Pathogenesis of Hypocalcemia in Primary Hypomagnesemia: Normal End-Organ Responsiveness to Parathyroid Hormone, Impaired Parathyroid Gland Function

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ABSTRACT Hypocalcemia is a frequent feature of hypomagnesemia in man and several other species. To elucidate the cause of this hypocalcemia, we have studied a child with primary hypomagnesemia and secondary hypocalcemia during magnesium supplementation when he was normomagnesemic and normocalcemic and after magnesium restriction for 16 days when he quickly became hypomagnesemic (0.5 meq/liter) and hypocalcemic (3.4 meq/liter) and had positive Chvostek's and Trousseau's signs.

Whether in the normomagnesemic or hypomagnesemic state, intravenous bovine parathyroid extract (PTE) 8 U. S. P. U/kg promptly caused transient increases in the urinary phosphate excretion, renal phosphate clearance and cyclic AMP excretion. The magnitudes of these responses were similar in the two states, and similar to those observed in a hypoparathyroid patient. When the patient was hypomagnesemic and hypocalcemic, intramuscular PTE, 8 U/kg at 8-h intervals for four doses promptly caused hypercalcemia. The findings indicate that the end-organs were responsive to parathyroid hormone.

The concentrations of serum parathyroid hormone (PTH) were normal in the normomagnesemic state ranging from 0.15 ng/ml to 0.40 ng/ml. Serum PTH did not increase in the hypomagnesemic state in spite of hypocalcemia. Indeed, PTH became unmeasurable in four consecutive samples at the end of the period of magnesium restriction.

The concentrations of serum calcitonin remained unmeasurable (< 0.10 ng/ml) throughout the study, implying that excess calcitonin was not the cause of hypocalcemia in magnesium depletion.

The findings in this study support our thesis that magnesium depletion causes impaired synthesis or secretion of parathyroid hormone. This impairment would account for the hypocalcemia observed in the hypomagnesemic state.

INTRODUCTION

In recent years, there has been interest in a condition called primary hypomagnesemia with secondary hypocalcemia (2–5). Convulsions are the presenting problem and severe hypomagnesemia and hypocalcemia are the two prominent abnormal biochemical findings. Onset is in the neonatal period. The defect is frequently persistent. Administration of magnesium promptly abolishes the symptoms, corrects hypocalcemia, and constitutes an effective long-term treatment. The basis of the defective magnesium homeostasis is still in question, but defective intestinal absorption of magnesium is the most likely cause.

The etiology of the hypocalcemia in primary hypomagnesemia is also not known. The present study was carried out in a child with this condition to elucidate the pathophysiology of this biochemical abnormality. Among the causes that might result in hypocalcemia are end-organ refractoriness to parathyroid hormone, de-

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TABLE I
Experimental Design

Phases	Biochemical status	Tests
High magnesium intake	Normomagnesemic and normocalcemic	Renal response to PTH (*a*) phosphate handling (*b*) cyclic-AMP-excretion Serum PTH assay Serum CT assay
Low magnesium intake	Hypomagnesemic and hypocalcemic	Calcemic response to PTH Renal response to PTH (*a*) phosphate handling (*b*) cyclic-AMP excretion Serum PTH assay Serum CT assay

fective parathyroid gland function, and excessive secretion of calcitonin. We have examined these three possibilities in our patient while in the normomagnesemic and hypomagnesemic states by assessing end-organ responsiveness to exogenous parathyroid hormone and by measuring the level of circulating parathyroid hormone and calcitonin. The results are consistent with the thesis derived from our earlier studies of magnesium-depleted hypocalcemic puppies (6), that magnesium depletion interferes with parathyroid gland function.

The patient. An 8 yr old boy with primary hypomagnesemia (HSC 554741) was studied. The features of his disease in early infancy and its clinical course until he was 3 yr old have been reported previously (2). On large oral supplements of magnesium, the patient is normomagnesemic and normocalcemic but he develops hypomagnesemia and hypocalcemia promptly if oral supplements of magnesium are withheld.

Up until the time of the study, he consumed regular food providing approximately 20 meq magnesium per day and received a daily oral supplement[1] of 72 meq magnesium. His plasma magnesium, calcium and inorganic phosphate levels were 1.5 meq/liter, 5.0 meq/liter, and 4.1 mg/100 ml, respectively. Serum parathyroid hormone ranged from 0.15 to 0.40 ng/ml (normal range for our immunoassay $<$ 0.15–0.60 ng/ml) and serum calcitonin was unmeasurable ($<$ 0.10 ng/ml) (normal range $<$ 0.10–0.38 ng/ml). The plasma sodium, potassium, and chloride, and the blood pH were normal.

METHODS

Experimental design. The experimental design is summarized in Table I.[2] Throughout the study, the patient consumed a constant diet consisting of natural foods of low magnesium content providing 5 meq of magnesium per day.[3] The study was carried out in two phases: while the patient received a high magnesium intake (90 meq/day) achieved by supplementing with 85 meq of magnesium daily (49 days), and while he received a low magnesium intake (5 meq/day) achieved by withholding the magnesium supplements (16 days). Magnesium supplementation was then reinstituted.

Serum parathyroid hormone (PTH)[4] and calcitonin concentrations were determined frequently throughout the study. The renal response to PTH was assessed in both phases of magnesium intake, and the calcemic response to PTH was assessed at the end of the period of low magnesium intake when the patient was severely hypomagnesemic and hypocalcemic.

Assessment of renal response to parathyroid hormone. Renal response to parathyroid hormone was assessed by determining renal phosphate handling and urinary excretion of adenosine 3′,5′-monophosphate (cyclic AMP) before and after intravenous injection of bovine parathyroid extract (PTE). The assessment was carried out in both phases of magnesium intake (Table I). Food was withheld during each test, commencing the previous evening. 2 h before starting urine collection, the patient was required to drink water, 20 ml/kg body wt, to induce diuresis. Subsequently, water intake was matched to urine output until the end of the test. Blood samples were obtained hourly throughout the test, and analyzed for inorganic phosphate and creatinine concentrations. Urine was collected hourly for 4 h, then PTE was injected and thereafter urine was collected half-hourly for 1 h and hourly for the remaining 3 h of the

[1] Magnesium chloride ($MgCl_2 \cdot 6H_2O$) 40.0 g; magnesium citrate, dibasic ($MgHC_6H_5O_7 \cdot 5H_2O$) 60.0 g; water to 1,000 ml. This solution provides approximately 0.8 meq magnesium per ml.

[2] The protocol for this investigation was accepted by the Committee on Human Experimentation of the University of Toronto and the implications were discussed in detail with the parents.

[3] This diet also provided relatively small amounts of calcium (13 meq), sodium (40 meq), potassium (24 meq), and phosphorus (420 mg) daily. However, the diet did not alter the patient's plasma concentrations of these minerals from the normal levels observed while he consumed regular foods.

[4] *Abbreviations used in this paper:* C_{cr}, creatinine clearance; C_p, renal phosphate clearance; CT, calcitonin; PTE, bovine parathyroid extract; PTH, parathyroid hormone; %TRP, percentage of filtered phosphate reabsorbed by renal tubules; $U_{c\text{-}AMP}$, urinary cyclic AMP excretion rate; U_p, urinary phosphate excretion rate.

test. Urine specimens were frozen at −20°C immediately after collection and subsequently analyzed for phosphate, cyclic AMP, and creatinine. The dose of PTE[5] was 8 U.S.P. U/kg body weight (total 160 U) diluted in 50 ml normal saline and injected intravenously over a 10 min period.

Urinary phosphate excretion rate (U_P), renal phosphate clearance (C_P), the percentage of filtered phosphate reabsorbed by the renal tubules (%TRP), creatinine clearance (C_{cr}), and urinary cyclic AMP excretion rate ($U_{c\text{-}AMP}$) were determined for each of the nine collection periods.

Assessment of calcemic response to parathyroid hormone. The calcemic response to PTE was assessed only while the patient was in the hypomagnesemic, hypocalcemic phase. Immediately following assessment of renal response to parathyroid hormone, that is 4 h after the intravenous PTE injection, PTE was administered intramuscularly (8 U/kg, 160 U/dose) every 8 h until the patient became hypercalcemic. Concentrations of plasma total and ionized calcium, and inorganic phosphate were measured 4 h after each dose of PTE.

Radioimmunoassay of parathyroid hormone. Serum PTH concentrations were measured by immunoassay (*7*), using an antiserum that gives greater sensitivity than the one described in the original report. The assay detects 0.15 ng of bovine PTH equivalent per milliliter of human serum. In each case, the recorded PTH concentration represents the mean of two separate determinations. Concentrations in plasma or serum from 200 normal, fasting humans ranged from unmeasurable (< 0.15 ng/ml) in 35% of subjects to 0.60 ng/ml (8). The range of serum PTH in children (ages 1–17 yr) did not differ significantly from that measured in adult subjects. No differences were detected between plasma and serum samples.

The following physiological observations confirm the validity of this immunoassay for human PTH:

(*a*) Serum PTH levels were at or above the upper limit of normal (0.60 ng/ml) in most individuals in the following groups of patients with hypocalcemia (Table II): (*1*) pseudohypoparathyroidism (four of six cases); (*2*) vitamin D-deficient rickets (two of two cases); (*3*) vitamin D-dependent rickets (one of two cases); (*4*) "dilantin rickets" (three of four cases); and (*5*) chronic renal insufficiency (12 of 15 cases).

(*b*) In one child with chronic renal insufficiency, infusion of EDTA for 2 h at a rate sufficient to lower the noncomplexed serum calcium concentration from 5.8 to 5.2 meq/liter caused an increase in serum PTH from 1.0 to 1.5 ng/ml. Serum PTH then decreased progressively to 0.60 ng/ml during a 4 h calcium infusion that increased the serum calcium to 6.1 meq/liter.

(*c*) The serum PTH concentration was greater than 0.60 ng/ml in 43 of 61 patients with surgically confirmed parathyroid adenoma or hyperplasia. The remaining patients had values between 0.30 and 0.60 ng/ml.

(*d*) Serum PTH increased from an unmeasurable concentration to 0.53 ng/ml when PTE was administered intramuscularly to our patient with primary hypomagnesemia in a dose sufficient to overcome hypocalcemia (Fig. 1, May 30).

(*e*) Serum PTH concentrations in our patient with primary hypomagnesemia decreased from 0.32 ng/ml to unmeasurable levels (two consecutive samples) when the plasma calcium was increased from normocalcemic (4.7 meq/liter) to hypercalcemic levels (5.8 meq/liter) during a 4 h infusion of calcium at a rate of 3.75 mg/kg per h (Fig. 1, April 7).

(*f*) In 22 patients with chronic renal failure and 8 nonazotemic hypocalcemic patients, infusion of calcium (3.75 mg/kg per h) for 4 h produced hypercalcemia, and the serum PTH concentrations decreased significantly to 2/3 of the preinfusion values (Table III).

(*g*) PTH was not measurable in the serum of eight hypercalcemic patients with nonparathyroid disease and cancer metastatic to the skeleton.

(*h*) PTH was not measurable in the serum of 12 patients with hypoparathyroidism.

Radioimmunoassay of calcitonin. Serum calcitonin (CT) was measured by an immunoassay homologous for the human peptide (9). Concentrations in plasma or serum from more than 200 normal fasting subjects ranged from unmeasurable (< 0.10 ng/ml) in most subjects to 0.38 ng/ml (8, 10). No differences were observed between samples from children (ages 4–16 yr) and adults.

TABLE II

Serum PTH Values in Hypocalcemic Patients

Diagnosis	No. of cases	Serum Ca	Serum PTH
		meq/liter	*ng/ml*
Pseudohypoparathyroidism	6	—	1.30
		—	1.20
		—	0.75
		—	0.65
		—	0.38
		—	0.35
Vitamin D-deficient rickets	2	4.0	1.10
		3.9	0.60
Vitamin D-dependent rickets	2	4.0	0.60
		3.7	0.25
"Dilantin rickets"	4	3.7	0.88
		4.4	0.75
		2.7	0.74
		3.4	0.35
Chronic renal insufficiency	15	4.0	1.90
		4.2	1.50
		2.7	1.40
		4.2	1.40
		2.8	1.30
		4.4	1.30
		3.9	1.30
		3.2	1.20
		3.9	1.20
		3.2	1.20
		2.6	0.90
		3.8	0.80
		4.4	0.50
		4.3	0.40
		3.9	0.40

[5] Para-Thor-Mone. Batch no. AL3KX75A, Eli Lilly & Co., Indianapolis, Ind.

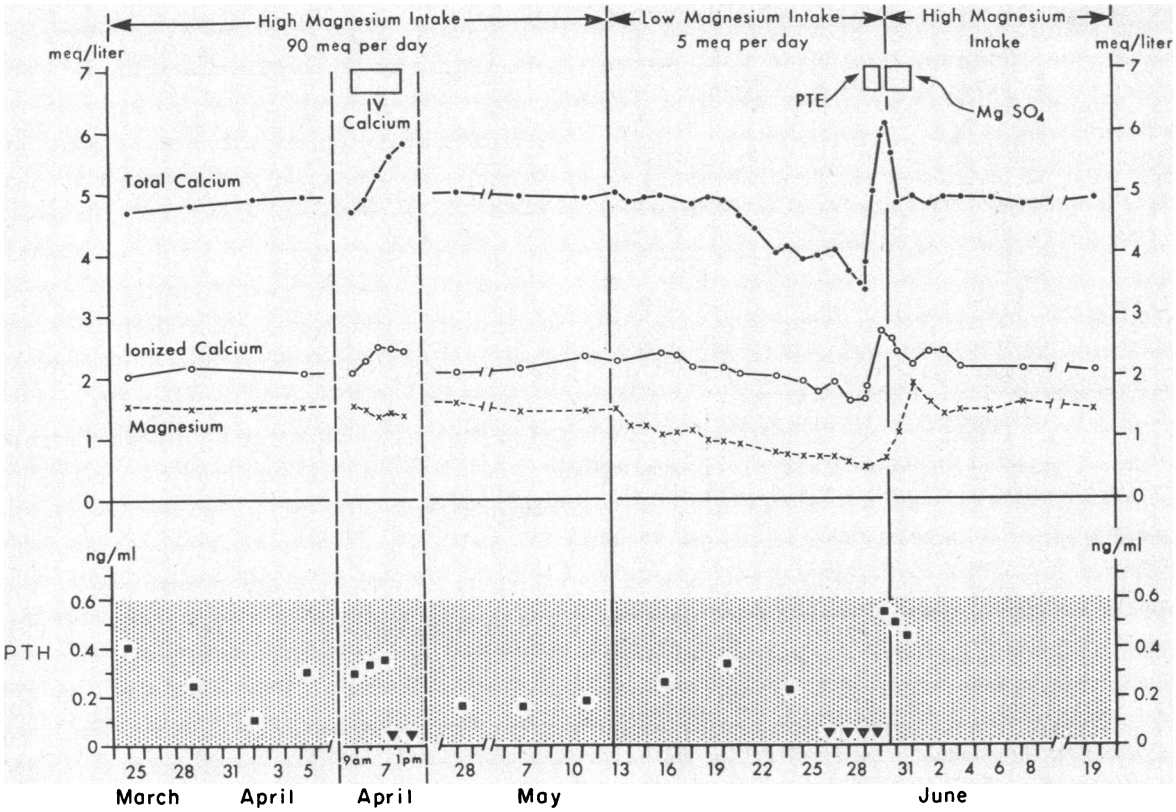

FIGURE 1 Concentrations of total and ionized Ca and Mg in plasma and PTH in serum while the patient was taking 90 meq and 5 meq of Mg per day. The limit of sensitivity of the PTH immunoassay is 0.15 ng/ml serum. Values below this level are unmeasurable and are denoted by ▼. The shaded area designates the normal range of serum PTH. IV Calcium denotes calcium gluconate infusion, 3.75 mg Ca/kg per h for 4 h; PTE denotes five doses of PTE, 160 U.S.P. U per dose (i.v. then i.m. × 4); $MgSO_4$ denotes five doses of 50% $MgSO_4$ i.m. (total 35 meq of Mg).

Chemical determinations. Concentrations of magnesium, calcium, ionized calcium, inorganic phosphate, and creatinine in plasma and of phosphate and creatinine in urine were determined by the methods described previously (2, 6). Other electrolytes were measured by standard methods.

The concentration of cyclic AMP in the urine was determined by the method of Brown et al. (11).

RESULTS

Serum PTH and CT concentrations and changes in plasma magnesium, total and ionized calcium, and inorganic phosphate. Fig. 1 shows the concentrations of plasma magnesium, total and ionized calcium, and serum

TABLE III

Effect of Calcium Infusion (3.75 mg/kg per h for 4 h) on Serum Calcium and PTH Values (Mean±SD)

	Serum Ca					Serum PTH				
	Before	After	Δ	*t*	*P*	Before	After	Δ	*t*	*P*
	meq/liter	*meq/liter*	*meq/liter*			*ng/ml*	*ng/ml*	*ng/ml*		
Patients with chronic renal failure (n = 22)	4.02 ±0.77	5.53 ±0.90	1.51 ±0.97	7.36	<0.01	1.10 ±0.39	0.80 ±0.29	−0.30 ±0.25	5.57	<0.01
Nonazotemic hypocalcemic patients (n = 8)	3.74 ±0.65	4.68 ±0.74	0.95 ±0.57	4.73	<0.01	0.59 ±0.21	0.37 ±0.14	−0.22 ±0.14	4.39	<0.01

PTH throughout the study. When the magnesium intake was high, the plasma magnesium concentration averaged 1.5 meq/liter, the plasma calcium 5.0 meq/liter, the ionized calcium 2.1 meq/liter, and the inorganic phosphate 4.0 mg/100 ml (not shown), these values being within the normal ranges. The concentrations of serum PTH were normal, ranging from 0.15 to 0.40 ng/ml. The serum CT in all samples was too low to measure (< 0.10 ng/ml). No increase in CT was noted during calcium infusion, a finding similar to that observed in many normal persons with the immunoassay employed (9).

When the patient was fed the low magnesium intake, the plasma concentrations of magnesium decreased steadily and reached 0.5 meq/liter by the 16th day of magnesium restriction (Fig. 1, May 28). The concentrations of total and ionized calcium remained normal during the 1st wk of the low magnesium intake, then declined steadily, reaching 3.4 and 1.6 meq/liter, respectively, on the 16th day. Plasma inorganic phosphate increased from 4.0 to 5.7 mg/100 ml (not shown). Because the Chvostek and Trousseau tests became strongly positive, magnesium restriction was not continued beyond the 16th day. The concentrations of serum PTH, determined several times while the patient was hypomagnesemic and hypocalcemic, remained low, and in the last four samples at the end of the period of magnesium restriction, PTH was unmeasurable.

Calcemic response to PTE. Just before ending the period of magnesium restriction, when the patient was severely hypomagnesemic and hypocalcemic, administration of five doses of PTE during a 28 h period increased the plasma calcium from 3.4 to 6.2 meq/liter and the ionized calcium from 1.6 to 2.7 meq/liter. Inorganic phosphate fell progressively from 5.7 to 4.0 mg/100 ml. The concentration of magnesium increased only slightly, from 0.50 to 0.64 meq/liter.

Renal responses to PTE. Fig. 2 shows the renal responses to PTE in the normomagnesemic and hypomagnesemic phases. While the patient was normomagne-

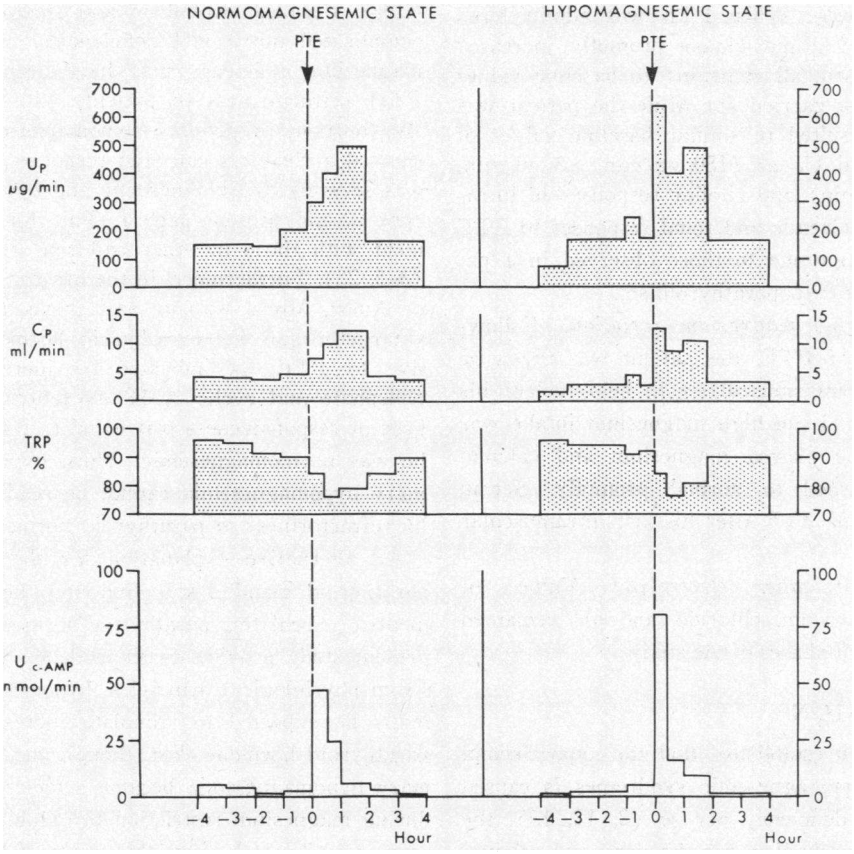

FIGURE 2 Renal responses to a single i.v. dose of PTE, 8 U.S.P. U/kg (total 160 U). During high magnesium intake, plasma Mg was 1.5 meq/liter, Ca 5.0 meq/liter, and Pi 3.6 mg/100 ml. During low magnesium intake, plasma Mg was 0.5 meq/liter, Ca 3.4 meq/liter, and Pi 5.7 mg/100 ml.

TABLE IV

End-Organ Responsiveness to PTH in Magnesium-Depleted Animals and Humans

Species	References	Calcemic response	Phosphaturic response	Cyclic-AMP-uric response
Rat				
In vivo	(14, 15)	+		
	(16)	+	+	+
In vivo and in vitro	(17)	±		
Dog, in vivo	(6)	+		
Monkey, in vivo	(18)	+		
Human				
Chronic alcoholism	(19, 20)	0	0	0
Steatorrhea	(19, 20)	0	0	0
	(21)	0	±	
	(22)	0	+	
Primary hypomagnesemia	(2–5)	+		
	(1, 23)	+	+	+

+ = response; 0 = no response; ± = diminished response.

semic, the baseline values for U_P, C_P, %TRP, and $U_{c\text{-}AMP}$ averaged 160 μg/min, 4.1 ml/min, 93%, and 3 nmol/min, respectively. When PTE was administered intravenously, U_P, C_P, and $U_{c\text{-}AMP}$ promptly increased and %TRP decreased, all returning to baseline values within 4 h. The test carried out while the patient was hypomagnesemic resulted in similar baseline values of U_P, C_P, %TRP, and $U_{c\text{-}AMP}$ (180 μg/min, 3.3 ml/min, 92%, and 3 nmol/min) and similar responses to intravenous PTE. The calcemic and renal responses to PTE were similar in magnitude to those observed in a patient with idiopathic hypoparathyroidism.

Serum PTH during magnesium repletion. Following administration of PTE, the patient was given 35 meq of magnesium intramuscularly to help replace his magnesium deficit and the high magnesium intake was again provided. The plasma magnesium and calcium concentrations returned to normal promptly. Serum PTH was 0.45 ng/ml, 33 h after his last intramuscular dose of PTE.

Concentrations of other electrolytes. Values of plasma sodium, potassium, chloride, and pH remained normal throughout all phases of the study.

DISCUSSION

Although it has been established that the hypocalcemia characteristic of hypomagnesemic syndromes is caused by the magnesium deficiency per se (2, 12, 13), the pathophysiological mechanism for the deranged calcium homeostasis has not been explained. A frequently suggested explanation is refractoriness of the end-organs to parathyroid hormone, implying a disturbance analogous to that proposed in pseudohypoparathyroidism. Table IV summarizes published data on end-organ responsiveness to parathyroid hormone in magnesium depletion. In magnesium-depleted rats, several studies showed normal calcemic (14–16) and renal (16) responses to PTE. In the study of MacManus, Heaton, and Lucas (17), bone of magnesium-depleted rats was reported to show diminished responsiveness to PTE in vivo and in vitro, but the significance of the findings is open to question because the increase of plasma calcium in response to PTE in the control rats was unusually small and because the use of nonfetal bone shafts in the in vitro experiment does not provide optimal conditions for action of parathyroid hormone. In puppies (6) and monkeys (18), quantitative comparisons indicated that the calcemic responses of magnesium-depleted animals were equal to those of magnesium-supplemented controls. In the human, hypomagnesemic adults with chronic alcoholism or steatorrhea were refractory to the calcemic action of PTE (19–22); response to the phosphoraturic action of PTE was variable (no response [19, 20], partial response [21] or full response [22]). Because alcoholism and steatorrhea may cause complicated nutritional deficiencies, factors other than magnesium deficiency may have been involved in the PTH refractoriness of patients with these conditions. By contrast, in primary hypomagnesemia, our patient showed a clear-cut calcemic response to PTE when he was severely hypomagnesemic and hypocalcemic (reference 2, and present paper). This finding is in agreement with those in other children with this condition (3–5, 23). Furthermore, in the present study and in that of Anast, Mohs, Kaplan, and Burns (23), the kidney, a target organ for parathyroid hormone, was responsive to PTE, as indicated by increases in urinary phosphate and cyclic AMP excretion; indeed, the degree of responsiveness was equal to that observed when he was normomagnesemic. Thus, hypocalcemia in primary hypomagnesemia cannot be readily accounted for by refractoriness to parathyroid hormone.

An alternative explanation for the hypocalcemia is parathyroid gland dysfunction and the serial measurements of circulating parathyroid hormone obtained in the present study provide direct evidence about this aspect. From physiological principles, hypocalcemia would normally be expected to stimulate increased secretion of parathyroid hormone. Yet, though our patient with primary hypomagnesemia became sufficiently hypocalcemic during magnesium restriction to exhibit positive Trousseau and Chvostek signs, his serum PTH did not rise; on the contrary, parathyroid hormone was no longer measurable by the end of the period of magnesium restriction. Our immunoassay was of sufficient sensitivity

to demonstrate increased circulating PTH in the majority of individuals with hypocalcemia of various causes. We interpret the observations in our hypomagnesemic patient to indicate that magnesium depletion resulted in impairment of synthesis or secretion of parathyroid hormone, causing, in effect, a form of hypoparathyroidism. This hypothesis fits our other observations in the patient during the phase of magnesium depletion—hypocalcemia, increasing plasma phosphate concentrations, unimpaired end-organ responsiveness to parathyroid hormone, no increase in the baseline rate of urinary cyclic AMP excretion (the last has been shown to increase in hyperparathyroid states [24–26]). Observations in magnesium-depleted puppies had previously led us to propose impaired PTH synthesis or secretion as the likely cause for hypocalcemia in that species (6). Recently, Anast et al. (23) have arrived independently at the same conclusion in an adolescent girl with chronic hypomagnesemia on the basis of results that are very similar to ours (27).

Theoretically, excess calcitonin secretion should be considered as a possible cause of hypocalcemia, and Rojo-Ortega, Bracht, and Genest (28) have advanced this thesis to explain their findings in magnesium-depleted dogs. We have previously presented our evidence in puppies against this theory (6). The finding in our patient that circulating calcitonin was consistently less than 0.10 ng/ml during the period of hypocalcemia provides direct evidence that the C cells do not play a role in the hypocalcemia of magnesium depletion.

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