Reports

Evidence for Parathyroid Failure in Magnesium Deficiency

Abstract. *Serum immunoreactive parathyroid hormone (IPTH) was low to nondetectable in spite of hypocalcemia in a patient with chronic magnesium deficiency. The administration of magnesium led to parallel increases in serum IPTH, serum calcium, and renal phosphate clearance. These findings support the view that magnesium depletion may result in impaired synthesis or release of parathyroid hormone in man, or both.*

Hypocalcemia is a frequent complication of magnesium deficiency in man. Characteristically, this form of hypocalcemia is resistant to calcium therapy but responds rapidly to the administration of magnesium salts. The underlying mechanism responsible for the hypocalcemia has not been elucidated although it has been the subject of a number of recent investigations (*1–4*).

The present study was undertaken to assess parathyroid function in a 21-year-old woman with chronic hypomagnesemia and hypocalcemia. At the age of 16 years the patient had a seizure disorder associated with hypocalcemia. A diagnosis of idiopathic hypoparathyroidism was made and treatment with vitamin D and calcium gluconate commenced. After 3 years of therapy, during which the serum calcium was usually found to be low normal, serum magnesium concentration was determined for the first time and found to be markedly depressed. Subsequent studies demonstrated that the hypomagnesemia was secondary to an isolated defect in the intestinal transport of magnesium (*5*), an entity previously described by other investigators (*6–8*). The intestinal absorption of fat, sugars, and calcium appeared to be unimpaired. The concentration of magnesium in red blood cells was 4.6 mg per 100 ml of packed red blood cells and in rectus muscle it was 72 mg per 100 g, dry, defatted weight. Both of these levels are low compared to published normal values (*9*) and indicate the presence of intracellular magnesium deficiency.

Observations made prior to the present study while the patient received no medication revealed that the serum magnesium level fluctuated between 0.35 and 1.05 mg per 100 ml (normal range, 1.6 to 2.6 mg per 100 ml), while the total serum calcium level was usually less than 8.5 mg per 100 ml. Serum sodium, potassium, chloride, bicarbonate, inorganic phosphate, alkaline phosphatase, and plasma protein concentrations were consistently normal. Although a strongly positive Chvostek's sign could frequently be elicited, neither tetany nor convulsions were observed.

The patient's parathyroid function was evaluated before and after the intramuscular administration of magnesium sulfate by making sequential measurements of serum immunoreactive parathyroid hormone (IPTH), total calcium, ionized calcium, magnesium, and renal phosphate clearances. Serum levels of IPTH were determined by a double-antibody radioimmunoassay technique developed in our laboratory (*10*). Human hyperparathyroid serum is used as a reference standard in the assay and the concentration of IPTH in unknown serum samples is expressed as microliter equivalents of the standard hyperparathyroid serum per milliliter (μlEq/ml). This assay is sensitive and reproducible and has proved to be an excellent tool for the evaluation of patients with parathyroid disorders. Total serum calcium and magnesium were determined by atomic absorption spectrophotometry (Perkin-Elmer 303), ionized calcium with the Orion flow-through electrode (model 88-20), and inorganic phosphorus by the method of Fiske and Subbarow (*11*) adapted to the Technicon Autoanalyzer.

The results are shown in Fig. 1. Baseline levels of serum magnesium, total calcium, and ionized calcium were all below the normal ranges. In the presence of persistent hypocalcemia and markedly depressed serum magnesium levels the serum IPTH levels were undetectable to low. The cautious administration of an oral phosphorus load (1 g of phosphorus as buffered phosphate salt every 8 hours) for 3 days led to a further decrease in serum total and ionized calcium but, even in the face of this, the serum IPTH level remained low. One week later intramuscular magnesium was administered in a dose of 100 mg of magnesium as magnesium sulfate every 8 hours. After 24 hours of magnesium therapy (indicated in Fig. 1 by the asterisks) the serum magnesium increased to within the low normal range, the serum total calcium and ionized calcium rose but were still below normal, the renal phosphate clearance increased, and there was a striking increase in serum IPTH to above the upper limit of the normal range. With the continuation of magnesium therapy the serum total calcium and ionized calcium levels progressively increased and then plateaued within the normal ranges; this increase in serum calcium was accompanied by a decrease in serum IPTH from above normal to the high and mid-normal range. When intramuscular magnesium was discontinued after 10 days of therapy, the serum magnesium fell precipitously to below normal over 48 hours while the serum total calcium and ionized calcium levels remained relatively stable for the next 2 weeks. During this time the serum IPTH remained within the lower limits of detectability. Eight weeks after magnesium therapy was discontinued the serum IPTH was nondetectable in the presence of hypocalcemia and profound hypomagnesemia. Several weeks later the study was repeated and very similar results were obtained.

The parallel changes in serum IPTH and calcium and in renal phosphate clearance following magnesium administration indicate that both immunochemical and biological activity of parathyroid hormone was increased. It has been well established that parathyroid hormone secretion is regulated primarily through a negative feedback control system whereby a decrease in extracellular calcium stimulates parathyroid secretion. Therefore, under circumstances of normal parathyroid gland responsiveness, a low serum calcium level should be associated with a high serum IPTH. In our magnesium-depleted patient, however, the serum IPTH was persistently undetectable to low in the presence of depressed serum total and ionized calcium levels. The intramuscu-

lar administration of magnesium sulfate resulted in a prompt increase in serum IPTH, the level of which appeared to be inversely related to the concentration of calcium in the peripheral circulation. The results of this study indicate that (i) the synthesis and/or secretion of parathyroid hormone may be impaired in the magnesium-deficient state in man, and (ii) magnesium administration rapidly restores the ability of the parathyroid gland to respond appropriately to the level of ionized calcium in blood. Another possibility is that magnesium deficiency causes increased destruction of parathyroid hormone; however, little is known concerning the normal metabolic fate of this hormone.

Calcium and magnesium behave similarly in biological systems. In some studies it has been shown that the administration of parathyroid extract produces an increase in the serum levels of both calcium and magnesium (*12*). The possibility, therefore, that the concentration of extracellular magnesium, like that of calcium, might influence parathyroid hormone secretion through a negative feedback control system has been investigated by other workers. Buckle *et al.* (*13*) demonstrated that when isolated thyroid and parathyroid glands of experimental animals were perfused in situ, the concentration of IPTH in the effluent plasma varied inversely with the magnesium concentration of the perfused blood. Pletka *et al.* (*14*) reported on the effects of magnesium on parathyroid hormone secretion in hypermagnesemic patients with chronic renal disease undergoing chronic hemodialysis. The serum IPTH decreased somewhat in patients on high magnesium dialysis while the IPTH increased as serum magnesium decreased from 4.0 to 2.3 mg per 100 ml in patients on low magnesium dialysis. Unfortunately, neither total nor ionized serum calcium levels were reported in this study, so the possible contribution of changes in serum calcium on IPTH secretion cannot be evaluated. The first of these studies (*13*) differs from ours in that the experimental animals were not magnesium deficient as was the patient who is the subject of this report. In the second study (*14*), the presence of magnesium depletion was not established. Therefore, these results may not be contradictory. It is conceivable that a magnesium-parathyroid negative feedback control system is normally operational and is responsive to acute changes in extracellular magnesium concentration. On the other hand, the development of a relatively severe degree of magnesium deficiency, with the level of intracellular magnesium possibly being a critical factor, may result in defective synthesis or diminished secretion of parathyroid hormone, or both.

The in vitro studies of Targovnik *et al.* (*15*) are consistent with this possibility. They observed that the release of parathyroid hormone from bovine parathyroid glands increased as the magnesium concentration in the media was decreased down to a level of 0.72 mg per 100 ml. Below this concentration, the secretion of parathyroid hormone was markedly diminished. In contrast to the studies of Targovnik *et al.*, Hamilton *et al.* (*16*) did not observe any change in the biosynthesis of parathyroid hormone in bovine parathyroid glands incubated in media containing concentrations of magnesium varying from 0.48 to 4.1 mg per 100 ml. It is apparent that further work needs to be done to establish a possible inverse relationship between the extracellular magnesium concentration and the secretion and synthesis of parathyroid hormone.

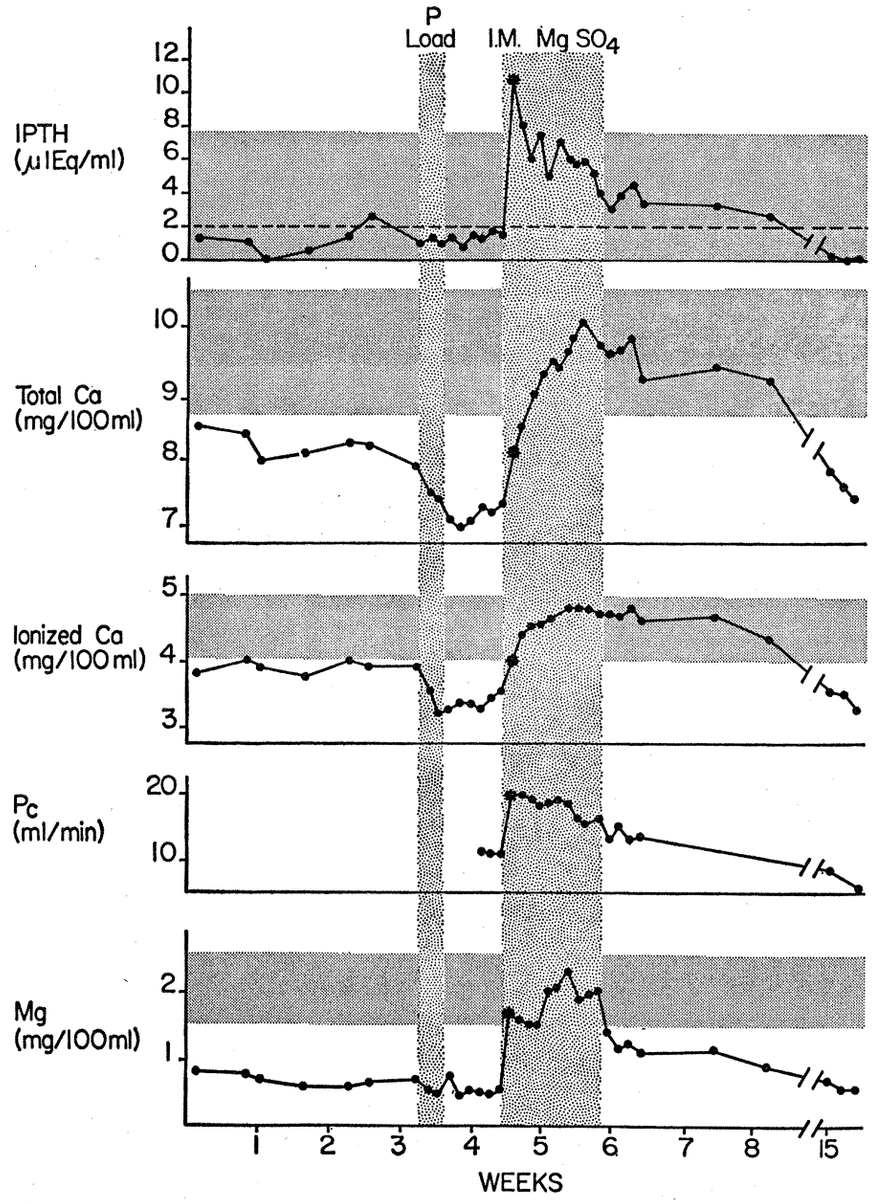

Fig. 1. Serum IPTH, total calcium, ionized calcium, magnesium, and renal phosphate clearance (*Pc*) before and after an oral phosphorus load and intramuscular magnesium sulfate (*I.M. $MgSO_4$*). The horizontal shaded areas indicate normal ranges. Normal ranges for serum total calcium, ionized calcium, and magnesium are defined as ± 2 S.D. of mean normal values established in our laboratory. Normal range of serum IPTH extends from nondetectable to + 2 S.D. of mean. Broken line indicates limits of detectability of the parathyroid hormone immunoassay.

It has been suggested that the hypocalcemia that occurs in magnesium deficiency is the result of diminished end-organ responsiveness to parathyroid hormone (*1*). However, normal end-organ responsiveness to parathyroid hormone has been observed in patients with primary hypomagnesemia (*8, 17*) as well as in magnesium-depleted dogs (*4*). If diminished end-organ responsiveness to parathyroid hormone were the cause of hypocalcemia in magnesium deficiency, elevated rather than low levels of serum IPTH would be an expected finding. In our patient, the levels of serum IPTH were consistently low in the presence of hypomagnesemia and hypocalcemia. Following the administration of intramuscular magnesium the serum IPTH increased and the parathyroid glands appeared to respond appropriately to the level of ionized calcium in blood. The results of this study, therefore, provide evidence for the occurrence of parathyroid failure in the magnesium-deficient state in man. The possibility of magnesium depletion should be considered in patients with unexplained hypocalcemia.

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Intestinal Uptake of Macromolecules: Effect of Oral Immunization

Abstract. *Animals were orally immunized with horseradish peroxidase and bovine serum albumin, and absorption of these antigens was studied. In comparison with controls, a consistent and significant decrease in peroxidase uptake was noted in both germ-free and conventional rats immunized with peroxidase; a similar decrease in serum albumin uptake was also noted in animals immunized with serum albumin. There was no difference in the uptake of an unrelated macromolecule. These observations suggest that local immunization interferes specifically with the intestinal uptake of macromolecular antigens.*

Although the intestinal absorption of intact macromolecules (*1, 2*) and the ability of such molecules to induce both a local and systemic immune response has been demonstrated under certain natural and experimental conditions (*2–4*), the effect of immunization on intestinal uptake of macromolecules has not been fully investigated (*5*). In previous studies from this laboratory, the absorption of horseradish peroxidase (HRP) by the small intestine of the rat was investigated using everted gut sacs in vitro (*6*), as well as using jejunostomy infusion (*7*) and instillation into ligated ileal loops in vivo (*8*). These studies indicated that an exogenous macromolecule was taken up by pinocytosis into the membranous subcellular system of the rat small intestinal absorptive cells and that the macromolecules were subsequently transported into the extracellular space of the lamina propria and from there into the lymph (*7, 8*). The present study was designed to investigate the effect of active oral immunization on the absorption of HRP and bovine serum albumin (BSA) by rat small intestinal segments in vitro.

Ten 40-day-old female germ-free rats (CD^R strain, Charles River Breeding Laboratories) were orally immunized according to the method of Crabbé *et al.* (*4*) by exposure to HRP (1 mg/ml) in the drinking water for a 10-day period; the rats were studied 2 weeks after the start of immunization. Two sets of 12 adult white female conventional Sprague-Dawley rats weighing approximately 175 g were fed either HRP (1 mg/ml) or BSA (1 mg/ml) in drinking water for 2 weeks and studied 1 week later. At the time of study, animals fasted 24 hours were subjected to a laparotomy under ether anesthesia, and the small intestine was removed. Five-centimeter everted gut sacs (*9*) were prepared and incubated for 60 minutes at 37°C in oscillating flasks containing oxygenated Krebs-Ringer bicarbonate solution and 10 μM HRP (Sigma, 250 purpurogallin units per milligram of protein). After incubation, the serosal contents were drained from the sacs and the concentration of HRP was determined enzymatically. A 0.1-ml sample of test solution was mixed with 2.9 ml of a reaction mixture containing 0.003 percent H_2O_2 in phosphate buffer (0.1M, *p*H 6.0) and 0.025 ml of an aqueous solution of *o*-dianisidine, dihydrochloride (10 mg/ml). With a Gilford recording spectrophotometer, the rate of increase in optical density at 460 nm was determined. At dilutions of HRP standard solutions below 10 μM, the relation between enzyme activity and enzyme protein concentration was not linear. However, when serial dilutions of standard solutions were assayed in phosphate buffer containing 1 percent BSA, a linear relation was noted (*10*). The concentration of enzyme protein in sac fluid was determined from a standard curve relating enzyme activity to enzyme protein. The viability of gut sacs during the test period was monitored by measuring active transport of L-[^{14}C]histidine. In order to permit comparisons of uptake by sections of intestine with different surface areas, uptake was expressed as picomoles of HRP per milligram of mucosal protein per hour.