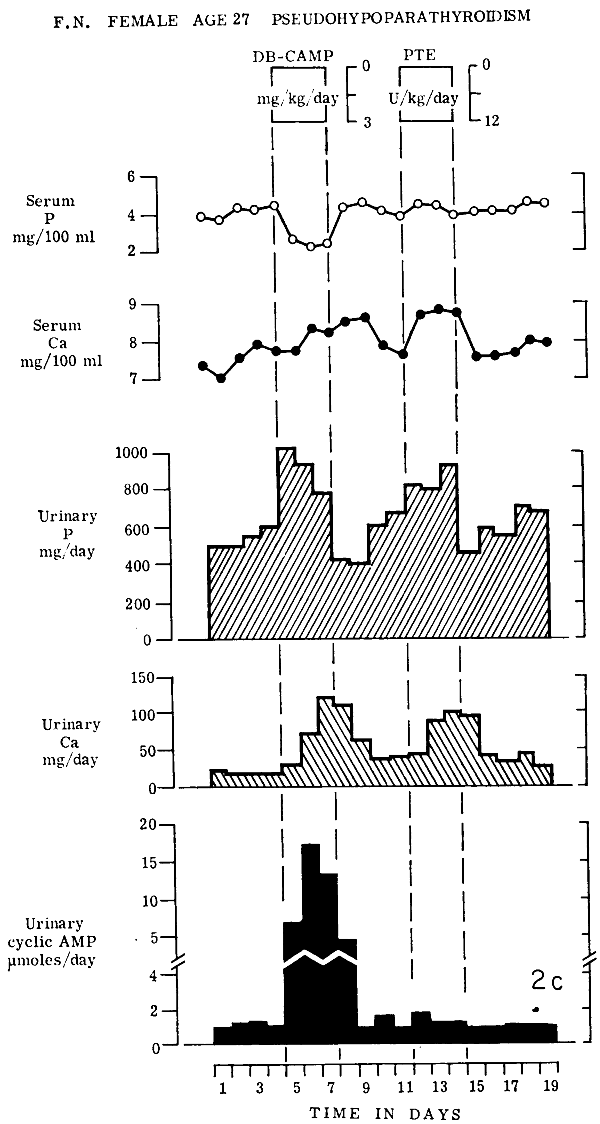


FIGURE 2 The effects of dibutyryl cyclic adenosine 3′,5′-monophosphate (DB-CAMP) and parathyroid extract (PTE) on serum and urinary calcium (Ca) and phosphorus (P) and on urinary cyclic AMP in patients J. B. (*2a*) and J. C. (*2b*) with pseudohypoparathyroidism.

First, PTH activates adenyl cyclase in both rat kidney (7, 8) and rat skeletal tissue (9) and increases urinary cyclic AMP in man (4, 10) and laboratory animals (11). Second, PTH stimulates the accumulation of cyclic AMP in fetal rat calvaria (12). Third, some of the effects of PTH on calcium and phosphorus metabolism (13–16) and on urinary hydroxyproline (13) are reproduced in laboratory animals and man by dibutyryl cyclic AMP. Moreover, resorption of fetal long bones cultured in vitro is also stimulated by this derivative of cyclic AMP (17).

The present findings in patients with surgical HP provide further evidence that the effects of PTH on calcium and phosphorus metabolism can be reproduced in man with dibutyryl cyclic AMP (16). They also suggest that the cellular response to cyclic AMP in this regard is present in PHP. Thus, the defect in these patients appears to be limited to the adenyl cyclase system in the PTH-sensitive target organs. As already noted, it cannot be determined with certainty whether the lesion is an absence or abnormality of the enzyme, the receptor for PTH or both (4).



The effects of DB-CAMP and PTE on serum and urinary calcium (Ca) and P and on urinary cyclic AMP in patient F. N. (*2c*) with pseudohypoparathyroidism. In J. C. (*2b*), urinary Ca was less than 1 mg/day on days 1 through 5, 10, 11, and 15 through 18.

That the response mechanism to cyclic AMP is intact in PHP is of more than theoretical interest. It is possible that derivatives of cyclic AMP could be developed for use in the treatment of both PHP and HP. Similarly, inhibitors of the enzyme phosphodiesterase, which converts cyclic AMP to adenosine monophosphate, might also be utilized to increase intracellular cyclic AMP in patients with HP or PHP who have an incomplete defect. Such treatment could be of particular value in patients with abnormal elevation of the serum phosphorus.

Each of the three patients with PHP appeared to have a renal defect as regards the PTH-activated adenyl cyclase system. With PTE there were no increases in urinary cyclic AMP (normally, the increase in urinary cyclic AMP after PTE is of renal origin [10]), serum phosphorus changed either equivocally (patient J. C.) or not at all (patients J. B. and F. N.) (it fell in each of them with dibutyryl cyclic AMP). As noted already, changes in mean C_P were considerably less with PTE than with dibutyryl cyclic AMP. There were no transient decreases in urinary calcium to indicate an augmentation in the renal tubular reabsorption of calcium (18, 19). In contrast, this effect of PTE on urinary calcium was clearly demonstrated on the 1st day of PTE administration in both patients with surgical HP (Fig. 1*a*, day 5 and Fig. 1*b*, day 12). This effect of PTE is a highly reproducible one in HP (19). No transient decrease in urinary calcium was demonstrated with dibutyryl cyclic AMP in any of the patients with PHP or HP. Since urinary calcium can be augmented by sodium infusion (20), such a decrease could have been obscured by the saline used to administer the cyclic nucleotide which could have inhibited the tubular reabsorption of calcium. The saline in this manner could have accounted for some of the increases in urinary calcium. However, increases in urinary calcium persisted after the infusions and were also usually associated with increases in serum calcium.

On the other hand, PTE, in the dose given, augmented serum and urinary calcium and urinary phosphorus in each of the patients with PHP. It is possible that smaller doses would not have elicited these responses and that these patients are relatively refractory and not absolutely refractory to PTE or PTH (3). These changes apparently resulted from enhanced bone resorption and increased C_P. The increases in urinary phosphorus were not accompanied by falls in serum phosphorus, as noted already, and were generally associated with increases in serum and urinary calcium. It would appear that there are at least two possible explanations to account for the PTE responsiveness of osseous tissue in PHP: either there is a partial defect in the adenyl cyclase system of bone in this syndrome or some but not all of the effects of PTH on skeletal tissue are mediated through the adenyl cyclase system. It has been demonstrated previously that some degree of PTH responsiveness may occur in some patients with PHP but not in others (21–26). Indeed, some patients develop osteitis fibrosa cystica presumably as a result of secondary increases in circulating parathyroid hormone (22, 27). In any event, it should be stressed that the hypothesis indicating that the skeletal and renal defects are similar has yet to be proved.

Chase, Melson, and Aurbach (4) demonstrated that PTH given acutely did not increase urinary cyclic AMP in patients with PHP. In 9 of 13 such patients there was no response. In the other four patients, the response was barely discernible. In contrast, PTH readily increased urinary cyclic AMP in HP. In the present studies, urinary cyclic AMP did not increase in response to PTE in the three patients with PHP even when it was given in rather large doses for 3 days but urinary cyclic AMP readily increased in the two patients with surgical HP. These results taken together provide further evidence that a demonstration that PTE or PTH does not augment urinary cyclic AMP is the best available means for establishing the diagnosis of PHP. Again, it is to be emphasized that increases in urinary cyclic AMP after PTE or PTH are derived almost entirely from the kidney (10, 11). The test may be of particular help when patients are only partially refractory to PTE as regards their calcium and phosphorus metabolism.

Finally, urinary "cyclic AMP" increased when dibutyryl cyclic AMP was given. Recovery of the administered nucleotide as urinary "cyclic AMP" ranged from 2.3 to 7.6%. The assay utilizes the binding affinity of rabbit muscle kinase for cyclic AMP (6). The affinity of the kinase for monobutyryl cyclic AMP is 1/5th and for dibutyryl cyclic AMP is 1/250th that for cyclic AMP. Since the preparation which was administered contained no detectable cyclic AMP or monobutyryl cyclic AMP on thin-layer chromatography (see Methods), it is likely that dibutyryl cyclic AMP was converted to monobutyryl cyclic AMP or cyclic AMP in vivo. It has been reported that an esterase in dog liver removes the acyl group in the 2′-0 position (28). Studies of the metabolism of dibutyryl cyclic AMP in man utilizing ^{3}H-dibutyryl cyclic AMP are in progress in this laboratory.

The means by which PTH and cyclic AMP exert their effects have not been determined. Preliminary evidence suggests that the action of cyclic AMP in mediating PTH effects on the kidney may be through activation of a kinase (29). The lack of affinity of the rabbit skeletal muscle kinase raises some interesting questions as to the mechanism of action of dibutyryl cyclic AMP. It is recognized, of course, that affinity of a cyclic nucleotide for the kinase of one tissue in vitro may not necessarily be related to activation of the kinase of another tissue in vivo. Dibutyryl cyclic AMP is resistant to conversion to adenosine monophosphate by phosphodiesterase in other tissues (30). It could act by inhibiting phosphodiesterase (31) which would allow accumulation of endogenously generated cyclic AMP. The latter explanation is difficult to accept in the case of PHP unless it is assumed that there is some cyclic AMP in cells normally responsive to PTH which is derived from an adenyl cyclase system other than the defective one.

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