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Pathogenesis of secondary hyperparathyroidism

EDUARDO SLATOPOLSKY, ALEX BROWN, and ADRIANA DUSSO

Renal Division, Washington University School of Medicine, St. Louis, Missouri

Pathogenesis of secondary hyperparathyroidism. Secondary hyperparathyroidism is a universal complication in patients with chronic renal failure. Hyperplasia of the parathyroid glands is typically seen in these patients. In early renal failure, alteration in vitamin metabolism, decreased levels of calcitriol and moderate decreases in ionized calcium may allow greater synthesis and secretion of PTH. As the disease progresses, there is a decrease in the number of vitamin D receptors (VDR) and calcium receptors (CaR). The decreased number of VDR and CaR makes the parathyroid glands more resistant to calcitriol and calcium. Phosphorus induces hyperplasia of the parathyroid glands independent of calcium and calcitriol, and by a post-transcriptional mechanism increases PTH synthesis and secretion. Experimental work in uremic rats demonstrated that if the animals are fed a high-phosphorus diet, they not only developed secondary hyperparathyroidism but parathyroid cell hyperplasia. If the diet is then reduced in phosphorus, the levels of PTH return to normal. However, the parathyroid cell hyperplasia persists and no apoptosis is seen. Thus, the control of the three most important factors, calcium, calcitriol and phosphorus, is critical to prevent the development of secondary hyperparathyroidism and hyperplasia of the parathyroid glands.

Hyperplasia of the parathyroid glands and high levels of serum parathyroid hormone (PTH) are among the most consistent pathogenetic factors affecting divalent ion metabolism in patients with chronic renal failure. Increased serum levels of PTH occur even in patients with mild to moderate renal impairment, recognized as early as the 1960s [1, 2]. Serum ionized calcium, 1,25-dihydroxyvitamin D_3 (1,25D), and phosphorus are the three main regulators of PTH in man. (Fig. 1)

ALTERATIONS IN VITAMIN D METABOLISM ON PTH SECRETION AND PARATHYROID GLAND GROWTH IN RENAL FAILURE

The control of PTH gene transcription by 1,25D is mediated by the vitamin D receptor (VDR), a protein with high affinity and specificity for the vitamin D hormone.

Korkor [3] demonstrated that parathyroid glands (PTG) from patients with chronic renal failure contained one-third the number of VDR compared to parathyroid adenomas. Merke et al [4] found that in rats, six days after subtotal nephrectomy, parathyroid glands contained only half the number of receptors compared to PTG of sham-operated controls. Similar results were found by Brown et al in dogs [5]. Although it has not been previously proven that VDR plays a role in suppressing PTH synthesis and determining the set-point for calcium, it is possible that the reduced VDR number in the parathyroid glands of uremic patients renders the glands less responsive to the inhibitory action of 1,25D. $1,25(OH)_2D_3$ plays an important role in the content of the VDR in several tissues. Binding of 1,25D stabilizes the VDR and the half-life of the receptor is increased [6]. In the PT glands and kidney, 1,25D also up regulates VDR mRNA. This data is consistent with the view that 1,25D regulates its own receptor in parathyroid cells. $1,25(OH)_2D_3$ may be an important regulator of parathyroid cell growth and low levels of 1,25D may allow parathyroid cells to proliferate. Studies by Szabo et al [7] in experimental animals with renal failure suggest that 1,25D administration suppresses parathyroid hyperplasia, perhaps through changes in serum calcium. However, once established, PT hyperplasia was not reversed by short-term 1,25D treatment. Fukuda et al [8] have provided evidence for decreased 1,25D receptor density in patients with parathyroid hyperplasia. These investigators studied the VDR distribution of surgically excised PTG obtained from dialysis patients. They classified the parathyroid glands as exhibiting nodular or diffuse hyperplasia. They found a lower density of the VDR in PTG showing nodular hyperplasia than in those showing diffuse hyperplasia. A significant correlation was found between VDR density and the weight of the parathyroid gland. In other words, the higher the levels of PTH in serum, the greater the degree of parathyroid gland hyperplasia and the lower the density of the VDR. This study provides a rational basis for understanding the difficulties in suppressing secondary hyperparathyroidism (SH) when the PTH levels are extremely high (>1500). In addition, it is important to emphasize that in the PTG there is a component of the secretory mechanism that can not be suppressed by either high calcium or pharmacological

Key words: chronic renal failure, calcium, calcitriol, phosphorus, parathyroid hormone.

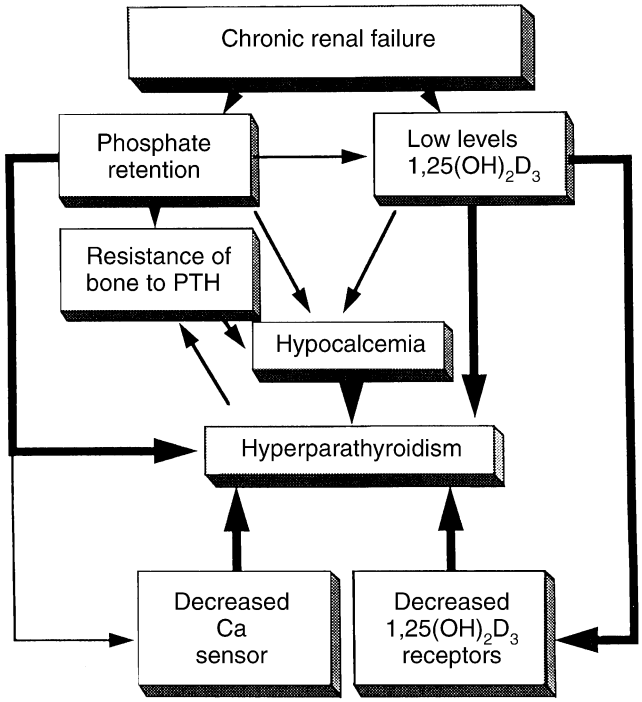

Fig. 1. Schematic representation of the factors involved in the pathogenesis of secondary hyperparathyroidism.

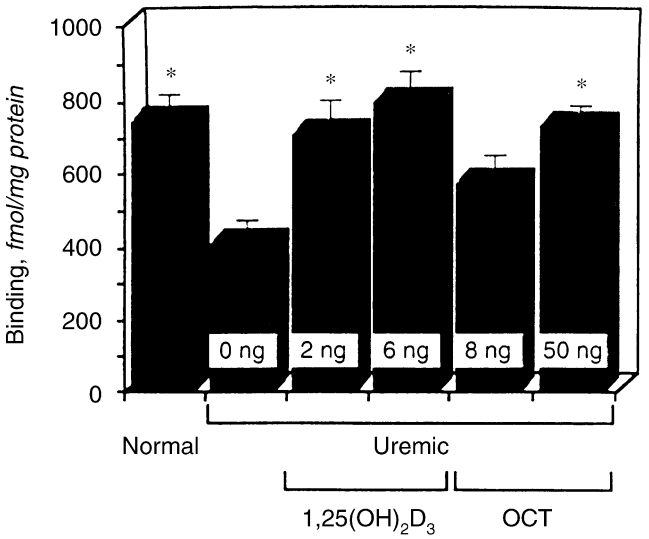

Fig. 2. Effects of $1,25(OH)_2D_3$ and 22-oxacalcitriol (OCT) on vitamin D receptor content in parathyroid glands. Normal control rats ($N = 9$), uremic control rats ($N = 15$), uremic rats receiving 2 or 6 ng of $1,25(OH)_2D_3$ ($N = 15$ for each group), and uremic rats receiving 8 or 50 ng of OCT ($N = 15$ for each group). $^*P < 0.05$ vs. uremic control. (Reproduced with permission from 11).

doses of 1,25D [9,10]. Although this component of secretion represents a small percentage of the total amount of PTH in normal individuals, it becomes extremely important in patients in which the PTG are 50–100 times the normal size. Recently, Denda et al [11] showed a strong correlation between serum 1,25D levels and VDR binding activity in the parathyroid glands of uremic rats suggesting that the decreased VDR content may be related to low levels of 1,25D. Moreover, low doses of 1,25D (2 or 6 ng three times per week) or the administration of 22-oxa-calcitriol, a noncalcemic analog of vitamin D, increased the vitamin D receptor content in the PT glands to normal levels (Fig. 2). Resistance to 1,25D in chronic renal failure also appears to be mediated by impaired VDR transcriptional activity. Patel et al [12] had presented evidence that components in the blood of renal failure patients diminished the ability of the VDR receptor to bind vitamin D responsive elements in the DNA of target genes. Intestinal VDR receptor from uremic rats was found to elute from DNA cellulose at a lower salt concentration, indicating a reduced affinity for DNA. Nuclear uptake studies performed *in vitro* showed that intestinal VDR from uremic rats was taken up to a lesser extent than the vitamin D receptor from normal rat intestine. When normal VDR was incubated with uremic plasma ultra-filtrate nuclear uptake *in vitro* was inhibited. Patel et al [13] determined the effect of uremic plasma ultra-filtrate on the binding of VDR to a known vitamin D responsive element (VDRE) using electrophoretic mobility shift assays (EMSA). Analysis of the data indicates that the ultra-filtrate did not affect the affinity of the binding but rather reduced a fraction of the VDR capable to binding to the VDRE. $1,25(OH)_2D_3$ may also be critical in the full responsiveness of the parathyroid glands to calcium. Regulation of the calcium receptor of the rat parathyroid gland by 1,25D has been reported by some authors [14]. Vitamin D deficiency decreased and 1,25D treatment increased the calcium sensing receptor mRNA.

Clinical observations suggest that therapy with 1,25D to control PTH is difficult in chronic renal failure patients having hyperphosphatemia. The nature of this resistance to 1,25D is unclear and may involve several alterations in the parathyroid gland such as VDR content, calcium receptor expression and/or activation of the parathyroid cell cycle.

THE ROLE OF CALCIUM

Evidence exists for an intrinsic abnormality of the PTG in uremia that leads to a disordered calcium-regulated PTH secretion and insensitivity to the suppressive effect of calcium on PTH secretion in glands obtained from patients with chronic renal failure [15]. These observations suggest that one mechanism for the increased PTH levels in chronic renal failure may be a shift in the set-point for calcium regulated PTH secretion in addition to the increase in the mass of parathyroid tissue. These abnormalities are also manifested by an increase in the

calcium concentration required for the inhibition of the adenylate cyclase activity in membranes prepared from hyperplastic PTG obtained from patients with chronic renal failure [16]. Although Brown et al [15] clearly demonstrated this abnormality of the set-point for calcium *in vitro* in parathyroid tissue obtained from patients with chronic renal failure, when studies were performed *in vivo*, the results proved controversial since several authors were unable to demonstrate an abnormal set-point. This might in part relate to the way PTH is secreted: it is partly rate-dependent ($\Delta Ca/dt$) and partly pulsatile. The initial secretory response of the PTG to hypocalcemia occurs within 1–3 minutes, suggesting that calcium acts directly on the plasma membrane. The mechanism of this effect may be related, at least in part, to an effect of calcium on parathyroid cell membrane potential. An additional effect of calcium secretion may also be a consequence of the regulation of the amount of hormone available for secretion as a result of the calcium dose dependent regulation of hormone degradation within the parathyroid gland. Brown et al [17] cloned a calcium receptor localized in bovine parathyroid cell membranes. Parathyroid cells possess a calcium sensing mechanism that recognizes trivalent and polyvalent cations such as (neomycin) and regulates PTH secretion by changes in phosphoinositide turnover and cytosolic calcium. This receptor features a large extracellular domain containing clusters of acidic amino acid that are possibly involved in calcium binding. The extracellular domain is coupled to a cellular seven membrane-spanning domain similar to the G-protein coupled receptor superfamily. Pollack et al [18] demonstrated that mutations of the human calcium sensing receptor caused familiar hypocalciuria, hypercalcemia, and severe neonatal hyperparathyroidism.

Calcium can also regulate PTH gene transcription. Okazaki et al [19] identified a negative regulatory element located 3.5 kb upstream from the transcriptional start site in the PTH gene that modulates the transcriptional suppression of PTH by extracelluar calcium. Thus, several factors can lead to elevated serum parathyroid hormone levels in man. This includes 1) an increase in tissue mass either from cell hypertrophy and/or hyperplasia; 2) an increase in the set-point for calcium; and 3) a change in the degree of suppression by calcium throughout the calcium sensitive range (e.g., slope of the suppression line). Kifor et al [20], using immunochemistry techniques, demonstrated a 59% decrease in calcium-sensor receptor in parathyroid glands of uremic patients. Similar results were demonstrated in adenomas of parathyroid glands obtained from patients with primary hyperparathyroidism. These investigators concluded that the degree of calcium receptor reduction would be sufficient to account, at least in part, for the altered sensitivity to calcium observed in secondary hyperparathyroidism. Gogusev et al [21] obtained similar results in patients with primary and secondary hyperparathyroidism. *In situ* hybridization techniques and histomorphometry demonstrated a significant decrease in the number of calcium receptors in both abnormal parathyroid tissues. In addition, they observed the lowest calcium receptor expression in nodular portion of the gland, is the most pathological one, as far as decrease in the number of calcium and vitamin D receptors. Thus, it appears that the more rapidly proliferating nodules have the greatest decrease in VDR and calcium receptor and are likely resistant to suppression by calcium and 1,25D.

ROLE OF PHOSPHORUS ON THE REGULATION OF PTH SYNTHESIS AND SECRETION

Reiss et al [22] reported that the administration of one gram of elemental phosphorus led to an increase in serum phosphorus, a decrease in ionized calcium and an increase in serum PTH in normal subjects. Studies in experimental renal failure in dogs have shown that a restriction of dietary phosphorus in proportion to the decrease in GFR can prevent the development of secondary hyperparathyroidism [23]. In addition to a decrease in the levels of ionized calcium, high serum phosphorus decreases the production of $1,25(OH)_2D_3$. As renal disease advances and GFR falls below 25 ml/min, hyperphosphatemia is present in most patients and hypocalcemia is more directly related to the markedly increased levels of serum phosphorus. Although high concentrations of serum phosphorus (7–9 mg/dl) precipitate calcium in soft tissue, the mechanism by which hyperphosphatemia affects the concentration of ionized calcium is not known. It may decrease the release of calcium from bone [24] and/or affect the activity of the renal enzyme 1-hydroxylase that converts 25-hydroxy vitamin D to $1,25(OH)_2D_3$. Since reduced renal mass may limit the production of 1,25D in advanced renal insufficiency, we performed studies in uremic dogs to clarify the mechanism by which dietary phosphate restriction improves secondary hyperparathyroidism [25]. After six months of renal insufficiency the dogs developed significant secondary hyperparathyroidism. Thereafter, we gradually decreased the amount of phosphorus in the diet from 0.9 to 0.6, 0.45, and finally 0.3%. In addition, the amount of calcium in the diet was reduced from 1.6 to 0.6% to prevent the development of hypercalcemia. As expected, by restricting dietary phosphorus there was a concomitant decrease in the levels of serum phosphorus and since dietary calcium was decreased from 1.6 to 0.6% there was also a mild decrease in ionized calcium. Although no change was observed in the levels of 1,25D, there was a significant decrease in the levels of PTH. Thus, this study clearly indicates that phosphorus per se, inde-

pendent of the levels of serum calcium and 1,25D has an important effect on the regulation of PTH secretion.

CURRENT STUDIES

More recently, Naveh-Many et al [26] demonstrated that parathyroid cell proliferation was greatly enhanced by a high phosphorus diet and markedly decreased by a low phosphorus diet in rats with 5/6 nephrectomy. The number of cells expressing proliferating cell nuclear antigen (PCNA) was increased by a high phosphorus diet. The authors concluded that hypocalcemia, hyperphosphatemia and uremia led to parathyroid cell proliferation and hypophosphatemia completely abolished PT hyperplasia in uremic rats. Similar results were found by Wang et al [27].

We studied in great detail the effect of phosphorus on PTH secretion *in vivo* in rats with chronic renal insufficiency and *in vitro* with disperse bovine parathyroid cells, primary cultures of bovine parathyroid cells and intact parathyroid glands from normal rats [28]. It appears that in either short-term studies with dispersed cells or in primary culture of bovine parathyroid cells phosphorous does not affect PTH secretion. We speculate that cell to cell contact or cell–matrix interaction may be necessary for the effect of phosphorus on PTH secretion. This concept is supported by our studies with intact parathyroid glands from normal rats. Short-term experiments (1 hr) did not show any effect of high (2.8 mM) phosphorus in the medium on the secretion of PTH. Thus, we performed long-term incubations over a period of 6 hr (Fig. 3). After 3 hr of incubation there was a significant increase in PTH secretion by the glands incubated in a medium containing 2.8 vs. 0.2 mM P. These results suggest that phosphorus alters the rate of PTH synthesis and subsequently secretion. There was no effect of phosphorus on PTH/GAPDH -mRNA after 24, 48 or 72 hr of incubation, consistent with our finding *in vivo* [28]. These results suggest that the effect of phosphorus is post-transcriptional. This was confirmed by other investigators [29] performing nuclear "run off" studies. Almaden et al [30] also have shown a direct effect of phosphorus on PTH secretion from whole rat parathyroid glands *in vitro*. These investigators have shown that, in a medium containing 1 or 2 mM P, an increase in calcium from 0.6 to 1.35 mM reduced PTH secretion by 63%, while in the presence of 4 mM P, the same increase in calcium inhibited PTH secretion by only 25%. Consistent with our studies, Nielsen et al [31] demonstrated a direct effect of phosphorus on PTH release from bovine parathyroid tissue slices. However they found no effect on dispersed bovine parathyroid cells. It would seem that cell to cell contact or cell–matrix interaction is crucial for the effect of phosphorus on PTH secretion. Thus, there is now agreement in the literature based on these *in vitro* studies

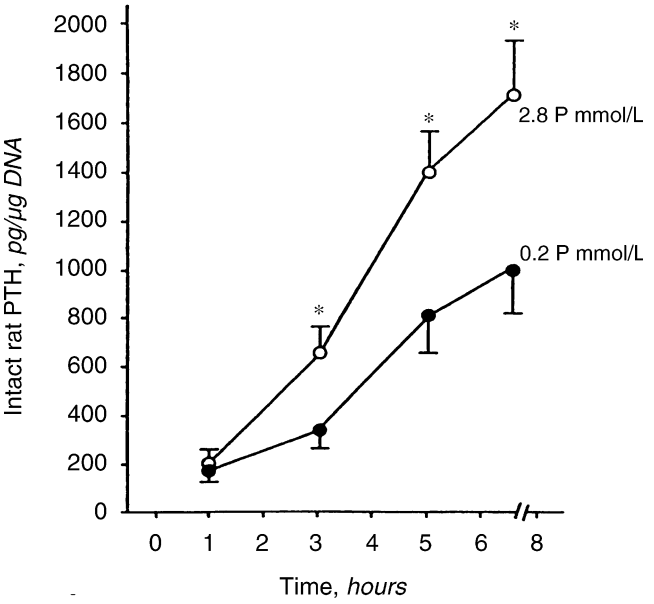

Fig. 3. Time course for PTH secretion by normal intact parathyroid glands, incubated in a low (0.2 mM) (○) ($N = 8$) or high (2.8 mM) (●) ($N = 8$) phosphorus in the media ($P < 0.05$). The effects of phosphorus were not evident until 3 hr. (Reproduced with permission of *J Clin Invest* 28).

about a direct effect of phosphorus on PTH synthesis and secretion. In the past five years, several groups of investigators have further advanced our knowledge on the effect of phosphorus in the development of secondary hyperparathyroidism and chief cell hyperplasia in animal models of chronic renal failure. Our studies also have demonstrated an effect of phosphorus on parathyroid cell growth. Similar results were reported by Naveh-Many [26]. Yi et al [32] studied the effect of phosphorus restriction on secondary hyperparathyroidism and parathyroid hyperplasia in 5/6 nephrectomized rats. The amount of phosphorus in the diet varied from 0.6 and 0.3 to 0.1%. The investigators found that the rats fed a 0.3% phosphorus diet did not develop secondary hyperparathyroidism; serum levels of calcium, phosphorus and $1,25(OH)_2D_3$ were no different from those seen in normal rats. Their results suggest that secondary hyperparathyroidism and parathyroid cell proliferation can be completely prevented by a mild dietary phosphorus restriction (0.3%) and that such effects may not depend upon changes in serum concentration of calcium, or $1,25(OH)_2D_3$. The time course for the effect of phosphorus on parathyroid gland weight was further studied by Denda et al [33]. These investigators demonstrated that in rats on a high phosphorus diet there was a 60–80% increase in the size of the parathyroid gland by 3–5 days after inducing uremia. Although the glands further increased after one month of renal insufficiency most of the growth occured in the first week. There was an increase in parathyroid

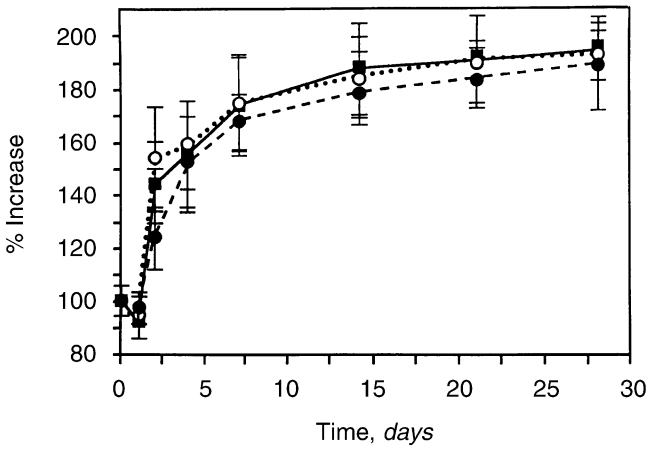

Fig. 4. The effects of a high phosphorus diet on parathyroid gland weight, protein and DNA in uremic rats. Weight, ■; protein, ○; DNA, ●. (Modified from [33]).

gland weight, protein and DNA indicating hyperplasia was the main factor responsible for the parathyroid cell growth (Fig. 4). Takahashi et al (abstract; *J Am Soc Nephrol* 8:580A, 1997) demonstrated that once the parathyroid glands were enlarged in uremic rats, a low phosphorus diet returned the parathyroid hormone to normal. However, the parathyroid glands remained enlarged. These investigators demonstrated that in uremic rats, two weeks of a high phosphorus diet significantly increased serum parathyroid hormone from 32 to 160 pg/ml, and the size of the parathyroid gland increased from 0.7 ± 0.06 to 1.20 ± 0.09 μg/g of body weight ($P < 0.001$). When the uremic rats on a high phosphorus diet were switched to a low phosphorus diet for only one week, both serum phosphorus and PTH returned to normal. However, the size of the glands did not decrease and there was no apoptosis. The intracellular PTH content was similar in the two groups of uremic rats. These results suggest that 1) the parathyroid gland PTH content correlates only with parathyroid gland weight and DNA regardless of the phosphate content of the diets; 2) the low phosphorus diet does not affect the synthesis of PTH in glands with established chief cell hyperplasia; and 3) the effect of the low phosphorus diet on PTH content in the parathyroid gland is independent of the mechanism regulated by ionized calcium. Thus, by switching the diet from high phosphorus to low phosphorus, the glands remained increased in size, but the levels of serum PTH returned to normal indicating that there is hyperplasia of the parathyroid glands without secondary hyperparathyroidism. Currently, we are studying at a molecular level potential mechanism(s) responsible for the dissociation between the histologic changes in the parathyroid gland and the secretory mechanisms of parathyroid hormone with phosphorus restriction. As we described above, it is not known how phosphorus induces PTH synthesis or parathyroid gland hyperplasia. Since Denda et al [33] demonstrated that the effect of phosphorus on parathyroid cell growth in uremic rats is very rapid, i.e., most of the growth occurring in the first five days, protooncogenes such as c-*fos*, c-*jun* and PRAD-1 [34–36] may play a role in the growth of the parathyroid glands. It is well known that normal parathyroid cells rarely divide and the great majority of cells remain in a quiescent state (G_0). Nevertheless, they have the potential to proliferate in response to mitogenic stimuli such as uremia, high phosphorous or low calcium. Parathyroid cell proliferation results from the cell commitment to abandon G_0, cross the restriction point and complete the mitotic cycle. This decision is made in early G_1 and depends upon the net balance between two opposite forces: 1) the cyclin and cyclin-dependent kinases, responsible for the cell progression through the consecutive phases of the cycle, and 2) the levels of inhibitors of the activity of the cyclin-cdk complexes. One important inhibitor of the cell cycle is P_{21}. In our laboratory Dusso et al (abstract; *J Am Soc Nephrol* 9:564A, 1998) have demonstrated that the levels of P_{21} mRNA and protein are increased after two days of uremia in animals maintained on a low phosphorus diet, suggesting a role for P_{21} in the suppression of parathyroid cell proliferation by a low phosphorus diet.

Two recent observations may provide further insight into how phosphorus affects PTH synthesis/secretion. Tasumi et al [37] cloned a Na^+–dependent Pi cotransporter (PiT-1) from rats parathyroid glands. The amount of PiT-1 mRNA in parathyroid glands of vitamin D-deficient rats was reduced compared with that in normal animals, but increased markedly after the administration of $1,25(OH)_2D_3$. Since 1,25D suppresses PTH secretion and phosphorus enhances PTH secretion, the physiological role of the Na^+-dependent P_i cotransporter in the regulation of PTH secretion is unclear. Mollem et al [38] demonstrated that parathyroid cytosolic proteins bind the 3′-untranslated region (UTR) of the PTH mRNA. These parathyroid proteins showed increased binding in hypocalcemia and decreased binding in hypophosphatemia. The decreased binding will enhance the degradation of the PTH mRNA and therefore shorten the life of the transcript during hypophosphatemia.

Thus, in the past 20 years numerous investigators have provided strong evidence for the action of phosphorus on PTH secretion. There is agreement that the mechanisms of action of phosphorus on PTH synthesis and secretion is post-transcriptional. Moreover, the rate of parathyroid cell proliferation also is greatly enhanced by phosphorus. Unfortunately, the absence of a parathyroid cell line is slowing the progress in understanding the molecular mechanism(s) involved in the regulation of PTH secretion and parathyroid gland hyperplasia by phosphorus.

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Reprint requests to Eduardo Slatopolsky, M.D., Washington University School of Medicine, Renal Division, 660 South Euclid Avenue, St. Louis, Missouri 63110, USA.

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