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Experimental secondary hyperparathyroidism and the therapeutic application of vitamin D analogs

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Experimental secondary hyperparathyroidism and
the therapeutic application of vitamin D analogs

by

Negussie Fedessa Bussa

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
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For the Graduate College

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1995

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DEDICATION

I dedicate this work for those whom I missed:

1. My little sister Yodit Bussa, who passed away on November 24, 1974 with a brief illness
2. My brother Bakala (Tesfaye) Bussa who passed away on November 30, 1985 in Massawa, Eritrea from internal bleedings due to heavy beatings in the prison by Ethiopia's military junta.
3. My elder brother Worku Duresso Minta, who was forced to be on the war front by the military junta and was killed in the war in April, 1991 in Northern Shoa, Ethiopia.
4. My other elder brother Haile Michael Mulata Fedhasa, Navy officer, who was killed on the Sudan-Ethiopian border by Tigrean army of Ethiopia.

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ABSTRACT

The two major pathophysiologic mechanisms responsible for the development of secondary hyperparathyroidism (SHPT) in advanced renal insufficiency are phosphorus retention and low levels of 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃].

The actions of the hormonal form of vitamin D extend beyond its role in mineral homeostasis. Recently, analogs of vitamin D that are less calcemic, but retain the therapeutically useful properties of 1,25-(OH)₂D have been developed.

This study was designed to determine if a high phosphorus diet could be the main contributor for increasing plasma phosphate and parathyroid hormone (PTH) and reducing the production of renal 1,25-(OH)₂D₃ in renal failure (reduced renal cell mass). Another objective was to determine if vitamin D analogs could be used as an effective drug in lowering plasma PTH and increase calcium during SHPT.

One hundred twelve 60-day-old male rats of Harlan-Sprague-Dawley (weighing 400-450 gm) were divided into 7 groups: SLP, SHP, NLP, NHP, NHP-A, NHP-B, and NHP-C. SLP and SHP were sham (S) operated groups, whereas the rest were unilaterally nephrectomized (N). The rats were fed two synthetic diets with low (LP) or high (HP) phosphorus levels. Three groups of rats: NHP-A, NHP-B, and NHP-C were simultaneously given an oral

administration of 18 ng/day of 1,25-(OH)₂D₃ (A), 18 ng/day of 1,25-(OH)₂D₂ (B) or 2 μg/day of 1,25,28-(OH)₃D₂ (C), respectively, every day for 30 days.

After 30 days all rats were made unconscious with CO₂-O₂ (50%:50% vol/vol) and decapitated. Blood samples were collected and plasma was analyzed for inorganic phosphorus, calcium, 1,25-(OH)₂D₃, PTH and creatinine.

The results of this study indicate that sham and nephrectomized rats fed the high dietary phosphorus lost 9.50 and 24.72%, respectively, of their initial body weights. Plasma phosphate increased significantly as dietary phosphorus increased. The treatment with vitamin D analogs lowered plasma phosphate, PTH and raised plasma calcium and 1,25-(OH)₂D₃. Among the vitamin D analogs used, 1,25-(OH)₂D₃ was found to be superior. These changes indicate that vitamin D analogs may be effective in SHPT.

INTRODUCTION

Secondary hyperparathyroidism (SHPT) is a common finding in patients with chronic renal failure (Coburn and Slatopolsky, 1991; Kubrusly *et al.*, 1993). It is caused by decreases in the plasma concentration of ionized calcium (Ca^{2+}) evoked by recurring increases in the plasma concentration of inorganic phosphorus (P_i) and phosphorus-mediated suppression of renal 25-hydroxyvitamin D-1 α -hydroxylase (1 α -OHase) that decreases the plasma concentration of 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃] (Portale *et al.*, 1984).

Elevated levels of parathyroid hormone (PTH) are now well recognized as an important uremic toxin in human medicine. Toxic effects of PTH on the brain that bring about depression have been studied extensively in the dog (Akmal *et al.*, 1984; Massry, 1985b). High plasma PTH will slow peripheral nerve conduction velocity (Akmal and Massry, 1990). The anemia of renal failure is largely related to erythropoietin deficits and excess PTH is one of the causes of these deficits and contributes to uremic anemia (Massry, 1983). A variety of leukocyte malfunctions including failures of the immune response have been reported to be caused by excessive PTH in uremia (Klinger *et al.*, 1990).

Several reports indicate that PTH causes an increased influx of calcium from blood and raises levels of

intracellular Ca^{2+} . High cellular calcium activates various enzymes that destroy cell membranes, proteins and nucleic acids (Fawthrop et al., 1991). Therefore, in all sites this accumulation of calcium injures mitochondria, disrupting energy production, before cells are killed (Nagode et al., 1992).

A PTH-mediated interference with the response of pancreatic insulin-secreting islet cells to a dietary intake of glucose appears to be responsible for much of the carbohydrate intolerance seen in uremic individuals (Fadda et al., 1990). Thus, the absence of adequate insulin to facilitate lowering of blood glucose may be one mechanism of the inappetence common in uremia (Nagode et al., 1992).

The relationship between the hyperplastic, hypersecreting parathyroid gland and the damaged kidney in the uremic patients forms a vicious cycle, as the damaged kidneys can produce hypersecretion of PTH (Nagode et al., 1992). Because renal tubular cells have high concentrations of the receptor for PTH, the kidney is affected early in PTH toxicity and marked calcium influx into tubular cells causes their death (Tan et al., 1989). Later, calcium phosphate precipitating within tubular lumen contributes to the renal damage (Lau, 1989). With loss of more renal tissue, hyperparathyroidism (HPT) worsens and the cycle continues (Nagode et al., 1992).

Uremic HPT is usually treated with derivatives of vitamin

D which can suppress PTH secretion by two mechanisms: indirectly through the stimulation of intestinal calcium absorption and thereby raising plasma calcium (Brickman et al., 1974) and directly through actions on PTH gene transcription (Silver et al., 1985). However, the effectiveness of the vitamin D derivatives is limited by the degree to which plasma concentrations of calcium can safely be raised (Lee et al., 1994). There has been considerable interest in ways of maximizing the direct, noncalcemic effect on PTH synthesis, particularly in use of intermittent injections of high dose of vitamin D derivatives (Lee et al., 1994).

The main objectives of this study were to investigate whether high phosphorus diets in conjunction with reduced renal cell mass (unilateral nephrectomy) are the primary factors responsible for increased plasma phosphate and PTH and decreased plasma calcium and $1,25-(OH)_2D_3$ which are the main features of SHPT. Furthermore, $1,25-(OH)_2D$ possesses direct suppressive action on PTH synthesis and decreases serum levels of PTH in most uremic patients with SHPT. Therefore, attention was directed towards determining if some of the vitamin D analogs could be used as an effective drug treatment against SHPT.

REVIEW OF LITERATURE**Parathyriod Hormone**Synthesis and secretion

In the rat, parathyroid glands (PTGs) are a single pair embedded in the cranial part of the thyroid glands. They possess two types of cells: the chief and the oxyphil cells. The chief cells are predominantly involved in the synthesis and secretion of PTH (Roth and Raisz, 1966). PTH is a single-chain polypeptide composed of 84 amino acids with a molecular weight of 9500 Daltons (Breslau, 1992). The amino acid sequence has been determined in several species and there is a high degree of similarity amongst species, particularly in the amino-terminal region of the molecule (Habener and Potts, 1990).

The genes for human (Hendy et al., 1981), bovine (Gordon and Kemper, 1980), rat (Heinrich et al., 1984), pig (Schmeizer et al., 1987) and chicken (Khosla et al., 1988) PTH have all been cloned and shown to have two introns and three exons (Kronenberg et al., 1986).

The primary RNA transcript transcribed from both the introns and exons is processed and translated into PreProPTH which is a polypeptide of 115 amino acids with a molecular weight of 13000 Daltons. This prehormone is short-lived, being rapidly cleaved to a smaller peptide, a ProPTH.

ProPTH is composed of 90 amino acids and has a molecular weight of 10200 Daltons. A second enzymatic cleavage of the 6 amino acid extension at the amino terminus of the molecule results in the final secretory product: 1-84 PTH (Breslau, 1992).

The translational product of the PTH mRNA is PreProPTH, which is the precursor of ProPTH. PreProPTH like any other proteins that are to be exported from a cell is synthesized on ribosomes bound to the rough endoplasmic reticulum. This process facilitates its specific transport into the cistern of the rough endoplasmic reticulum and directs it along the secretory pathway of the cell (Silver and Naveh-Many, 1994). During the synthesis, pre or signal sequence is cleaved from the precursor protein and the two amino-terminal methionines are removed enzymatically.

As the nascent chain continues to grow, the hydrophobic amino-terminal sequence of PreProPTH emerges and associates with the endoplasmic reticulum in accord with the signal hypothesis (Von Heijne, 1983).

A signal peptidase acts enzymatically to cleave the glycyl-lysine bond and removes the 23-amino acid signal sequence as the protein traverses the membrane of the rough endoplasmic reticulum, leaving the intermediate precursor, ProPTH, in the lumen of the endoplasmic reticulum (Dorner and Kemper, 1978).

The ProPTH product moves to the Golgi apparatus where the dibasic residues at the end of the pro sequence direct the enzymatic cleavage of the pro sequence. After synthesis, PTH is packed in secretory vesicles and may then be secreted, stored or degraded intracellularly. However, since the parathyroid cell is not rich in secretory granules, as much as 90% of the newly generated PTH is neither secreted nor stored but proteolytically degraded within the cell (Morrissey et al., 1980). Furthermore, in the peripheral circulation, the intact PTH is cleaved into two major fragments (particularly in the liver and probably in the kidney), N-terminal (1-34) and C-terminal (53-84). The N-terminal fragment retains all the biological activities, whereas the C-terminal has no known function (Breslau, 1992).

Regulations of synthesis and secretion

Regulation of PTH production is exerted at the stages of secretion, intracellular catabolism, transcription and proliferation. The secretion of PTH from its secretory granules is regulated within seconds to minutes and the major regulatory factor is calcium. The regulation of PTH gene transcription is a major site of PTH regulation which occurs within hours and can continue for prolonged periods. The major regulators of transcription are $1,25-(OH)_2D_3$, which decreases PTH gene transcription and hypocalcemia which increases PTH

gene transcription. However, parathyroid cell proliferation is the final site of regulation and this occurs after the stimulus of prolonged hypocalcemia, but $1,25\text{-(OH)}_2\text{D}_3$ very well might have a role in preventing parathyroid cell proliferation. However, in vivo this property of $1,25\text{-(OH)}_2\text{D}_3$ is not established (Silver and Naveh-Many, 1994).

Rupp et al. (1990) analyzed the human PTH promoter region and identified a number of consensus sites which included a sequence resembling cyclic adenosine monophosphate (cAMP) responsive element. Demay et al. (1990) identified DNA sequences in human PTH gene that bind $1,25\text{-(OH)}_2\text{D}_3$ receptor and further examined nuclear extracts containing $1,25\text{-(OH)}_2\text{D}_3$ receptor for the binding to sequences in the 5'-flanking region of the human PTH (hPTH) gene. Several reports indicated that the transcription of PTH gene is regulated by calcium, $1,25\text{-(OH)}_2\text{D}_3$ and estrogen. However, a low extracellular calcium concentration is the major secretagogue for PTH and calcium was also shown to regulate PTH gene expression both in vitro and in vivo.

Russell et al. (1983) cloned the bovine cDNA for PTH and maintained bovine parathyroid cells in primary culture in the presence of different calcium concentration. They reported that a high calcium concentration (2.5 mM, which in vitro is all ionized and therefore equivalent to 5.0 mM in vivo) led to a decrease in PTH mRNA levels after 16 hours with a maximum

effect at 72 hours. However, they indicated that a low calcium level has no effect. Brookman et al. (1986) also studied the effect of calcium on PTH mRNA levels in primary cultures of parathyroid cells. They showed that a low calcium level (0.4 mM) increased and that a high calcium (3.0 mM) level decreased PTH mRNA levels with a similar time sequences as in Russell et al. (1983). A study conducted by Naveh-Many et al. (1989) in rats, showed that small decreases in serum calcium from 2.6 to 2.1 mM/l led to large increases in PTH mRNA levels reaching three fold that of controls. A high serum calcium had no effect on PTH mRNA levels even at concentrations as high as 6.0 mM/l.

Thus, physiologically PTH biosynthesis is maximally suppressed with respect to calcium, and the gland is geared to respond to decreases in serum calcium but not to increases in serum calcium. Yamamoto et al. (1989) also studied the in vivo effect of calcium on PTH mRNA levels in rats. Their findings confirmed that calcium regulated PTH mRNA levels with hypocalcemia for 48 hours and their rats with high serum calcium (2.9 to 3.4 mM) had the same PTH mRNA levels as those with normal serum calcium (2.4 to 2.6 mM) in agreement with the results of Naveh-Many et al. (1989). Thus, the in vivo results on the effect of calcium on the PTH gene expression are obviously the physiologically relevant observation; however, the differences between the in vivo and in vitro

systems need to be explained.

In vitro studies with primary cultures of parathyroid cells are complicated by the loss of calcium responsiveness with time in culture (LeBoff et al., 1983; MacGregor et al., 1983) and also because high calcium levels encourage the growth of fibroblasts at the expense of parathyroid cells. Therefore, studies on the effect of calcium using primary cultures of parathyroid cells are only valid at time periods when the cells are still responsive to changes in serum calcium.

Sherwood et al. (1968) reported an inverse linear relationship between calcium concentration and PTH secretion. Mayer et al. (1976) and Mayer and Hurst (1978) found that the relationship was sigmoidal and in addition there was a nonsuppressible portion of PTH secretion even at very high serum calcium levels. Furthermore, Brent et al. (1988) confirmed the inverse sigmoidal relationship between PTH and serum total and plasma ionized calcium in humans which was applicable for rapid and slow changes in serum calcium.

Johansson et al. (1987) measured cytoplasmic calcium concentration of single parathyroid cell and have shown a sigmoidal relationship between sustained changes in cytoplasmic calcium and extracellular calcium concentration in the 5 to 3 mM range. The sustained change in calcium concentration is due to the influx of extracellular calcium through

calcium channel and perhaps other transduction mechanisms such as activation of protein kinase A (PKA) and protein kinase C (PKC) (Silver and Naveh-Manly, 1994).

Thus, PTH secretion and perhaps also PTH gene transcription might, therefore, respond proportionately to the sigmoidal change in cytoplasmic calcium concentration.

However, Brown (1983) has analyzed in detail the sigmoidal relationship between calcium and PTH secretion defining the so-called set-point which is the calcium concentration producing half of the maximal inhibition of secretion which is a useful parameter in the analysis of parathyroid tissue from patients with SHPT due to renal failure. It was suggested (Brown et al., 1982) that there was a shift in the set-point to the right indicating a relative insensitivity to calcium. In addition, Leboff et al. (1983) and Nygren et al. (1988) have shown that parathyroid cells in culture, when rapidly proliferating, are also relatively insensitive to a high calcium showing the importance of rigorously defining in vitro conditions for any study on PTH regulation.

Parathyroid cells have stereospecific, high affinity receptors for $1,25-(OH)_2D_3$, similar to the receptors found in the classic target sites for $1,25-(OH)_2D_3$, namely intestine and bone (Minghetti and Norman, 1988), and thus the parathyroid is a target organ for $1,25-(OH)_2D_3$.

Russell et al. (1984) and Silver et al. (1985)

demonstrated that addition of $1,25\text{-(OH)}_2\text{D}_3$ and other metabolites to bovine parathyroid cells in primary culture decreased PTH mRNA levels to 50% of control at 48 to 96 hours with no effect on actin mRNA as a control. Naveh-Many et al. (1990) performed nuclear transcript run-off experiments in order to determine whether the $1,25\text{(OH)}_2\text{D}_3$ regulation of PTH mRNA levels was mediated transcriptionally. They found that there was a dramatic reduction in PTH transcription in $1,25\text{(OH)}_2\text{D}_3$ -treated rats to 10% of control rats, whereas β -actin transcription in the same $1,25\text{(OH)}_2\text{D}_3$ -treated rats was 100% of control rats. Therefore, these in vivo results established that $1,25\text{(OH)}_2\text{D}_3$ regulates PTH mRNA levels through its effect on PTH gene transcription and that the parathyroid cell is an important target organ for $1,25\text{(OH)}_2\text{D}_3$ (Silver and Naveh-Many, 1994).

Naveh-Many et al. (1990) injected $1,25\text{(OH)}_2\text{D}_3$ in rats and measured the levels of the vitamin D receptor (VDR) mRNA and PTH mRNA in parathyroid tissue. They showed that $1,25\text{(OH)}_2\text{D}_3$ in physiologically relevant doses led to an increase in VDR mRNA levels in the PTGs in contrast to the decrease in the PTH mRNA levels. Therefore, $1,25\text{(OH)}_2\text{D}_3$ increases the expression of its receptor's gene in the PTG which would result in increased VDR protein synthesis and increased binding of $1,25\text{(OH)}_2\text{D}_3$. This ligand-dependent receptor up-regulation would lead to an amplified effect of $1,25\text{(OH)}_2\text{D}_3$ on the PTH gene and

might help explain the dramatic effect of $1,25-(\text{OH})_2\text{D}_3$ on the PTH gene (Silver and Naveh-Many, 1994).

In summary, Ca^{2+} and $1,25-(\text{OH})_2\text{D}$ are the major controllers of PTH synthesis and secretion. It is believed that calcium mainly controls the secretion of PTH whereas, $1,25-(\text{OH})_2\text{D}$ regulates the PTH gene transcription.

Physiological actions

PTH acts on bone and kidney to maintain plasma levels of Ca^{2+} and decreasing circulating concentrations of phosphate.

PTH regulates the levels of calcium and phosphate in blood by modulating the activities of specific cells in bone and kidney (Hadley, 1992). PTH stimulates: 1) the release of calcium and phosphate from bone; 2) reabsorption of calcium and phosphate from glomerular filtrate and 3) the renal synthesis of $1,25-(\text{OH})_2\text{D}_3$, thereby increasing intestinal absorption of calcium and phosphate. Thus, the net result of these actions is to raise the level of blood calcium and lower the level of blood phosphate (Breslau, 1992; Hadley, 1992).

However, blood calcium, in turn, is the major regulator of PTH secretion and rise in blood calcium decreases PTH secretion. Thus, the mutual regulatory interactions of PTH and calcium serve to keep the blood level of calcium constant, despite moderate fluctuations in diet, bone metabolism and renal function (Kronenberg, 1993).

PTH has a complex and still poorly understood action on bone. Even though PTH causes a release of phosphate from bone, PTH administration leads to a fall in the blood levels of phosphate, because of the phosphaturia caused by PTH. This phosphaturia reinforces the effect of PTH on bone, because low levels of blood phosphate independently lead to resorption of bone (Hadley, 1992). Phosphate is normally reabsorbed from glomerular filtrate both in the proximal and distal convoluted tubules. However, reabsorption at both these sites is inhibited by PTH (Bringham et al., 1989).

PTH and vitamin D interact in a number of complex ways. In the kidney, PTH activates 1α -OHase, which in the proximal convoluted tubule catalyzes the synthesis of the most active metabolite of vitamin D, $1,25-(OH)_2D_3$, which, in turn, is a potent inducer of intestinal calcium absorption (Garabedian et al., 1972; Kronenberg, 1993).

In the proximal convoluted cell of the kidney, phosphate is transported into the cell against an electrochemical gradient. The ATP used to accomplish this task does so indirectly; ATP fuels the sodium pump, which drives sodium from the cell. Sodium then travels back into the cell in response to the concentration gradient established by the sodium pump and phosphate transport is coupled to the entry of sodium back into the cell (Cheng and Sacktor, 1981). However, PTH blocks this sodium-dependent phosphate cotransport

(Kronenberg, 1993).

In the distal tubule, presumably PTH has a similar effect on phosphate transport and stimulates calcium reabsorption against an electrochemical gradient (Cheng and Sacktor, 1981).

Molecular mechanisms

Bone and kidney are the two target organs of PTH. However, while there is a PTH receptor in the osteoblast, there is no known PTH receptor in the osteoclast. Intact PTH does not act directly in the cytoplasm of its target cells but binds to specific receptors on the surface of target cells. This binding triggers the release of cytoplasmic second messenger that then mediates the multiple distal effects of PTH.

However, binding of PTH to its receptors requires only the first 34 residues of the PTH molecule which contains 84 amino acids (Hadley, 1992).

Recently, several reports have identified new mechanisms for the generation of intracellular signals by the PTH receptor. However, these reports indicated the existence of multiple forms of the PTH receptor (Breslau, 1992).

But, Hadley (1992) proposed a two-receptor model of PTH which each leads to activation of phospholipase C (PLC) or adenylate cyclase (AC) although a one-receptor model with coupling through different types of G proteins to various effector elements has not been excluded (Caulfield and

Rosenblatt, 1990; Dunlay and Hruská, 1990).

The best described pathway so far is the PTH activation of PLC. According to this model, the PTH receptor is coupled via a G protein to PLC. Binding of PTH to its receptor leads to activation of PLC with the subsequent hydrolysis of phosphatidylinositol,4,5-bisphosphate (PIP₂) to inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ generation leads to the release of intracellular calcium stores, which produces an increase in the intracellular calcium concentration. DAG activates PKC. Both IP₃ metabolites and PKC may play a role in returning the intracellular calcium concentration back to base line, by stimulating the movement of calcium from the intracellular to the extracellular compartments and by sequestering calcium within intracellular organelles (Meltzer *et al.*, 1982; Yamaguchi *et al.*, 1987; About-Samra *et al.*, 1989; Hadley, 1992).

The other equally important pathway is PTH activation of AC which leads to the generation of cAMP. The cAMP produced by AC then binds to the regulatory subunit of PKA. The free catalytic subunit then phosphorylates specific serine and threonine residues in target proteins (Hadley, 1992; Breslau, 1992; Kronenberg, 1993).

In summary, various reports have shown that PTH is capable of stimulating both AC and PLC signal transduction pathways to manipulate several physiological changes in its target

tissues.

Vitamin D Metabolism and Mechanisms of Actions

Vitamin D metabolism

The importance of vitamin D in cellular physiology was realized early in medical history from clinical studies on rickets (Lowe et al., 1992).

Vitamin D is a a prohormone that becomes a required nutrient only in the absence of adequate exposure to sunlight (Reinhardt et al., 1988), and it is one of the principal factors required for normal calcium and phosphorus homeostasis as well as for the proper development and maintenance of bone (Norman and Henry, 1993; MacDonald et al., 1994).

Vitamin D is a seco-steroid in which the B ring structure is cleaved (Walters, 1992). Exposure of the skin to the ultraviolet radiation present in sunlight converts 7-dehydrocholesterol to previtamin D₃ and this previtamin isomerizes to vitamin D₃, at body temperature and is transported to the liver for further metabolism (Reinhardt et al., 1988). However, dietary vitamin D₂ and D₃ are absorbed in the small intestine, where they enter the lymphatic circulation (Reinhardt et al., 1988).

In the liver, the enzyme 25-hydroxylase (25-OHase) converts the vitamin to the inactive hormonal precursor 25-

hydroxyvitamin D (25-OHD) (Walters, 1992), which is the major circulating form of vitamin D (Reinhardt et al., 1988).

Vitamin D and its metabolites circulate in the blood primarily bound to and in equilibrium association with the vitamin D-binding protein (DBP) that contains a single, high affinity, vitamin D specific binding site (Reinhardt et al., 1988; Walters, 1992). With the normal concentration of the binding protein in plasma, less than 10% of the available binding sites usually are occupied (Fraser, 1980).

Thus, 25-OHD is transported in the blood bound to DBP and taken up by the kidney, where it can be metabolized further to more than 30 known metabolites (Horst, 1986; Reinhardt et al., 1988).

25-OHD is 1α -hydroxylated exclusively in the kidney by the renal 1α -OHase to the biologically active form, $1,25-(OH)_2D$ (Walters, 1992), and regulated through a variety of factors by the need for calcium and phosphorus (Reinhardt et al., 1988). Thus, both hydroxylases are cytochrome P_{450} containing enzymes and 1α -OHase activity is tightly controlled by a number of ionic and endocrine factors including stimulation by PTH and reduced plasma Ca^{2+} or inhibition by $1,25-(OH)_2D$ in a classic negative feedback loop (Walters, 1992; Norman and Henry, 1993).

Under conditions of adequate plasma Ca^{2+} levels, 25-OHD is alternatively converted to $24,25-(OH)_2D$ by the reciprocally

regulated renal P_{450} dependent 24-hydroxylase (24-OHase) enzyme (Walters, 1992; Norman and Henry, 1993). Furthermore, Brommage and DeLuca (1985) and Haussler et al. (1988) indicated that 24-hydroxylation is the first in a series of steps leading to the inactivation of $1,25-(OH)_2D$ and important for the elimination of 25-OHD. Thus, the circulating level of $1,25-(OH)_2D$ is dependent not only on the rate of formation of this hormone, but also on the rate of $1,25-(OH)_2D$ degradation (Reinhardt et al., 1988; Goff et al., 1992).

$1,25-(OH)_2D$ can be cleared from the circulation by biliary excretion as polar glucuronide and sulfated metabolites of $1,25-(OH)_2D$ (Kumar, 1984) and by C-23/C-24 side chain oxidation (Lohnes and Jones, 1987; Goff et al., 1992). Thus, raising the blood $1,25-(OH)_2D$ concentration by treatment of animals with large doses of $1,25-(OH)_2D$ rapidly induces 24-OHase in many tissues including the kidney and intestine (Goff et al., 1992).

Boyle et al. (1971) found out that the in vivo production of $1,25-(OH)_2D$ to be related to the concentration of calcium in the medium and Omdahl and DeLuca (1977) reported that an increased plasma $1,25-(OH)_2D$ concentrations occur in response to a low calcium diet.

However, removal of the parathyroid glands prevents the increase of $1,25-(OH)_2D$ in response to low calcium diet and calcium may act indirectly via PTH to control production of

1,25-(OH)₂D (Garabedian et al., 1972). Other reports indicate that PTH increases the 1 α -OHase activity by a cAMP mediated mechanism (Fraser and Kodicek, 1973; Horiuchi et al., 1977; Reinhardt et al., 1988; Walters, 1992).

Goff et al. (1992) reported that phosphorus deficiency seems to prevent the up-regulation by 1,25-(OH)₂D of 24-OHase activity in the intestine.

Hypophosphatemia increases 1,25-(OH)₂D levels, even in parathyroidectomized animals (Tanaka et al., 1973; Hughes et al., 1975; Steele et al., 1975). Furthermore, Baxter and DeLuca (1976) found a low phosphorus diet increased 1 α -OHase activity in chickens. The stimulatory ability of the low phosphorus diet, however, was small compared to that of the low calcium diet. In contrast, Edelstein et al. (1978) found a decrease in plasma 1,25-(OH)₂D concentration in rats fed low phosphorus diet. Thus, there may be differences among species in the ability of a low phosphorus diet to stimulate 1,25-(OH)₂D production. Reports by Ribovich and DeLuca (1978) and Summerville et al. (1978) suggest that low phosphorus diet fed to rats may stimulate the intestinal accumulation of already existing 1,25-(OH)₂D presumably by inducing VDRs.

Once calcium and phosphorus needs have been met, as reflected by plasma calcium and phosphate concentrations, inhibition of the 1 α -OHase will occur probably through 1,25-(OH)₂D. Thus, Omdahl et al. (1980) indicated that 1,25-(OH)₂D

acts by controlling the synthesis and turnover of the 1α -OHase, in addition to modulating the enzymes endogenous activity. Haussler et al. (1980) demonstrated specific binding of $1,25-(OH)_2D$ in chick parathyroid glands as well as in rat pituitary glands.

Thus, these studies suggest that the possibility of feedback regulation mechanisms similar to that of many steroid hormones that involve the pituitary gland.

Target organs

The $1,25-(OH)_2D$ hormone participates in stringent regulatory system that governs mineral homeostasis, primarily through coordinating actions at several mineral regulating organs (MacDonald et al., 1994).

The classical target organs of the hormone are intestine bone, kidney, and parathyroid gland. Since the functions of $1,25-(OH)_2D$ hormone extends beyond mineral homeostasis, there are several non-classical target organs.

Lowe et al. (1992) have compiled lists of vitamin D target organs. Although tissue responsiveness to steroid hormones, such as $1,25-(OH)_2D$, is dependent on the concentration of the $1,25-(OH)_2D$ in the extra- and intracellular milieu (Reinhardt and Horst, 1989; Goff et al., 1992), target tissues of $1,25-(OH)_2D$ must have three key components in order for their genes to be regulated by a steroid hormone such as $1,25-(OH)_2D$

(Norman and Henry 1993). These include: 1) a protein receptor for the $1,25\text{-(OH)}_2\text{D}$ that contains both a unique binding domain for $1,25\text{-(OH)}_2\text{D}$ and a DNA binding domain that allows the occupied receptor to locate the genes in the nucleus of the cell it regulates, 2) hormone responsive element (HRE) that consists of specific sequences of DNA nucleotides that facilitate/promote an interaction between the occupied receptor and the genes to be regulated (either upward or downward) in that particular cell, and 3) access to $1,25\text{-(OH)}_2\text{D}$ (usually the hormone is delivered through blood bound to DBP)

Vitamin D receptor

The steroid hormone $1,25\text{-(OH)}_2\text{D}$ exerts its biological actions in target tissues via interactions with specific high affinity intracellular receptors (Haussler, 1986; Lowe et al., 1992).

Evidence has been presented for a soluble factor of approximately 50 to 70 kDa that interacts with the intestinal metabolite of vitamin D in a saturable fashion (Haussler and Norman, 1969). Several studies (Lawson et al., 1971; Holick et al., 1971; Brumbaugh and Haussler, 1975) have shown that this soluble factor facilitated $1,25\text{-(OH)}_2\text{D}$ association with chromatin. Further evidence for the involvement of functional receptors in $1,25\text{-(OH)}_2\text{D}$ action was demonstrated in clinical

studies by Pike et al. (1984). These studies provided the scientific foundation and the first biochemical evidence for the VDR, the hormone receptor that mediates most of the biological actions of vitamin D (Lowe et al., 1992; Walters, 1992; Norman and Henry, 1993; MacDonald et al., 1994; Brown et al., 1994).

It is clear that VDR functions as a ligand induced transcription factor in mediating the genomic effects of 1,25-(OH)₂D. It does so by interacting with specific DNA sequence elements or vitamin D responsive elements (VDREs) within vitamin D responsive genes in important target tissue such as bone, intestine, parathyroid gland and kidney (MacDonald et al., 1994).

Important functional domains of the VDR have been identified including regions involved in DNA binding, hormone binding, phosphorylation and protein-protein interactions (MacDonald et al., 1994). Further evidence indicates that VDR heterodimerization with other nuclear factors is required for DNA interaction and VDR mediated transcription. Thus, the VDR could be dissected into two discrete, functionally independent subdivisions: one involved in DNA interaction and another in binding the 1,25-(OH)₂D ligands (Allegretto et al., 1987; MacDonald et al., 1994).

The precise location of the VDR gene within human and rat genomes has been determined (MacDonald et al., 1994). Southern

blot analysis of DNA from human-Chinese hamster cell hybrids localized the VDR gene to human chromosome 12 (Faraco et al., 1989). This was later extended to 12q by means of somatic cell hybrid mapping (Szpirer et al., 1991) and further refined to the 12q 13-14 region with in situ hybridization and linkage analysis (Labuda et al., 1992). Similarly, the VDR sequence was localized to chromosome 7 of the rat genome (Szpirer et al., 1991). In both the human and rat genomes, the VDR gene is located within regions where distantly related DNA binding proteins also map, including transcription factor SP-1 and the gamma isoform of the retinoic acid receptor (RAR) (Ishikawa et al., 1990; Szpirer et al., 1991; Mattei et al., 1991). This clustering of related DNA binding proteins may not be purely by chance, and their close proximity might suggest that these proteins were derived from some common ancestral gene (MacDonald et al., 1994).

Biological mechanisms of vitamin D

Historically, over the past two decades much research has documented the extensive involvement of 1,25-(OH)₂D in generating biological responses via genomic pathways involving the nuclear receptors (Pike, 1991).

Recently, however, evidence has emerged that 1,25-(OH)₂D may also have the capability to generate biological responses via activation of voltage-dependent Ca²⁺ channels that are

coupled via appropriate signal transduction pathways to the generation of biological responses (Caffrey and Farach-Carson, 1989; de Boland *et al.*, 1990).

Like any steroid hormone $1,25-(\text{OH})_2\text{D}$ binds to a specific cytosolic receptor in the intestine (Reinhardt *et al.*, 1984; Reinhardt and Horst, 1989; Goff *et al.*, 1990; Goff *et al.*, 1993; Beckman *et al.*, 1994). The hormone-receptor complex then is transported to the nucleus, associates with the chromatin, and results in the *de novo* production of mRNA for calcium binding protein (CaBP) (Walters, 1992; Lowe *et al.*, 1992).

However, given the unexpectedly widespread distribution of VDRs and the well known effects of $1,25-(\text{OH})_2\text{D}$ on Ca^{2+} translocation in intestine, Walters (1992) developed the hypothesis that $1,25-(\text{OH})_2\text{D}$ and its receptor regulate intracellular calcium homeostasis and, thus, play a role in regulating the specialized intracellular effects of calcium. These postulated effects of $1,25-(\text{OH})_2\text{D}$ could occur by direct regulation of cellular calcium levels (by effects at the cell membrane), by affecting calcium sequestration/release cycles in endoplasmic reticulum or other organelles by affecting CaBPs by indirect effects modulating the functions of hormones and other agonists which act via membrane receptors, or by other unknown mechanisms (Walters, 1992).

The above hypothesis was tested directly by investigating $1,25-(\text{OH})_2\text{D}$ effect on net $^{45}\text{Ca}^{2+}$ uptake in isolated or cultured

cells as reviewed by Walters (1992) and reported by Walters et al. (1987) in adult rat ventricular cardiac muscle, in TM_4 mouse Sertoli cells by Akerstorm and Walters (1992), in chick embryo myoblast by de Boland and Boland (1985) and in mouse mammary gland by Mezzetti et al. (1988). These reports indicate that in spite of distinct differences in the pattern of $1,25-(OH)_2D$ induction of $^{45}Ca^{2+}$ -uptake across tissues, these studies are important in establishing biological effects of the hormone in these tissues and in providing evidence in support of the general hypothesis that $1,25-(OH)_2D$ regulates intracellular calcium homeostasis (Walters, 1992).

Several reports (reviewed by Nemere and Norman, 1991) described $1,25-(OH)_2D$ -mediated effects in opening calcium channels in several tissues. This apparent rapid response seems to be linked to activation of PKC and may occur through nongenomic mechanisms. However, Walters (1992) reported that there are at least two Ca^{2+} -related phenomena: a rapid $1,25-(OH)_2D$ mobilization of Ca^{2+} which serves as a signal transduction event and the ultimate and sustained $1,25-(OH)_2D$ effect on the cell which would involve net transcellular Ca^{2+} translocation in intestinal (and other epithelial) cells and altered intracellular Ca^{2+} homeostasis in symmetrical cells.

Further studies demonstrated that the mechanism of the $1,25-(OH)_2D$ enhanced calcium entry involved modulation of dihydropyridine sensitive L type Ca^{2+} channels and activation

of the $\text{Na}^+/\text{Ca}^{2+}$ exchange pathways. However, this effect required protein synthesis (Russell et al., 1990).

Although not as thoroughly investigated as effects on calcium transport, $1,25\text{-(OH)}_2\text{D}$ also stimulates phosphate translocation in intestine (Marx et al., 1983; Karsenty et al., 1985; Cross and Peterlik, 1988), kidney (Kumar, 1986; Kurnik et al., 1987). This phenomena has been briefly investigated in two non-classical systems in which $1,25\text{-(OH)}_2\text{D}$ has been shown to increase phosphate uptake in skeletal muscle and cultured myoblasts and in seminiferous tubules (Eliaschewitz et al., 1982; Teresita and Ricardo, 1991). However, in cultured chick embryonic myoblasts the effect of $1,25\text{-(OH)}_2\text{D}$ involved the Na^+ -linked phosphate uptake component and required protein and RNA synthesis (Teresita and Ricardo, 1991).

Several reports indicate that $1,25\text{-(OH)}_2\text{D}$ markedly increases the levels of one of several vitamin D regulated CaBPs in its classical tissues (Walters, 1992). The CaBP-Ds thus induced include the CaBP- $\text{D}_{28\text{k}}$ in kidney and in chicken intestine (Heizmann and Hunziker, 1990; Gross and Kumar, 1990), CaBP- $\text{D}_{9\text{k}}$ in mammalian intestine (Christakos et al., 1989; Gross and Kumar, 1990), a paralbumin-like CaBP- $\text{D}_{12\text{k}}$ in mammalian skin (Schelling et al., 1987), now termed epidermal CaBP (Rizk-Rabin and Pavlovitch, 1988), the 6 kDa vitamin K-dependent bone gla protein known as osteocalcin (Walters,

1992), and a 20 kDa integral membrane protein found initially in rat intestinal brush border (Kowarski *et al.*, 1987).

Because of the heterogeneity of known CaBP-Ds and the lack of vitamin D-dependent CaBPs in some 1,25-(OH)₂D target tissues there is not an obligatory relationship between 1,25-(OH)₂D effects and CaBP induction (Walters *et al.*, 1991). Although, vitamin D-induced CaBPs may not be present in all 1,25-(OH)₂D targets, vitamin D related CaBPs are present in numerous tissues. Their distribution does not necessarily correlate with the presence of VDRs, and they are not always vitamin D regulated.

In fact, numerous factors such as age and development, Ca²⁺ and phosphate levels, sex steroids and glucocorticoids are now known to participate in CaBP-D regulation (Varghese *et al.*, 1988). Moreover, Christakos *et al.* (1989), reviewed that levels of the CaBPs are altered in several non-vitamin D related disease states.

Thus, taken collectively, these results further support the concept that these interesting proteins may serve functions other than simply mediating vitamin D effects. However, several investigators have noted the close relationship between CaBP_{D28K} and intestinal Ca²⁺ transport and further concluded that CaBP_{D28K} and CaBP_{D9K} play a role in 1,25-(OH)₂D-induced transport and/or a role in buffering intracellular calcium pools during these events (Feher, 1983;

Bronner and Stein, 1988; Bronner, 1989 and 1990).

In the intestine, $1,25\text{-(OH)}_2\text{D}$ stimulation of calcium translocation involves, in part, stimulation of the epithelial cell Ca-Mg-ATPase (Ghijsen and Van Os, 1982; Zelinski et al., 1991). Although the enhanced activity may be due in part to binding and activation by the CaBP-Ds, recent evidence suggests a role for $1,25\text{-(OH)}_2\text{D}$ in regulating mRNA levels for this protein (Zelinski et al., 1991). Moreover, there seem to be correlations between the cellular locations and magnitude of activation of Ca-ATPase and the site and induction of Ca transport in intestine and kidney (Gross and Kumar, 1990).

Although this response has not yet received a great deal of attention in nontraditional targets, $1,25\text{-(OH)}_2\text{D}$ has been shown to increase Ca ATPase activity in isolated cartilage cells (Lidor and Edelstein, 1987), and in vascular smooth muscle cells (Kawashima, 1988).

Alkaline phosphatase activity consists of at least three glycoprotein isozymes predominantly located in plasma membranes of enterocytes, nephron and osteoblasts (McKenna et al., 1979). The activity of these enzymes is thought to correlate with calcium and phosphate metabolism and their levels are considered to reflect osteoblast activity and bone turnover (Duda et al., 1988; Nagata et al., 1989; Fukayama and Tashjian, 1990).

In addition to the description of possible interactions

between the CaBP-Ds and alkaline phosphatases, $1,25-(\text{OH})_2\text{D}$ treatment has been shown to increase alkaline phosphatase activity in intestinal epithelial cells and osteoblasts (Leathers and Norman, 1985). However, blockade of alkaline phosphatase response does not abolish the response of the intestinal brush border calcium transport to $1,25-(\text{OH})_2\text{D}$ (Gross and Kumar, 1990).

Alkaline phosphatase regulation by $1,25-(\text{OH})_2\text{D}$ has been reported in colonic carcinoma cells (Giuliano and Wood, 1991), chondrocytes (Schwartz et al., 1988), breast cancer cells (Mulkins and Sussman, 1987), and uterine endometrial cells (Lieberherr et al., 1984). However, studies in the breast cancer cells demonstrated that treatment with $1,25-(\text{OH})_2\text{D}$ increased the levels of the alkaline phosphatase isozymes found in normal breast tissue (the bone, liver, kidney form) but decreased the activity of the embryonic isozyme (Mulkins and Sussman, 1987).

The other important biological mechanism of $1,25-(\text{OH})_2\text{D}$ is the control of its own receptor (Reinhardt and Horst, 1989; Goff et al., 1990). Binding of the $1,25-(\text{OH})_2\text{D}$ -VDR complex to DNA regulatory sites results in control of transcription of various gene products (Minghetti and Norman, 1988; Goff et al., 1990) and regulation of VDR number is an important mechanism for modulating cellular responsiveness to $1,25-(\text{OH})_2\text{D}$ as the biological activity of $1,25-(\text{OH})_2\text{D}$ in cells has

shown to be proportional to cell VDR number (Dokoh et al., 1984; Chen et al., 1986; Reinhardt and Horst, 1989; Goff et al., 1990).

Several hormones (retinoic acid, glucocorticoid and estrogen), various physiological states (age, pregnancy and lactation), and dietary calcium restriction have been shown to alter tissue VDR number, thus, modulating tissue responsiveness to $1,25-(OH)_2D$ (Reinhardt and Horst, 1989; Goff et al., 1990). In addition to stimulating the synthesis of VDR, $1,25-(OH)_2D$ also stimulates the synthesis of enzymes responsible for $1,25-(OH)_2D$ inactivation (Reinhardt and Horst, 1989). Thus, report by Reinhardt and Horst (1989) indicates that self-induced target tissue deactivation of $1,25-(OH)_2D$ regulates $1,25-(OH)_2D$ occupancy of VDR and ultimately the biopotency of $1,25-(OH)_2D$ in target cells. However, Goff et al. (1990) reported that under physiological conditions, increased plasma concentrations of $1,25-(OH)_2D$ do not result in up-regulation of tissue VDR concentration, and that dietary calcium restriction must induce some factor(s) that results in down regulation of VDRs in the kidney.

In addition to induction of 24-OHase, there are reports of $1,25-(OH)_2D$ inhibition of 1α -OHase in several tissues (Walters, 1992). It has been proposed that degradative enzymes such as vitamin D_{23} and D_{24} hydroxylases may be important regulators of cellular responsiveness to $1,25-(OH)_2D$ because

of their ability to metabolize 1,25-(OH)₂D to inactive forms (Napoli and Horst, 1983; Reinhardt and Horst, 1989).

The therapeutic potential of vitamin D analogs

The actions of the hormonal form of vitamin D extend beyond its role in mineral homeostasis (Brown et al., 1994). Receptor for this hormone has been identified in dozens of different target cells that respond to 1,25-(OH)₂D with a diverse range of biological actions including effects on cell differentiation and proliferation and the control of other hormonal systems (Walters, 1992). However, a major limitation of 1,25-(OH)₂D therapy is that the effective doses for many of these applications cause hypercalcemia (Brown et al., 1994). Hence, this has precluded the use of 1,25-(OH)₂D for treatment of malignancies and limited the use of vitamin D analog for psoriasis and HPT (Brown et al., 1994).

However, analogs of vitamin D that are less calcemic, but retain the therapeutically useful properties of 1,25-(OH)₂D, may provide a wider application of vitamin D therapy (Kubrusly et al. 1993). Thus, many vitamin D analogs with therapeutic potential have been developed in recent years (Brown et al., 1990; Bikle, 1992). Analog with low calcemic activity represent the latest generation of clinically useful vitamin D compounds (Brown et al., 1994).

Theoretically a vitamin D analog that is able to suppress

PTH without effects on plasma calcium and phosphate levels would be an ideal tool to control SHPT (Slatopolsky et al., 1984; Kubrusly et al., 1993; Liou et al., 1994; Lee et al., 1994).

As reviewed by Brown et al. (1994), 1,25-(OH)₂D₃ administration regulates PTH by several mechanisms. It can directly inhibit synthesis at the level of transcription and up-regulates the VDR in parathyroid cell to enhance its own activity. There is substantial evidence that treatment with 1,25-(OH)₂D₃ in chronic renal disease may result in severe hypercalcemia and consequent suppression of PTH secretion (Slatopolsky et al., 1984). Furthermore, it can mediate the suppressive action of calcium by raising serum calcium and by increasing the sensitivity of the PTGs to calcium (Brown et al., 1994). Thus, 1,25-(OH)₂D₃ is widely used for the treatment of SHPT in patients with advanced renal failure in the United States (Brown et al., 1994).

Although a slight elevation in serum calcium by 1,25-(OH)₂D₃ can be beneficial in correcting the chronic hypocalcemia, the reduced capacity to eliminate excess calcium into the urine makes these patients very susceptible to hypercalcemia with 1,25-(OH)₂D₃ therapy (Brown et al., 1994). Furthermore, while there is increasing evidence that treatment of patients with less advanced renal failure may prevent the hyperplasia of the parathyroid glands there is a concern that

hypercalcemia may promote nephrocalcinosis and accelerate the loss of renal function which ultimately prevents the use of $1,25-(\text{OH})_2\text{D}_3$ in early stages of renal disease (Brown et al., 1994).

Vitamin D analog with lower calcemic activity that can suppress PTH may provide a safer alternative to $1,25-(\text{OH})_2\text{D}_3$ therapy in patients with moderate renal failure (Brown et al., 1994). However, another complication in these patients is hyperphosphatemia. High serum phosphate decreases serum ionized calcium and inhibit production of $1,25-(\text{OH})_2\text{D}_3$, the two important factors in controlling PTH.

In addition, dietary phosphate restriction, independent of changes in calcium and $1,25-(\text{OH})_2\text{D}_3$ can reduce PTH levels (Lopez-Hilker et al., 1990). Thus, the ideal vitamin D analog would also have low phosphatemic activities as well (Brown et al., 1994).

Several of the vitamin D analogs with low calcemic activity have been found to be nearly as effective as $1,25-(\text{OH})_2\text{D}_3$ in suppressing PTH secretion by cultured parathyroid cells. These include 22-oxa- $1,25-(\text{OH})_2\text{D}_3$ (OCT) (Evans et al. 1991; Kubrusly et al., 1993), $1,25-(\text{OH})_2-16\text{-ene-23-yne-D}_3$, $1,25-(\text{OH})_2-24\text{-dihomo-D}_3$ and $1,25-(\text{OH})_2-24\text{-trihomo-22-ene-D}_3$ (Brown et al., 1994) and $1,25,28-(\text{OH})_3\text{D}_2$ (Goff et al., 1993).

Brown et al. (1989) reported that OCT, despite its rapid clearance in vivo, could suppress PTH mRNA and low, submaximal

doses of OCT and $1,25\text{-(OH)}_2\text{D}_3$ produced comparable inhibition. Furthermore, OCT has also been shown to suppress serum PTH levels in uremic rats and dogs (Fukagawa et al., 1991).

However, in every case the $1,25\text{-(OH)}_2\text{D}_3$ -treated animals had elevated calcium that could account for the lower PTH or PTH mRNA. Just as important as its low calcemic effects, OCT was found to be less phosphatemic than $1,25\text{-(OH)}_2\text{D}_3$ with lower activity on intestinal phosphate absorption and bone phosphate mobilization (Finch et al., 1993). Thus, OCT appears to offer major therapeutic advantages over $1,25\text{-(OH)}_2\text{D}_3$ in the treatment of SHPT in renal failure patients (Brown et al., 1994).

Administration of vitamin D analogs

Several reports have indicated that $1,25\text{-(OH)}_2\text{D}_3$ administered intravenously rather than orally may result in a greater delivery of the vitamin D metabolite to peripheral target tissues other than the intestine and allow a greater expression of biological effects of $1,25\text{-(OH)}_2\text{D}_3$ in peripheral tissues (Slatopolsky et al., 1984; Liou et al., 1994; Lee et al., 1994).

However, recent studies have shown substantial degradation of $1,25\text{-(OH)}_2\text{D}_3$ in the intestine. Hence, Napoli et al. (1983) and Slatopolsky et al. (1984) reasoned that while oral administration of $1,25\text{-(OH)}_2\text{D}_3$ may increase intestinal calcium absorption, the degradation of the vitamin D metabolite may

impair the delivery of $1,25-(OH)_2D_3$ to other peripheral target organs and thus limit the expression of direct biological effects.

Furthermore, Liou et al. (1994) have compared the effect of oral versus intravenous calcitriol administration on SHPT in chronic hemodialysis patients. They concluded that both intravenous and oral treatments resulted in a significant decrement in blood levels of PTH. However, this PTH suppressive effect was more pronounced with intravenous therapy and could not be totally explained by either the higher dose or elevated serum calcium.

A comparison of oral and intravenous alfacalcidol in treatment of uremic HPT by Lee et al. (1994) showed that both routes were equally effective in suppressing HPT but they suggested that further controlled studies to find out the advantages of intravenous over oral vitamin D therapy.

Calcium and Phosphate Homeostasis

Maintenance of calcium homeostasis

The integrated actions of PTH on distal convoluted tubular calcium reabsorption, bone resorption, and $1,25-(OH)_2D_3$ -mediated intestinal calcium absorption are responsible for the fine regulation of the serum Ca^{2+} concentration (Broadus, 1993).

Distal convoluted tubular calcium reabsorption and osteoclastic bone resorption are the major control points in minute to minute serum calcium homeostasis; of these two processes, the effect of PTH on the distal convoluted tubule is quantitatively the most important. Therefore, together, these effects constitute a classical "short-loop" feedback system on the parathyroid chief cell.

Adjustment in the rate of intestinal calcium absorption via the PTH-1,25-(OH)₂D axis represents a classical "long-loop" feedback system (Broadus, 1993).

During hypocalcemia, a series of physiological adjustments occur, leading to new steady state. A moderate increase in the secretion rate of PTH results in: 1) increased calcium reabsorption from the distal convoluted tubule; 2) increased mobilization of calcium and phosphorus from bone; and 3) increased synthesis of 1,25-(OH)₂D, which participates with PTH in bone resorption and increases the efficiency of calcium and phosphorus absorption in the intestine (Broadus, 1993).

The increased circulating concentration of PTH resets the renal tubular phosphate threshold/glomerular filtration rate (TmP_i/GFR) at a lower level, so that the increased amount of phosphorus mobilized from bone and absorbed from the intestine is quantitatively excreted into urine. Thus, in the new steady state, serum calcium has returned to normal, serum phosphorus is unchanged or slightly reduced and a state of mild secondary

HPT and efficient intestinal mineral absorption exists and the initial requirement for calcium mobilization from the skeleton is largely replaced by the enhanced absorption of calcium in the intestine (Broadus, 1993).

The systemic mechanism for the prevention of hypercalcemia involves an inhibition of PTH and $1,25\text{-(OH)}_2\text{D}$ synthesis, with a reduction in calcium mobilization from bone, absorption from the intestine and reclamation from the distal convoluted renal tubule. However, the bottleneck in the defense against hypercalcemia is the limited capacity of the kidney to excrete calcium.

Limitations in the theoretical ability of the kidney to combat hypercalcemia include: 1) the fact that abnormalities in distal convoluted tubular reabsorption are actually in the genesis of hypercalcemia in a number of conditions (e.g., primary HPT); 2) the fact that a degree of renal impairment frequently accompanies many hypercalcemic conditions; and 3) the fact that an increased calcium concentration inhibit the ability of the renal tubule to conserve water, which may lead to a vicious cycle of dehydration, prerenal azotemia and worsening hypercalcemia (Broadus, 1993).

Maintenance of phosphate homeostasis

The kidney plays the dominant role in systemic phosphate homeostasis and maintains the serum phosphorus concentration

at a value close to the TmP_i/GFR .

However, because of the normal efficiency and lack of fine regulation of phosphorus absorption in the intestine, only in unusual circumstances (e.g., prolonged use of phosphate-binding antiacids) is the systemic supply of phosphorus a limiting factor in phosphate homeostasis. Thus, most disorders associated with chronic hypophosphatemia and/or phosphorus depletion in humans result from either intrinsic (e.g., familial hypophosphatemic rickets) or extrinsic (e.g., primary HPT) alterations in TmP_i/GFR . Similarly, most conditions of chronic hyperphosphatemia result from intrinsic (e.g., renal impairment or HPT) abnormalities in the renal threshold for phosphorus. However, acute hypophosphatemia most commonly results from the flux of extracellular phosphate ions into soft tissues.

The sequence of events initiated in the face of a hypophosphatemic challenge include: 1) stimulation of 1,25-(OH)₂D synthesis in the kidney; 2) enhanced mobilization of phosphorus and calcium from bone; and 3) hypophosphatemia induced increase in TmP_i/GFR (the exact mechanism is unknown).

The increased circulating concentration of 1,25-(OH)₂D leads to increases in phosphorus and calcium absorption in the intestine and provides an additional stimulus to phosphorus and calcium from bone. The increased flow of calcium from bone and the intestine results in an inhibition of PTH secretion

which diverts the systemic flow of calcium into the urine and further increases TmP_i/GFR . Thus, net result of this sequence of adjustment is a return of the serum phosphorus concentration to normal without change in the serum calcium concentration (Broadus, 1993).

The defense against hyperphosphatemia consists largely of a reversal of the sequence of adjustments described above. The principal humoral factor that combats hyperphosphatemia is PTH. An acute rise in the serum phosphorus concentration produces a transient fall in the concentration of serum ionized calcium and a stimulation of PTH secretion, which reduces TmP_i/GFR and leads to a readjustment in serum phosphorus and calcium concentrations. Hence, a prolonged rise in the serum phosphorus concentration results in: 1) an intrinsic downward adjustment in TmP_i/GFR that is independent of PTH; and 2) a persistent increase in PTH secretion that can ultimately lead to chief cell hyperplasia. If hyperphosphatemia is prolonged and severe (e.g., as occurs in chronic renal insufficiency), the degree of SHPT is sufficient to lead to the typical finding of parathyroid bone disease.

Hyperparathyroidism

All HPT disorders are characterized by elevated circulating levels of PTH that arise from augmented PTH

production/secretion per cell and/or from an expanded number of functioning PTH producing cells (Galbraith and Quarles, 1993).

However, the variable underlying pathophysiology of increased PTH secretion permits subclassification of HPT into primary and secondary disorders.

Primary HPT is characterized functionally by an abnormal synthesis and secretion of PTH in excess of calcium homeostatic needs and morphologically by adenomatous transformation or diffuse hyperplasia of PTH secreting cells. Furthermore, it is pathophysiology associated to the loss of normal feedback control of PTH secretion by extracellular calcium (Bilezikian, 1993).

SHPT, in contrast, is an an acquired disorder representing a normal physiological response to perturbations in calcium metabolism (Galbraith and Quarles, 1993). However, tertiary HPT is a term used to describe patients with sustained SHPT who develop elevated levels of serum calcium, whereas the term refractory SHPT defines a second subset of patients with severe SHPT without hypercalcemia that displays non-suppressible PTH secretion after correcting the inciting metabolic abnormalities. Thus, in both tertiary and refractory HPT, the PTG has reached a hyperfunctioning state that no longer responds appropriately to physiological regulation (Galbraith and Quarles, 1993).

Secondary Hyperparathyroidism and Renal Insufficiency

SHPT is a universal complication of chronic renal insufficiency frequently found in patients with end-stage renal disease and is an important cause of morbidity in patients on maintenance dialysis (Massry et al., 1980; Slatopolsky et al., 1984; Massry, 1986; Lee et al., 1994; Liou et al., 1994).

PTH acts upon the proximal convoluted cell to increase the production of $1,25-(OH)_2D_3$ (Garabedian et al., 1972; Slatopolsky et al., 1984; Johnson and Kumar, 1994), which in turn increases intestinal calcium absorption. Therefore, it seems possible that a negative feedback system could operate to regulate PTH secretion (Slatopolsky et al. 1984). In severe renal insufficiency the lack of $1,25-(OH)_2D_3$ could thus be a factor in maintaining the hypersecretion of PTH (Slatopolsky et al., 1984; Liou et al., 1994; Johnson and Kumar, 1994). Thus, the principal cause of HPT is loss of activity of the renal 1α -OHase enzyme and impaired production of $1,25-(OH)_2D_3$ from its precursor 25-OHD (Kubrusly et al., 1993; Lee et al., 1994).

Therefore, chief cell hyperplasia of the PTG and high levels of PTH are among the earliest alterations in mineral metabolism observed in patients with chronic renal

insufficiency (Slatopolsky *et al.*, 1992).

An important mechanism for the increased PTH levels in chronic renal failure may be a shift in the set-point (at which 50% maximum PTH secretion is achieved) for calcium regulated PTH secretion in addition to an increase in the mass of parathyroid tissue. It has been indicated that the set-point for calcium in normal parathyroid cells is approximately 1.0 mM calcium, whereas in patients with SHPT, the set-point is increased to 1.26 mM calcium (Brown *et al.*, 1978 and 1982). In addition to the effect of 1,25-(OH)₂D₃ and serum calcium on the regulation of PTH secretion, phosphorus retention in chronic renal failure has shown to affect the degree of SHPT (Slatopolsky *et al.*, 1992).

Reports by Portale *et al.* (1984); Llach and Massry (1985) and Portale *et al.* (1986) indicated that restriction of dietary phosphorus increases the production of 1,25-(OH)₂D₃, thus, decreasing the level of immunoreactive PTH (iPTH). However, this mechanism may not be operative in advanced renal insufficiency because the decrease in renal mass may severely limit the production of 1,25-(OH)₂D₃.

Studies in dogs by Lopez-Hilker *et al.* (1990) suggested that reduction in dietary phosphate in advanced renal insufficiency improves SHPT by a mechanism which is independent of the level of 1,25-(OH)₂D₃ or serum ionized calcium. Several reports have provided evidence for reduced

plasma concentration of $1,25\text{-(OH)}_2\text{D}_3$ in mild or moderate chronic renal failure. This low plasma levels could be interpreted merely as the consequence of the reduced mass of renal tubular cells capable of producing $1,25\text{-(OH)}_2\text{D}_3$.

However, experimental and clinical data cast serious doubt on this simple explanation. Rather, a reduced circulating level of $1,25\text{-(OH)}_2\text{D}_3$ in early chronic renal failure might represent an adaptive response to a primary disturbance in Phosphate homeostasis, the exact nature of which has not yet been appreciated (Bonjour et al., 1992).

For many years, PTH has been considered to be the main regulator of plasma Phosphate homeostasis, principally because of its action on renal Phosphate reabsorption. However, PTH cannot function as the major regulator of Phosphate homeostasis, since the secretion of PTH is primarily controlled tightly by the Phosphate status itself (Bonjour and Fleisch, 1980).

Reports by Bonjour et al. (1982a) indicated that there are two very specific PTH-independent mechanisms that rapidly respond to variations in Phosphate status in such a way that they tend to maintain Phosphate homeostasis despite fluctuations either in the supply of dietary Phosphate or in its utilization for bone mineralization or cellular metabolism. Bonjour et al. (1992) indicated that these two mechanisms are the production of $1,25\text{-(OH)}_2\text{D}_3$ and the capacity

of renal tubule to respond to changes in Phosphate status with changes in Phosphate transport. Further, Bonjour et al. (1982b) and Insogna et al. (1983) indicated that both the renal production of $1,25\text{-(OH)}_2\text{D}_3$ and the capacity of tubular reabsorption are rapidly stimulated in response to a restriction in supply of Phosphate. These two systems are fully effective even in the absence of PTH. However, Bonjour et al. (1982a and b) reported that increased production of $1,25\text{-(OH)}_2\text{D}_3$, in response to Phosphate deprivation, does not stimulate tubular Phosphate transport under vitamin D-replete conditions. Similarly, there is no direct evidence indicating that the stimulation of tubular Phosphate transport during restriction is responsible for enhanced production of $1,25\text{-(OH)}_2\text{D}_3$ (Bonjour et al., 1992).

To understand Phosphate homeostasis in early chronic renal failure, it is also important to bear in mind that the concentration of extracellular Phosphate can be set at different levels according to needs of the organism, with Phosphate levels being mainly determined by the tubular reabsorption of Phosphate (Bonjour et al., 1992). Thus, when Phosphate needs are diminished, as, for instance, when there is a reduction in body growth and/or bone mineralization, $T_m\text{P}_i/\text{GFR}$, and also $1,25\text{-(OH)}_2\text{D}_3$ production will be set at a lower level. The reduced $T_m\text{P}_i/\text{GFR}$ and $1,25\text{-(OH)}_2\text{D}_3$ would lead to a fall in plasma Phosphate concentration and could be

considered, therefore, as physiologically appropriate for Phosphate homeostasis (Bonjour et al., 1992). This notion implies that the level of extracellular Phosphate itself is not the ultimate variable that the homeostatic system attempts to maintain constant, but rather the balance between the supply and utilization of Phosphate. This putative pool is most likely an intracellular one, but it is neither necessarily nor exclusively within the renal tubular cells endowed with the $1,25-(OH)_2D_3$ producing machinery (Bonjour et al., 1992). According to this concept, the two controlling elements of Phosphate homeostasis, namely, the adaptive tubular Phosphate transport and the $1,25-(OH)_2D_3$ producing system, would act in concert to maintain a constant critical intracellular Phosphate pool. Reports indicated further that these two renal controlling mechanisms would be physiologically linked to this "internally controlled pool" of Phosphate by a single specific signal which probably corresponds to a still unknown hormonal factor (Bonjour et al., 1992).

Along the notion of Phosphate homeostasis, it is important to consider the consequence of reduced renal mass on the plasma levels of $1,25-(OH)_2D_3$ before examining the situation in early chronic renal failure. However, in experimental studies, it has been shown that the plasma levels of $1,25-(OH)_2D_3$ do not fall significantly within the first few hours of days

following unilateral nephrectomy in the rat (Taylor et al., 1983).

Similarly, in healthy human kidney donors, the remaining kidney appears to compensate very rapidly to a 50% reduction in the renal mass (Lucas et al., 1986). Furthermore, studies in unilaterally nephrectomized rats have shown that the remaining kidney has a marked capacity to increase its production of $1,25-(OH)_2D_3$ in response to a reduction in the dietary intake of calcium (Taylor et al., 1983).

It is interesting to compare the pathophysiologic sequence with that proposed many years ago by Slatopolsky et al. (1966 and 1968) from which the very attractive "trade-off" hypothesis was developed to explain the reactive HPT observed in chronic renal failure (Bricker, 1972). Although, these two sequences differ in several aspects, they have in common one very important element: both theories consider a disturbance in Phosphate homeostasis, more precisely a Phosphate overload, as the primary alteration occurring in early chronic renal failure (Bonjour et al., 1992).

However, according to the earlier theory (Slatopolsky et al. 1966 and 1968; Bricker, 1972), Phosphate overload would be mainly in the extracellular compartment. The loss of nephrons and the consequent fall in GFR would result in transient increase in plasma Phosphate that would be rapidly corrected by an increase in PTH secretion. This in turn, would lead to a

reduction in T_mP_i/GFR . Thus, the first trade-off operation for maintaining Phosphate homeostasis would comprise the hypersecretion of PTH and the reduced renal mass and possibly the hyperphosphatemia would account for the insufficient production of $1,25-(OH)_2D_3$.

However, despite its attractiveness, there are several difficulties with this pathophysiologic concept: 1) plasma Phosphate does not increase in early or moderate chronic renal failure, but rather tends to be set at levels lower than normal (Wilson et al., 1985; Portale et al. 1984); 2) PTH is not essential for the reduction in T_mP_i/GFR observed in the course of chronic renal failure (Caverzasio et al., 1982); and 3) $1,25-(OH)_2D_3$ production cannot be considered as insufficient or defective in early chronic renal failure. Indeed, it is not maximal at normal intake of Phosphate since it can be further stimulated by a low diet (Portale et al., 1984).

According to the new theory developed, the overload of Phosphate would affect the intracellular rather than the extracellular compartment. The first consequence of this overload would be a compensatory reduction in $1,25-(OH)_2D_3$ production and T_mP_i/GFR . Thus, instead of PTH, the first element of the homeostatic system trade-off would be $1,25-(OH)_2D_3$, its reduced production counteracting the Phosphate overload (Bonjour et al., 1992). HPT would in this view be only a consequence of the low production of $1,25-(OH)_2D_3$ that

could lead to a lower plasma calcium, changes in the set-point of the PTH secretory process, and a reduction in the calcemic response to PTH (Llach *et al.*, 1975; Massry, 1985a; Lopez-Hilker *et al.*, 1986; Delmez *et al.*, 1989; Rodriguez *et al.*, 1991a and b). Feinfeld and Sherwood (1988) have also independently suggested that the decrease in $1,25-(\text{OH})_2\text{D}_3$ observed in early chronic renal failure was a consequence of a primary disturbance in Phosphate homeostasis.

In summary, PTH is a polypeptide hormone of 84 amino acids which utilizes the first 1-34 amino acids for its biological actions. Its signal transduction pathways are either cAMP or IP_3 . PTH maintains the extracellular calcium via bone resorption and by stimulating the activities of $1\alpha\text{-OHase}$ in the proximal convoluted tubule of the kidney. It has been suggested that the major regulators of PTH synthesis and secretion are Ca^{2+} and $1,25-(\text{OH})_2\text{D}$.

The vitamin D precursors are either obtained from the diet or synthesized in the skin. These are processed in the intestine, metabolized in the liver and finally the biologically active form, $1,25-(\text{OH})_2\text{D}$, is synthesized in the kidney. On top of many biological functions, $1,25-(\text{OH})_2\text{D}$ is primarily involved in the absorption of calcium and phosphate by stimulating the synthesis of CaBP and Ca-ATPase proteins in the intestinal cells.

The homeostasis of calcium and phosphate are maintained

primarily by the concerted actions of PTH and $1,25-(OH)_2D$. However, during renal failure, the production of $1,25-(OH)_2D$ is lowered due to reduced mass of renal tissue. On the other hand, high phosphorus diet leads to hyperphosphatemia and eventually inhibit 1α -OHase and raise plasma PTH. Low $1,25-(OH)_2D_3$ and hyperphosphatemia are among the features of SHPT.

Several reports indicated that phosphorus restriction and the use of some of the vitamin D analogs as a treatment might be the therapeutic alternative to lower excessive PTH levels and consequently restoring calcium and phosphate homeostases.

Therefore, the use of some of the vitamin D analogs with therapeutic potential in combating SHPT would be a choice provided that the effective doses of each one of these analogs are worked out. However, attention must be given to the level of dietary phosphorus whenever such therapies would be taken.

MATERIALS AND METHODS

Animals

Protocols for the use of animals were approved by the Institutional Animal Care and Use Committee of the USDA, Agricultural Research Service, National Animal Disease Center (NADC). One hundred twelve male Harlan Sprague-Dawley rats that were 60-day-old and on an average of 400 ± 50 gm body weight were used in this study.

All rats were housed individually under fluorescent lighting on a 12 hour on, 12 hour off lighting cycle during the entire study period (Lieualen et al., 1990).

Diets and Feeding

Upon arrival all rats were fed a standard laboratory rat chow (Diet C in this study) containing 1.01% calcium, 0.74% phosphorus, and 3.3 IU/gm vitamin D. All rats were fed this diet for two weeks of adaptation.

At the end of the two weeks period, that is at the beginning of experimentation, all rats were fed the experimental diets.

Two synthetic diets, namely Diet A and B containing low phosphorus (LP) and high phosphorus (HP) levels, respectively,

were purchased from Harlan Teklad, Madison, Wisconsin. The Ca, P, and vitamin D contents of all the diets (on an as fed basis) used in this study were as follows:

Diet	Ca	P	Na	K	Vitamin D
	(%)	(%)	(%)	(%)	(IU/gm)
A	0.48	0.40	0.20	0.90	5.0
B	0.35	3.22	1.32	2.00	5.0
C	1.00	0.74	0.28	1.08	3.3

In order to avoid overeating of the diets particularly by the sham groups, a fixed amount of the diets was weighed and offered every day until the end of experiment. Following unilateral nephrectomy, the rats were maintained on standard laboratory rat chow for 5 days for recovery. The feeding period was 30 days and water was offered ad libitum.

Body Weight Measurements

Body weight measurements (gm) were made for each rat at the beginning and at the end of the experiment. Body weight changes (%) were calculated from the differences between the beginning and end body weights expressed as a percentage.

Design of the Study

At the end of the two weeks period of adaptation, all rats were randomly picked and divided into 7 groups.

Group	Treatment	Diet	Vit. D Analog
SLP	Sham	Low P	----
SHP	Sham	High P	----
NLP	Nephrectomy	Low P	----
NHP	Nephrectomy	High P	----
NHP-A	Nephrectomy	High P	1,25-(OH) ₂ D ₃
NHP-B	Nephrectomy	High P	1,25-(OH) ₂ D ₂
NHP-C	Nephrectomy	High P	1,25,28-(OH) ₃ D ₂

Nephrectomy

The unilateral nephrectomy in this study was done as follows: Each rat was anesthetized with intraperitoneal injections of 60 mg/kg Ketamine and shaved ventro-dorsally and the entire left kidney was removed via a left paracostal approach (Lieuallen et al., 1990).

An incision was made into the abdominal cavity, down the side of the rat near to the last rib. The kidney was freed of connective tissue and was pulled out gently, preferably by grasping the peripheral fat. The adrenal gland which was attached loosely to the anterior pole of the kidney by

connective tissue and fat was gently freed by tearing the attachments. Then, a single ligature was placed around the renal blood vessels and the ureters as far from the kidney as possible, towards the midline, but without damaging or occluding any collateral blood vessels that may be encountered. Then, the ligature was tied securely with a double reef-knot and the blood vessels were transected next to the kidney which was removed. Finally, the incision was closed (Waynforth, 1980).

Oral Administration of Vitamin D Analogs

1,25-(OH)₂D₃, 1,25-(OH)₂D₂, and 1,25,28-(OH)₃D₂ (Figure 1) were provided by Dr J.P. Goff and Dr R.L. Horst. The concentration of these analogs was determined by UV absorption with molar extinction coefficient for D₂ analogs being 19600 and for D₃ analogs being 18300. The solutions were mixed with corn oil as carrier after each dose was adjusted to 200 μl. Then they were evaporated to dryness by Nitrogen gas to remove diethylether. The volume for each week administration was put in falcon blue tubes and stored at - 20°C. After each use, the remainder of the mixture was always maintained at - 20°C.

The vitamin D analogs were delivered to each rat by using 1 ml syringe orally every day between 9.00 to 10.00 am until the end of the experiment. The feeding of diets and vitamin D

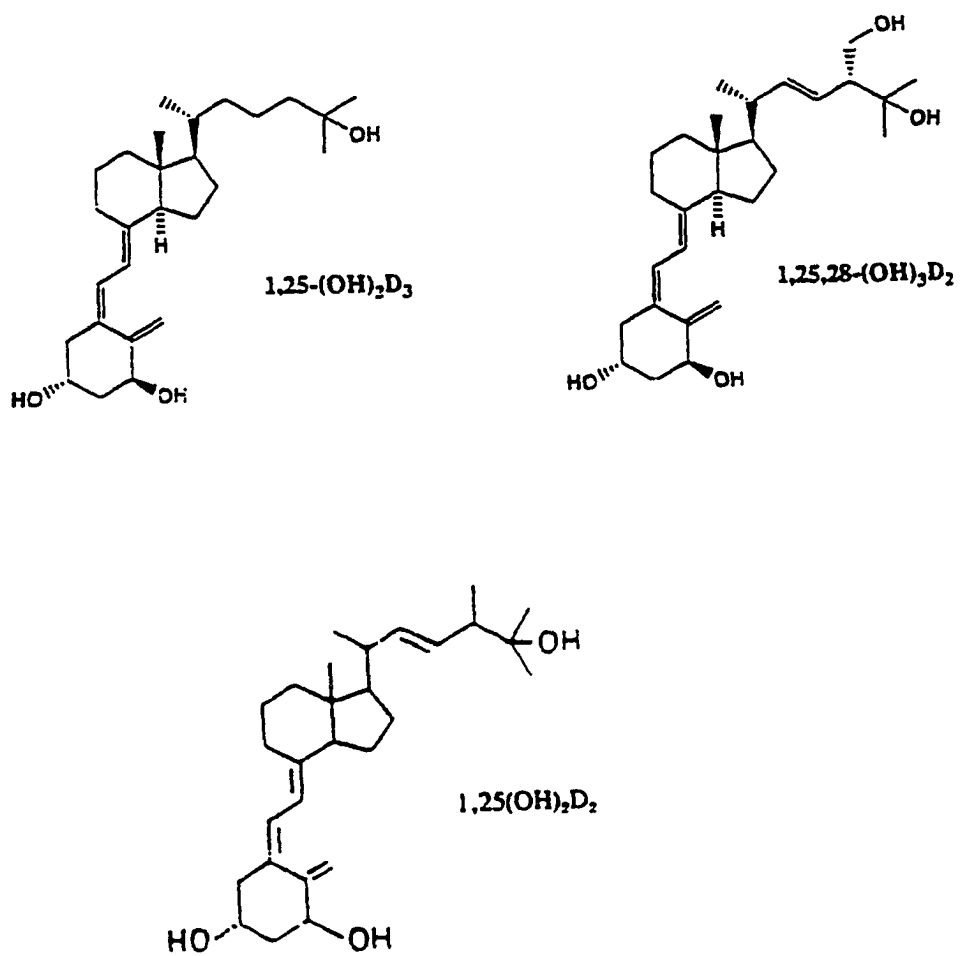


Figure 1. Structure of vitamin D analogs used in this study
(Adapted from Goff *et al.*, 1993, and Norman, 1979).

analog administration began the same day. The dose of each analog is as follows:

Vitamin D analog	Corn oil (μ l)	Dose/day
1,25-(OH) ₂ D ₃	200	18 ng
1,25-(OH) ₂ D ₂	200	18 ng
1,25,28-(OH) ₃ D ₂	200	2 μ g

Sample Collection and Plasma Analyses

After 30 days of feeding and oral treatment of vitamin D analog, all rats were weighed and rendered unconscious with CO₂-O₂ (50%:50% vol/vol) and decapitated. Blood sample was collected into tubes containing heparin and then it was centrifuged for 5 minutes at 2300 rpm. Plasma was aspirated and stored at 4°C until analysis.

Samples were analyzed for calcium, inorganic phosphorus, 1,25-(OH)₂D₃, PTH, and creatinine. The plasma calcium concentration was determined by atomic absorption spectrophotometry (Perkin-Elmer Corp. 1965). The plasma phosphorus concentration was determined by a colorimetric assay (Parekh and Jung, 1970). The plasma 1,25-(OH)₂D₃ concentration was determined by the method of Reinhardt et al. (1984). Intact PTH was determined by rat PTH immunoassay kit from Nichols Institute Diagnostics (1995) according to the methods of Nussbaum et al. (1987) and Reiss et al. (1968), and

the plasma creatinine was determined by creatinine kit from Sigma Diagnostics (1992) following the method of Chasson et al. (1961).

One-way analysis of variance (ANOVA) was employed to test the hypotheses that: 1) means from sham operated or unilaterally nephrectomized rats were the same; 2) means from rats fed low or high phosphorus diets were not different, and; 3) means from rats treated or not treated with vitamin D analogs were the same. Where the ANOVA were different Tukey-Kramer Multiple Comparisons Test was used to test for differences among the means according to Snedecor and Cochran (1980).

RESULTS

The mean body weight change (%) of eight groups of rats is presented in Table 1. The loss of body weight of SHP was higher ($P < 0.001$) than the SLP rats. SHP rats lost 9.50% of the initial body weight. But SLP rats gained 14.95% of the initial body weight. The mean body weight loss of NHP was higher ($P < 0.001$) than the NLP rats. NHP rats lost 24.72% of their initial body weight whereas NLP rats gained 17.12% of their initial body weight. The loss of body weight of the NHP-A and NHP-C groups was not significantly ($P > 0.05$) different from the NHP rats. But still their body weight losses were lower than the control rats. The body weight loss of NHP-B was significantly ($P < 0.01$) less than the control rats. NHP-A, NHP-B and NHP-C rats lost (%) 20.32 ± 2.03 , 13.87 ± 2.70 and 20.06 ± 2.41 , respectively, of their initial body weights. It is clear that rats on high phosphorus diets lost more body weight as compared than the control rats. But the application of vitamin D analogs were found to be helpful in reducing the losses in vitamin D treated rats. Particularly, the application of $1,25-(OH)_2D_2$ was found to be superior in reducing the body weight losses in NHP-B rats as compared to the other two vitamin D analogs.

Inorder to prove that feeding a high phosphorus diet to patients with renal failure (reduced renal tissue mass)

increases plasma phosphate, four groups of rats SLP, SHP, NLP and NHP were fed low or high phosphorus diets for 30 days (Figure 2). The mean plasma phosphate (mg/dl) of SHP rats was significantly ($P < 0.001$) higher than SLP rats. Similarly, the mean plasma phosphate of NHP rats was significantly ($P < 0.001$) higher than the NLP rats.

Three groups of rats NHP-A, NHP-B and NHP-C were orally administered three different kinds of vitamin D analogs for 30 days. The analog groups were compared to the NHP group. The result (Figure 3) indicates that NHP-B and NHP-C rats treated with $1,25-(\text{OH})_2\text{D}_2$ and $1,25,28-(\text{OH})_3\text{D}_2$, respectively, had significantly ($P < 0.001$) reduced plasma phosphate as compared to the control rats. Although plasma phosphate of NHP-A treated rats was not significantly different from the NHP rats, the treatment of $1,25-(\text{OH})_2\text{D}_3$ lowered plasma phosphate by 5% compared to the NHP rats.

The mean plasma calcium concentration (mg/dl) of SLP, SHP, NLP and NHP were 8.07 ± 0.08 , 6.70 ± 0.09 , 9.14 ± 0.09 and 7.19 ± 0.10 , respectively (Figure 4). The mean plasma calcium of SHP was significantly ($P < 0.001$) lower than SLP rats. Similarly, the mean plasma calcium of NHP rats was significantly ($P < 0.001$) lower than NLP rats.

The mean plasma calcium concentration (mg/dl) of NHP, NHP-A, NHP-B and NHP-C were 7.19 ± 0.10 , 8.29 ± 0.15 , 8.86 ± 0.16 and 8.50 ± 0.15 , respectively (Figure 5). The mean plasma

calcium concentration of NHP-A, NHP-B and NHP-C were significantly ($P < 0.001$) higher than the NHP rats. Therefore, the applications of vitamin D analogs proved to improve the conditions of SHPT by increasing the plasma calcium.

The mean plasma $1,25\text{-(OH)}_2\text{D}_3$ concentration (pg/ml) of SLP, SHP, NLP and NHP were 75.76 ± 3.70 , 50.30 ± 6.33 ; 98.17 ± 4.25 and 22.94 ± 2.51 , respectively (Figure 6). The mean plasma $1,25\text{-(OH)}_2\text{D}_3$ of SHP was significantly ($P < 0.01$) lower than the SLP rats. Similarly, the mean plasma $1,25\text{-(OH)}_2\text{D}_3$ of NHP was significantly ($P < 0.001$) lower than the NLP rats.

The mean plasma $1,25\text{-(OH)}_2\text{D}_3$ concentration (pg/ml) of NHP, NHP-A, NHP-B and NHP-C were 22.94 ± 2.51 , 36.76 ± 2.72 , 76.37 ± 5.59 and 30.52 ± 3.53 , respectively (Figure 7). The mean plasma $1,25\text{-(OH)}_2\text{D}_3$ of NHP-A and NHP-C were not significantly different from NHP rats. However, the mean plasma $1,25\text{-(OH)}_2\text{D}_3$ of NHP-B was significantly ($P < 0.001$) higher than the NHP rats. Thus, it seems likely that high phosphorus diet has inhibited the $1\alpha\text{-OHase}$ enzyme and consequently lowered plasma $1,25\text{-(OH)}_2\text{D}_3$ production.

The mean plasma PTH concentration (pg/ml) of SLP, SHP, NLP and NHP were 41.14 ± 2.15 , 131.20 ± 3.53 , 34.07 ± 1.16 and 267.16 ± 5.31 , respectively (Figure 8). The mean plasma PTH concentration of SHP rats was significantly ($P < 0.001$) higher than the SLP rats. Similarly, the mean plasma PTH of NHP was significantly ($P < 0.001$) higher than the NLP rats.

The mean plasma PTH concentration (pg/ml) of NHP, NHP-A, NHP-B and NHP-C were 267.16 ± 5.31 , 206.79 ± 8.95 , 207.16 ± 8.17 and 324.72 ± 12.84 , respectively (Figure 9). The mean plasma PTH of NHP-A and NHP-B were significantly ($P < 0.001$) lower than the NHP rats. But the mean plasma PTH of NHP-C was significantly ($P < 0.001$) higher than the control rats.

The mean plasma creatinine concentration (mg/dl) of SLP, SHP, NLP and NHP were 1.40 ± 0.03 , 2.21 ± 0.05 , 4.47 ± 0.02 and 4.50 ± 0.08 , respectively (Figure 10). The mean plasma creatinine of SHP was significantly ($P < 0.001$) higher than the SLP rats. However, there was no significant difference between NHP and NLP rats.

The mean plasma creatinine concentration (mg/dl) of NHP, NHP-A, NHP-B and NHP-C were 4.50 ± 0.08 , 6.05 ± 0.03 , 7.26 ± 0.21 and 6.77 ± 0.06 , respectively (Figure 11). The mean plasma creatinine of NHP-A, NHP-B and NHP-C were significantly ($P < 0.001$) higher than the NHP rats.

Table 1. Change in body weight (%) of sham, nephrectomized and nephrectomized and vitamin D analogs treated rats fed either high or low phosphorus diets for 30 days.

Treatments	Body weight change (%)*	P value
SLP	+ 14.95 ± 1.12	
SHP	- 9.50 ± 3.96	P<0.001
NLP	+ 17.12 ± 1.08	
NHP	- 24.72 ± 1.80	P<0.001
NHP-A	- 20.32 ± 2.03	ns
NHP-B	- 13.87 ± 2.70	P<0.01
NHP-C	- 20.06 ± 2.41	ns

+ = gain in body weight.

- = loss in body weight.

* = mean ± SEM (standard error of mean).

ns = not significant.

Figure 2. Mean plasma phosphate (mg/dl) of sham and nephrectomized rats fed either low or high phosphorus diets for a period of 30 days.

a = significantly different from SLP $P < 0.001$.

b = significantly different from NLP $P < 0.001$.

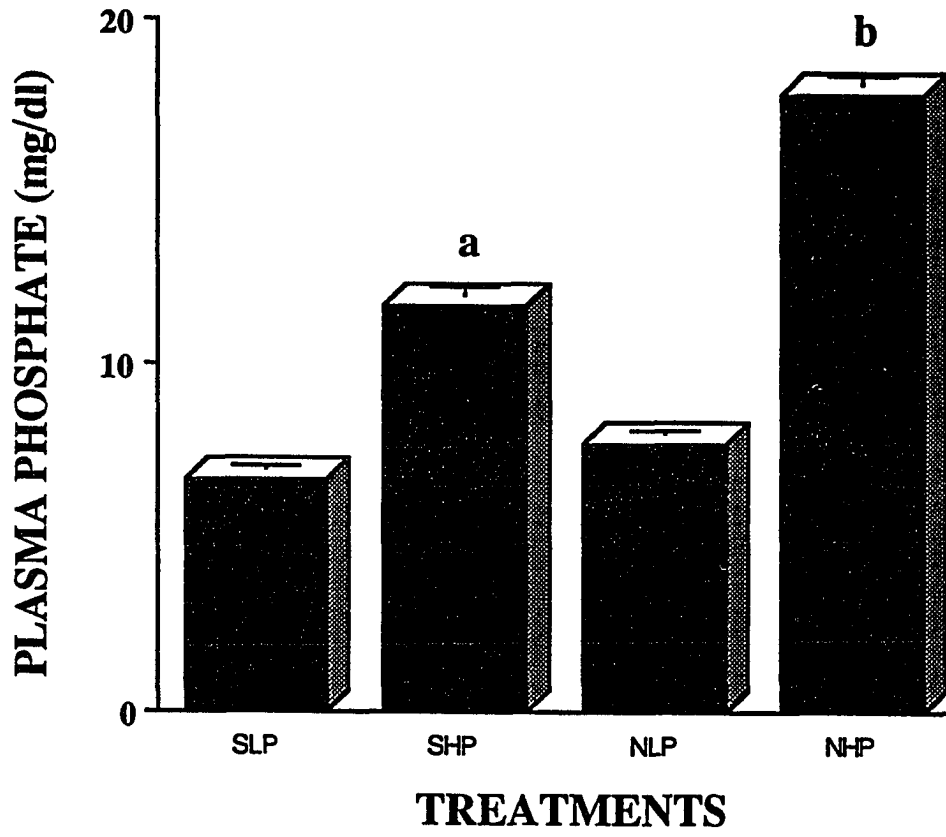


Figure 3. Mean plasma phosphate (mg/dl) of nephrectomized rats fed high phosphorus diets and treated with vitamin D analogs for a period of 30 days.
a = significantly different from NHP $P < 0.001$.

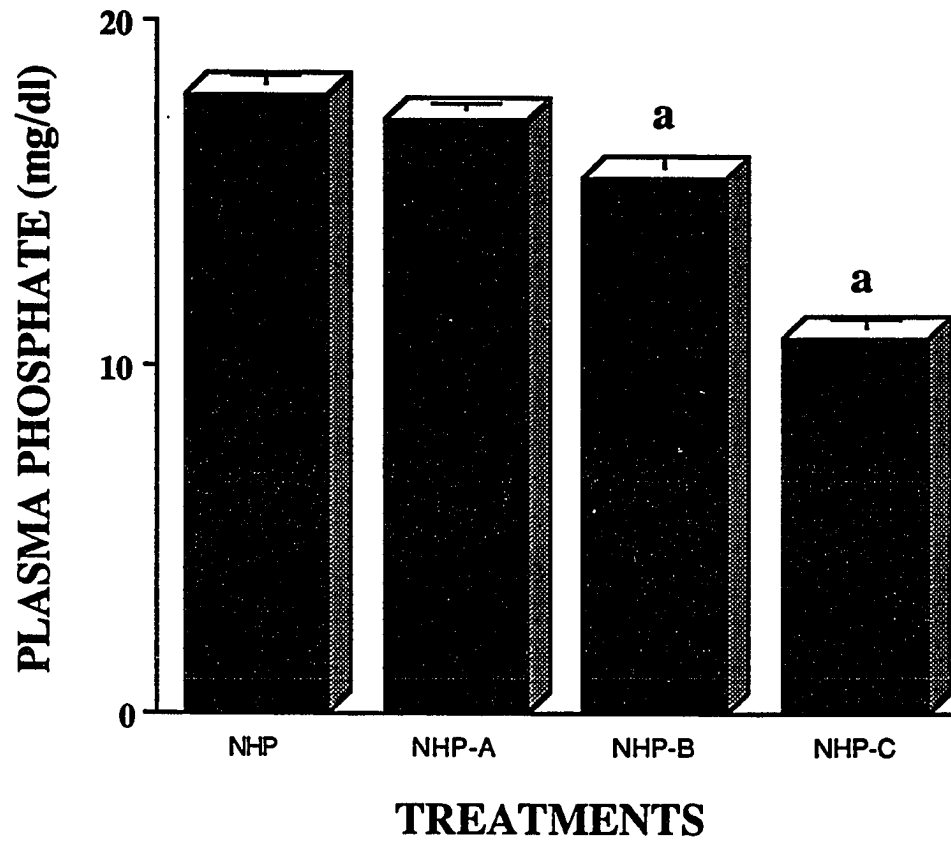


Figure 4. Mean plasma calcium (mg/dl) of sham and nephrectomized rats fed either low or high phosphorus diets for a period of 30 days.

a = significantly different from SLP $P < 0.001$.

b = significantly different from NLP $P < 0.001$.

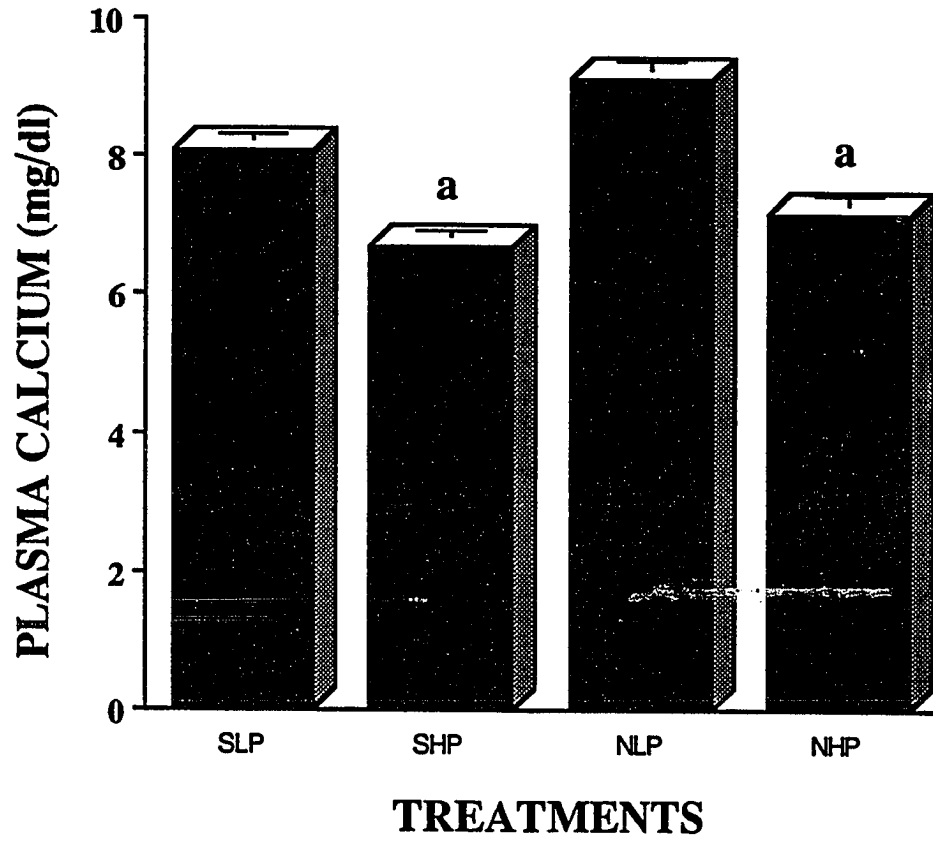


Figure 5. Mean plasma calcium (mg/dl) of nephrectomized rats fed high phosphorus diets and treated with vitamin D analogs for a period of 30 days.

a = significantly different from NHP $P < 0.001$.

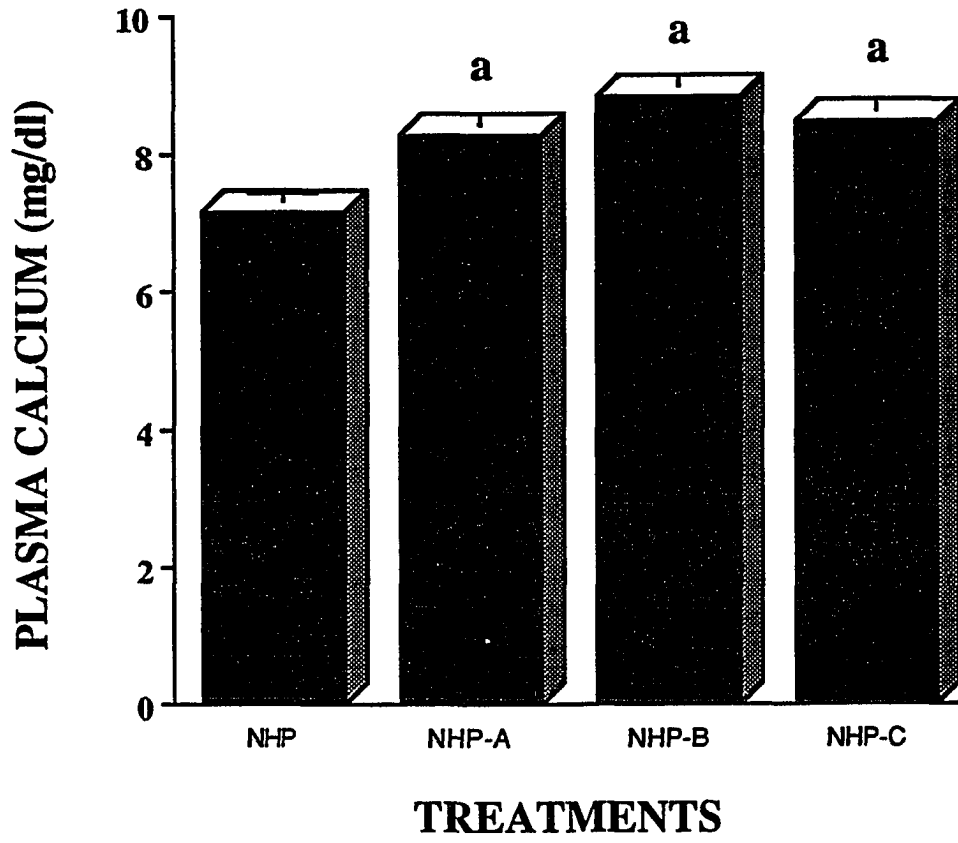


Figure 6. Mean plasma 1,25-(OH)₂D₃ (pg/ml) of sham and nephrectomized rats fed either low or high phosphorus diets for a period of 30 days.

a = significantly different from SLP P<0.001.

b = significantly different from NLP P<0.001.

1,25-DIHYDROXYVITAMIN D3 (pg/ml)

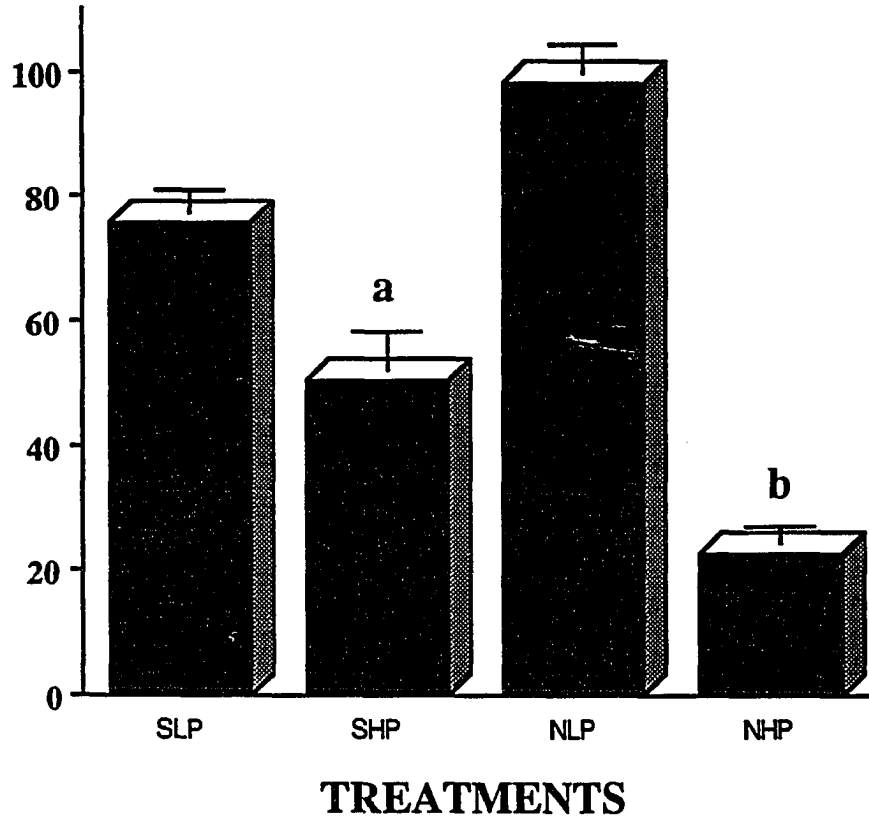


Figure 7. Mean plasma 1,25-(OH)₂D₃ (pg/ml) of nephrectomized rats fed high phosphorus diets and treated with vitamin D analogs for a period of 30 days.
a = significantly different from NHP P<0.001.

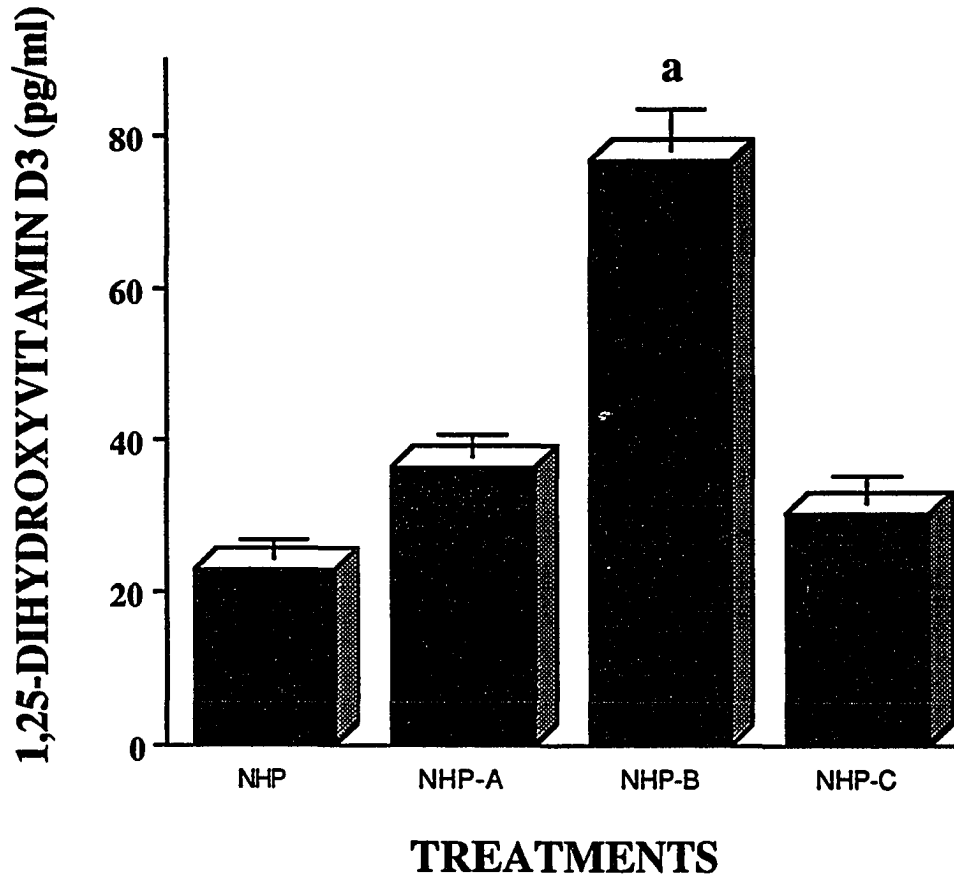


Figure 8. Mean plasma PTH (pg/ml) of sham and nephrectomized rats fed either low or high phosphorus diets for a period of 30 days.

a = significantly different from SLP $P < 0.001$.

b = significantly different from NLP $P < 0.001$.

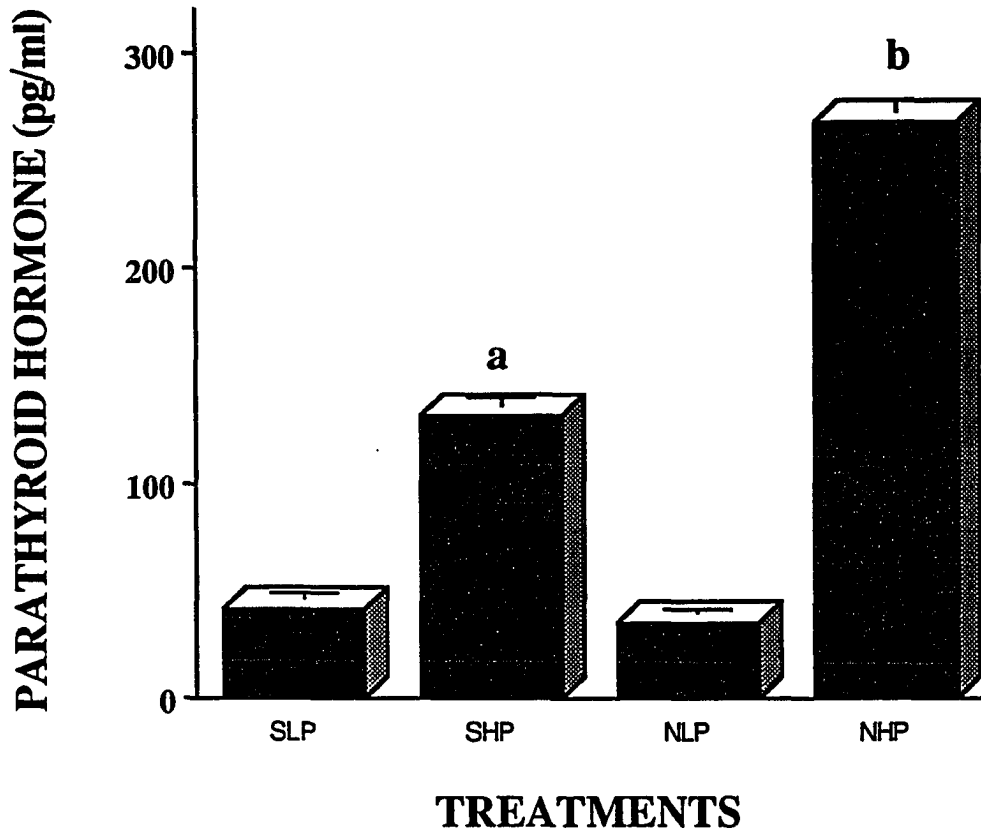


Figure 9. Mean plasma PTH (pg/ml) of nephrectomized rats fed either low or high phosphorus diets for a period of 30 days.

a = significantly different from NHP $P < 0.001$.

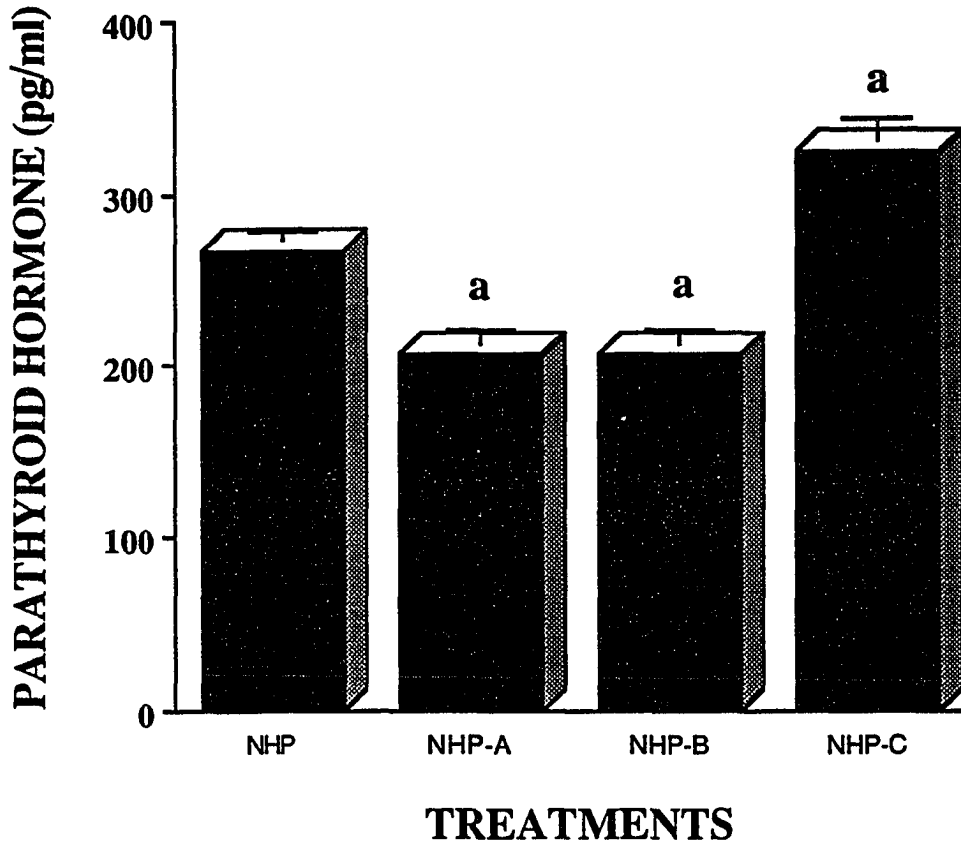


Figure 10. Mean plasma creatinine (mg/dl) of sham and nephrectomized rats fed either low or high phosphorus diets for a period of 30 days.
a = significantly different from SLP $P < 0.001$.

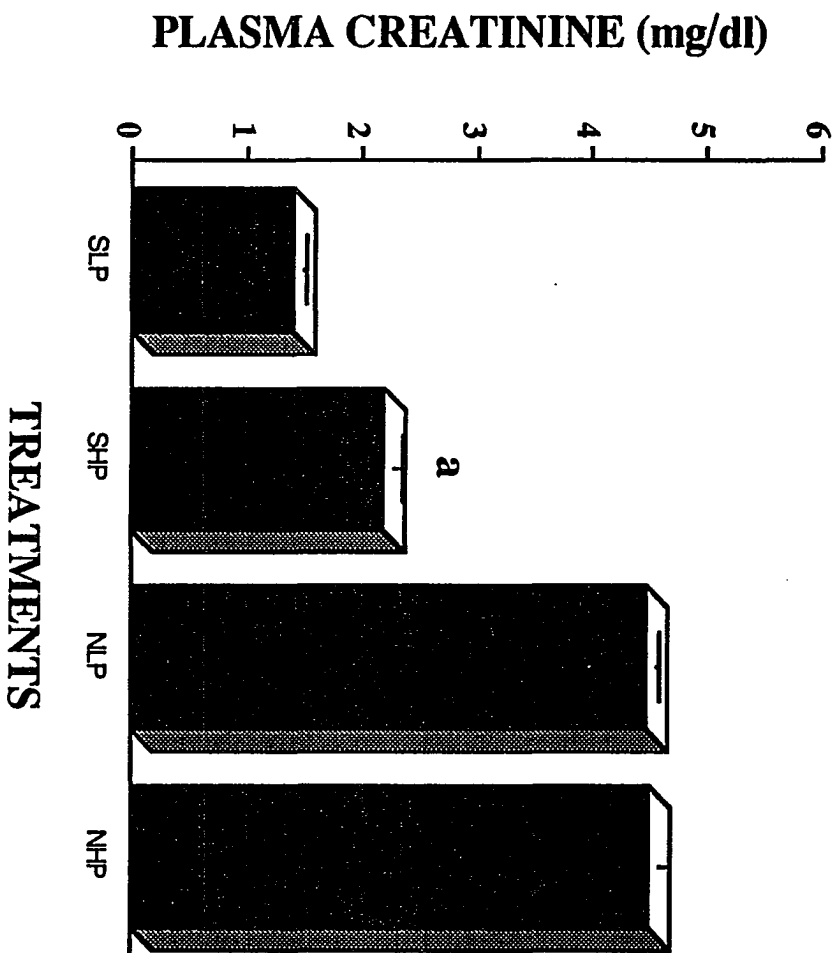
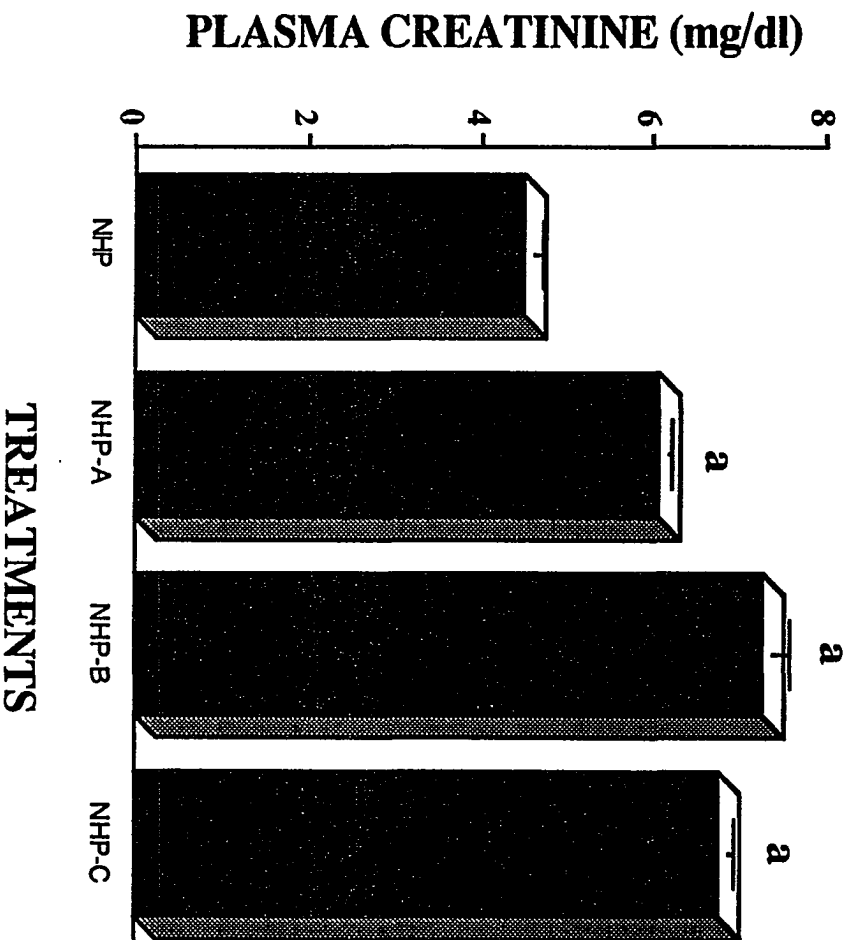


Figure 11. Mean plasma creatinine (mg/dl) of nephrectomized rats fed high phosphorus diets for a period of 30 days.

a = significantly different from NHP $P < 0.001$.



DISCUSSION

Results reported in this study are in agreement with many results reported earlier. Body weight losses were found to be higher in nephrectomized rats fed high phosphorus diets. Probably the main reasons could be loss of appetite compounded and dehydration due to loss of water to overcome the kidney overload with phosphates. The build up of phosphate further destroys the tubules and reduces T_mP_i/GFR and most importantly interferes with hormone secretion such as erythropoietin which is ultimately required for RBC synthesis. Furthermore, the functional losses of the kidney functions (filtration, secretion and reabsorption) also contributes to the increased water. The lowered reabsorption of the Na^+ ion from the kidney tubules cause for decreased blood volume and decreased systemic pressure. The high value of phosphate in the diets reduces the palatability of the diets which ultimately leads to lowered feed intake. From personal observations, most of the rats under the study did not like the high phosphorus diet and they preferred to drink more water. However, the loss of body weights in other studies were not observed. Lieuallen et al. (1990) did not see significant differences in weights between the different nephrectomized rats fed different calcium and phosphorus combinations in the diets.

Feeding high dietary phosphorus shown to increase the

plasma phosphate in this study. Sham operated and nephrectomized rats fed on high dietary phosphorus had high plasma phosphate as compared to rats fed on low phosphorus diets. Thus, feeding high dietary phosphorus to patients with renal failure would lead to hyperphosphatemia and ultimately worsen the situation of renal insufficiency. Support for the major role of phosphorus retention has been provided by several investigators who have shown that decreasing the amount of phosphorus in the diet in proportion to the fall in GFR prevents or ameliorates SHPT (Bricker et al., 1969; Slatopolsky et al., 1972; Lopez-Hilker et al., 1990). However, in this study treatments with vitamin D analogs were shown to reduce plasma phosphate and improve the situations. The reductions of plasma phosphate by the use of 1,25-(OH)₂D₂ and 1,25,28-(OH)₃D₂ were very significant. Although not statistically significant, 1,25-(OH)₂D₃ also reduced plasma phosphate. Thus, overall it is possible to say that treatments by vitamin D analogs lowered substantially phosphate levels and they were beneficial for the treatments of SHPT.

Plasma calcium in this study was found to be depressed by high levels of plasma phosphate. In both sham and nephrectomized rats, high dietary phosphorus showed to lower plasma calcium. This could be due to the fact that hyperphosphatemia results in hypocalcemia by several possible mechanisms including inhibition of the activity of the renal

enzyme of 1α -OHase which is already limited by the decrease in renal mass (Tanaka and DeLuca, 1973; Lopez-Hilker et al., 1990). The application of vitamin D analogs was found to be successful because plasma calcium was raised from lower levels to nearly normal levels. Thus, $1,25-(OH)_2D_3$, $1,25-(OH)_2D_2$ and $1,25,28-(OH)_3D_2$ were found to improve plasma calcium levels in NHP-A, NHP-B and NHP-C rats, respectively.

The production of $1,25-(OH)_2D_3$ in sham operated rats fed high dietary phosphorus was found to be lower than the SLP group which fed low dietary phosphorus. Similarly, in nephrectomized rats, those fed high dietary phosphorus were found to be lower in their $1,25-(OH)_2D_3$ production. Therefore, $1,25-(OH)_2D_3$ was found to be limited by two major factors in this study. These were hyperphosphatemia which inhibited 1α -OHase activities and the reduced renal cell mass which also limited the capacity of the proximal renal tubule to synthesis $1,25-(OH)_2D_3$. Portale et al. (1986) and Lopez-Hilker et al. (1990) reported that when dietary phosphorus was restricted and then supplemented, an increase and then a decrease in plasma $1,25-(OH)_2D_3$ was found. Furthermore, they reported that this phenomenon was associated with an increase and decrease in the production rate of $1,25-(OH)_2D_3$, respectively. Gray et al. (1977) also demonstrated in healthy women an increase in $1,25-(OH)_2D_3$ levels in response to dietary phosphorus restriction. In patients with severe and moderate renal

insufficiency, the occurrence of severe and moderate renal reductions in plasma concentrations of $1,25-(\text{OH})_2\text{D}_3$ presumably reflects a reduction in the renal synthesis of $1,25-(\text{OH})_2\text{D}_3$ (Portale *et al.*, 1984). The present study supported that in rats with renal insufficiency, the application of $1,25-(\text{OH})_2\text{D}_3$, $1,25-(\text{OH})_2\text{D}_2$ and $1,25,28-(\text{OH})_3\text{D}_2$ had improved the plasma levels of $1,25-(\text{OH})_2\text{D}_3$ greatly. Improvements were found to be 60, 232 and 33% more than the control for NHP-A, NHP-B and NHP-C, respectively.

The stimulatory effect of hyperphosphatemia to HPT was observed in this study. Sham rats fed high dietary phosphorus were tripled their PTH production compared to the control rats fed low phosphorus diets. Similarly, nephrectomized rats fed high dietary phosphorus had increased their PTH production more than seven-fold as compared to the control rats.

The therapeutic potentials of some of the vitamin D analogs were confirmed in this study by the fact that $1,25-(\text{OH})_2\text{D}_3$ and $1,25-(\text{OH})_2\text{D}_2$ reduced the PTH production by 23 and 22%, respectively. However, $1,25,28-(\text{OH})_3\text{D}_2$ was not likely to improve the situations.

The actions of phosphorus restriction or supplementation on PTH and $1,25-(\text{OH})_2\text{D}_3$ have been documented in several reports. Thus, phosphorus retention has long been considered to be important to the pathogenesis of the SHPT of chronic renal failure, and the resultant disabling renal

osteodystrophy (Silver and Naveh-Many, 1994). Slatopolsky and Bricker (1973) and later Lopez-Hiker et al. (1990) showed, in dogs with experimental chronic renal failure, that dietary phosphorus restriction prevented SHPT. Clinical studies have demonstrated that phosphorus restrictions in patients with chronic renal insufficiency were effective in preventing the increase in serum PTH levels (Portale et al., 1984; Lucas et al., 1986). But the mechanism of this effect was not clear, although at least part of it was considered to be due to changes in serum $1,25\text{-(OH)}_2\text{D}_3$ concentrations. Portale et al. (1984) performed metabolic studies in children with moderate renal insufficiency and showed that with normal dietary phosphorus there was a decrease in serum $1,25\text{-(OH)}_2\text{D}_3$ and an increase in serum PTH levels, which were prevented by restriction of dietary phosphorus and amplified by supplementation with phosphorus. Furthermore, they showed that in normal men, phosphorus was a physiological regulator of serum $1,25\text{-(OH)}_2\text{D}_3$ levels. It was therefore, assumed that phosphorus retention, which occurs even in early renal failure, was an important factor in the pathogenesis of the SHPT of chronic renal failure by virtue of its effect on serum $1,25\text{-(OH)}_2\text{D}_3$ levels and its direct reduction of serum calcium by formation of calcium phosphate in the serum, which is then deposited in bone and soft tissues (Silver and Naveh-Many, 1994).

It is evident that low levels of plasma $1,25-(OH)_2D_3$ also lead to hypocalcemia due to a decrease in absorption of calcium from the gastrointestinal tract and possibly by its contribution to the skeletal resistance to the calcemic action of PTH (Olgaard et al., 1982). However, clinical studies in patients with chronic renal failure have shown that a low phosphorus diet prevented an increase in PTH levels without an increase in serum $1,25-(OH)_2D_3$ levels (Lucas et al., 1986).

The occurrence of renal SHPT and hence uremia was shown by higher values of plasma creatinine in all rats on high phosphorus diets. This conditions might result from the concerted actions of reduced renal cell mass and high dietary phosphorus which lowered plasma $1,25-(OH)_2D_3$.

The increasing levels of plasma creatinine with high dietary phosphorus obtained in this study were in agreement with other findings reported. Both sham and nephrectomized rats fed high dietary phosphorus had more plasma creatinine as compared to the control rats fed low dietary phosphorus. Thus, in this study plasma $1,25-(OH)_2D_3$ levels varied inversely proportional to plasma creatinine, suggesting that in advanced renal insufficiency the reduced renal tissue mass is a major determinant of $1,25-(OH)_2D_3$ synthesis and/or secretion. Thus, it was suggested that in advanced renal failure, the significant decrease in renal mass may not allow dietary phosphorus restriction to increase $1,25-(OH)_2D_3$ synthesis

(Lopez-Hilker et al., 1990). However, Nagode et al. (1992) reported that there was a decrease of plasma creatinine after 1,25-(OH)₂D₃ therapy.

In this experiment with rats, some of the vitamin D analogs appear to have some therapeutic potentials for SHPT. A reduction in blood phosphate and PTH concentration and an increase in blood calcium are positive responses needed in SHPT. However, more intensive work would be required to determine their effective doses and confirmation before they are used for human therapies. Patients of SHPT are more likely suffer from damages caused by phosphate overload on the kidney tubules than the reduction of renal cell mass. Thus, care must be given to the levels of dietary phosphorus intake.

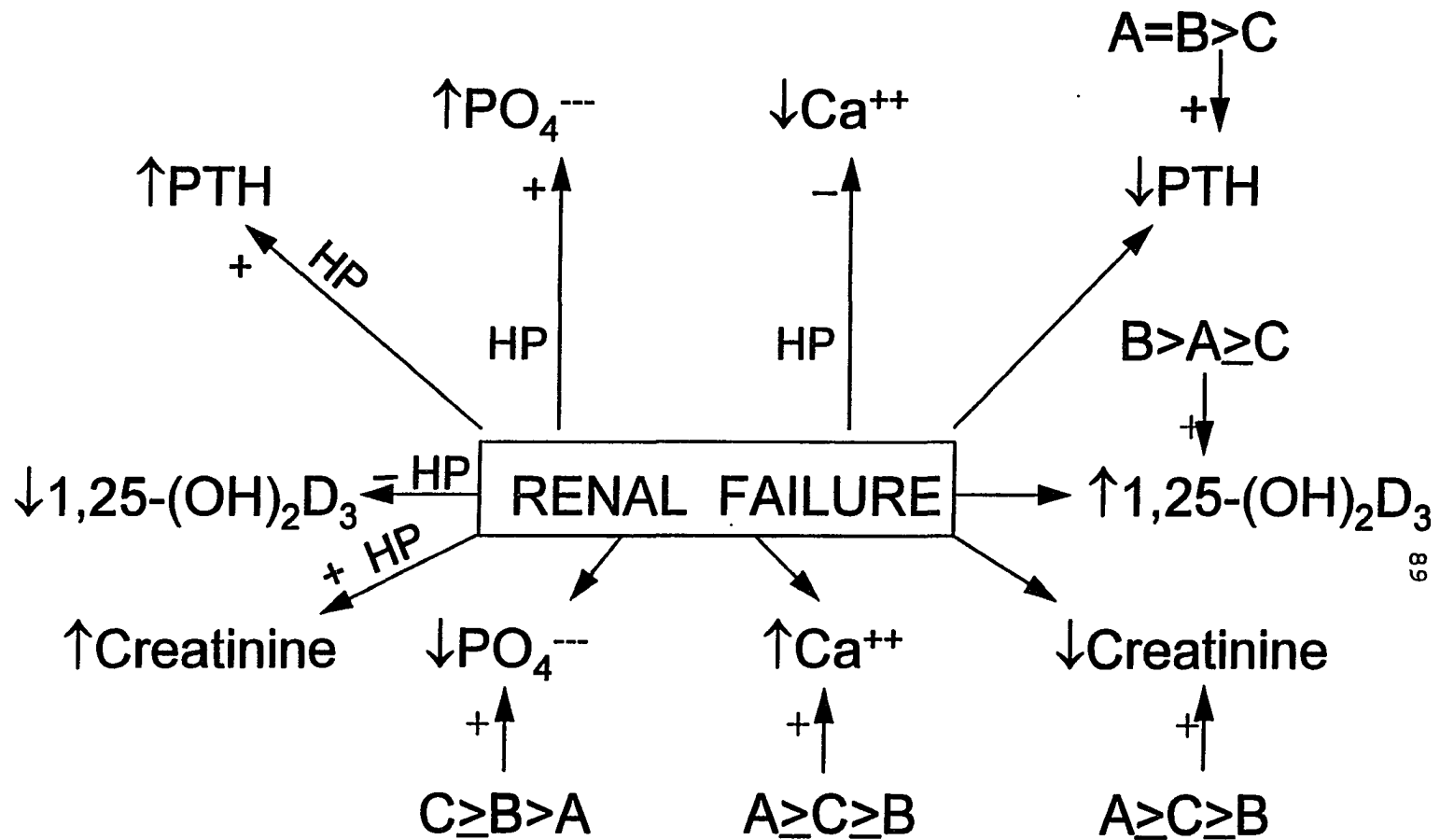


Figure 12. Summarized effect of high phosphorus diet (HP) and the treatment by vitamin D analogs during renal failure. A= 1,25-(OH)₂D₃; B= 1,25-(OH)₂D₂; C= 1,25,28-(OH)₃D₂; - = Decrease; + = Increase.

SUMMARY AND CONCLUSIONS

Plasma phosphate retention and reduction of renal 1,25-(OH)₂D₃ are the major factors that cause SHPT.

1. Sham and nephrectomized rats fed high phosphorus diets lost 9.50 and 24.72%, respectively, of their initial body weights.
2. Plasma phosphate increased substantially as dietary phosphorus increased and the application of vitamin D analogs did lower the levels of plasma phosphate.
3. Treatments by the vitamin D analogs raised the level of plasma calcium.
4. High plasma phosphate has more significant impact on 1,25-(OH)₂D₃ production than the reduction of renal cell mass.
5. Treatments by the vitamin D analogs did lower the levels of plasma PTH.
6. Among the vitamin D analogs, 1,25-(OH)₂D₃ was most likely appeared to be superior because of reduction of plasma phosphate, PTH and creatinine and improved plasma calcium and 1,25-(OH)₂D₃.
7. There was an inverse relationship between plasma creatinine and plasma levels of 1,25-(OH)₂D₃.

REFERENCES

- About-Samra, A.B., Juppner, H., Westerberg, D., Potts, J.T.Jr. and Segre, G.V. 1989. Parathyroid hormone causes translocation of protein kinase-Ca activity from cytosol to membrane in rat osteosarcoma cells. *Endocrinology* 124:1107.
- Akmal, M., Goldstein, D.A., Multani, S., and Massry, S.G. 1984. Role of uremic brain calcium and parathyroid hormone on changes in electroencephalogram in chronic renal failure. *Am. J. Physiol.* 246:F575.
- Akmal, M., and Massry, S.G. 1990. Role of parathyroid hormone in the decreased motor nerve conduction velocity of chronic renal failure. *Proc. Soc. Exp. Biol. Med.* 195:202.
- Allegretto, E.A. Pike, J.W., and Haussler, M.R. 1987. C-terminal proteolysis of the avian 1,25-dihydroxyvitamin D₃ receptor. *Bioch. Biophys. Res. Commun.* 147:479.
- Ankerstorm, V.L., and Walters, M.R. 1992. Physiological effects of 1,25-dihydroxyvitamin D₃ in the TM₄ sertoli cells line. *Am. J. Physiol.* 262:E884.
- Baxter, L.A., and DeLuca, H.F. 1976. Stimulation of 25-hydroxyvitamin D₃-1 α -hydroxylase by phosphate depletion. *J. Biol. Chem.* 251:3158.
- Beckman, M.J., Goff, J.P., Reinhardt, T.A., Beitz, D.C., and Horst, R.L. 1994. In vivo regulation of rat intestinal 24-hydroxylase: Potential new role of calcitonin. *Endocrinology* 135(5):1951.
- Bikle, D.D. 1992. Clinical counter point: vitamin D: New actions, new analogs, new therapeutic potential. *Endocrine Rev.* 13:765
- Bilezikian, J.P. 1993. Primary hyperparathyroidism. In: *Primer on the metabolic bone diseases and disorders of mineral metabolism. An official publication of the American society for bone and mineral research (2nd ed).* Raven Press, New York. pp.155.
- Bonjour, J.P., Caverzasio, J., and Fleisch, H. 1982a. The adaptive system of the tubular transport of phosphate. *Adv. Exp. Med. Biol.* 151:1.

- Bonjour, J.P., Caverzasio, J., Muhlbauer, R., Trechsel, U., and Troehler, U. 1982b. Are 1,25 (OH)₂D₃ production and tubular phosphate transport regulated by one common mechanism which would be defective in X-linked hypophosphotemic rickets? In the 5th proceeding of vitamin D, Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism. Walter de Gruyter, Berlin. pp.427.
- Bonjour, J.P., and Fliesch, H. 1980. Tubular adaptation to the supply and requirement of phosphate. In Massry, S.G. and Fliesch, H. eds. Renal Handling of Phosphate. Plenum, New York. pp.243.
- Bonjour, J.P., Caverzasio, J., and Rizzoli, R. 1992. Phosphate homeostasis, 1,25-dihydroxyvitamin D₃ and hyperparathyroidism in chronic renal failure. Trends Endocrinol. Metabolism. 3:301.
- Boyle, I.T., Gray, R.W., and DeLuca, H.F. 1971. Regulation by calcium in vitro synthesis of 1,25-dihydroxycholecalciferol and 21,25-dihydroxycholecalciferol. Proc. Natl. Acad. Sci. USA. 68:2131.
- Brent, G.A., LeBoff, M.S., Seely, E.W., Colin, P.R., and Brown, E.M. 1988. Relationship between the concentration and rate of change of calcium and serum intact parathyroid hormone levels in normal humans. J. Clin. Endocrinol. Metabolism. 67:944.
- Breslau, N.A. 1992. Calcium Homeostasis. In text book of Endocrine Physiology, (2nd ed), edited by Griffin, J.E., and Ojeda, S.R. Oxford University Press, New York. pp.276.
- Bricker, N.S. 1972. On the pathogenesis of the uremic state: An exposition of the trade-off hypothesis. N. Engl. J. Med. 286:1093.
- Bricker, N.S., Slatopolsky, E., Reiss, E., and Avioli, L.V. 1969. Calcium, phosphorus and bone in renal disease and transplantation. Arch. Intern. Med. 123:543.
- Brickman, A.S., Coburn, J.W., Massry, S.G., and Norman, A.W. 1974. 1,25-dihydroxyvitamin D₃ in normal man and patients with renal failure. Ann. Intern. Med. 80:161.

- Bringhurst, F.R., Zajac, J.D., Daggett, A.S., Skurat, R.N., and Kronenberg, H.M. 1989. Inhibition of parathyroid hormone responsiveness in clonal osteoblastic cells expressing a mutant form of 3', 5'-cyclic adenosine monophosphate-dependent protein kinase. *Mol. Endocrinol.* 3:60.
- Broadus, A.E. 1993. Physiological functions of calcium, magnesium, and phosphorus and mineral ion balance. in *Primer on the metabolic bone diseases and disorders of mineral metabolism* (2nd ed). An official publication of the American Society for Bone and Mineral Research. Raven Press, New York. pp.41.
- Brommage, R., and DeLuca, H.F. 1985. Evidence that 1,25-dihydroxyvitamin D₃ is the physiologically active metabolite of vitamin D₃. *Endocrine Rev.* 6:491.
- Bronner, F. 1989. Renal calcium transport: Mechanism and regulation: An overview. *Am. J. Physiol.* 257:F707.
- Bronner, F. 1990. Transcellular calcium transport. In: Bronner, F. (ed). *Intracellular calcium metabolism*. Alan R. Liss Inc, New York. pp.415.
- Bronner, F., and Stein, W.D. 1988. CaBP facilitates intracellular diffusion for Ca pumping in distal convoluted tubules. *Am. J. Physiol.* 255:F558.
- Brookman, J.J., Farrow, S.M., Nicholson, L., O'Riordan, J.L., and Hendy, G.N. 1986. Regulation by calcium of parathyroid hormone mRNA in cultured parathyroid tissue. *Bone Miner. Res.* 1(6):529.
- Brown, E.M. 1983. Four-parameter model release and extracellular calcium concentration in normal and abnormal parathyroid tissue. *J. Clin. Endocrinol. Metab.* 56:572.
- Brown, A.J., Dusso, A., and Slatopolsky, E. 1994. Selective vitamin D analogs and their therapeutic applications. *Nephrol.* 14(2):156.
- Brown, E.M., Wilkson, R.E., Eastman, R.C., Pallotta, J., and Marynick, S.P. 1982. Abnormal regulation of parathyroid hormone by calcium in secondary hyperparathyroidism due to chronic renal failure. *J. Clin. Endocrinol. Metab.* 54:172.

- Brown, A.J. Finch, J.L., Lopez-Hilker, S., Dusso, A., Ritter, C., Pernalete, N., and Slatopolsky, E. 1990. New active analogues of vitamin D with low calcemic activity. *Kidney Int. Suppl.* 29:S22.
- Brown, A.J., Ritter, C.S., Finch, J.L., Morissey, J., Martin, K.J., Murayama, E.; Nishii, Y., and Slatopolsky, E. 1989. The noncalcemic analogues of vitamin D. 22-Oxacalcitriol suppresses parathyroid hormone synthesis and secretion. *J. Clin. Invest.* 84:728.
- Brown, E.M., Brennan, M.F., Hurwitz, S., Windeck, K., Max, S.J., Spiegel, E.M., Koehler, J.O., Garadner, D.G., and Aubrach, G.D. 1978. Dispersed cells prepared from human parathyroid glands. Distinct calcium sensitivity of adenomas vs primary hyperplasia. *J. Clin. Endocrinol. Metab.* 46:267.
- Brumbaugh, P.F., and Haussler, M.R. 1975. Specific binding of $1\alpha,25$ -dihydroxycholecalciferol to nuclear components of chick intestine. *J. Biol. Chem.* 250:1588.
- Caffrey, J.M. and Farach-Carson, M.C. 1989. Vitamin D₃ metabolites modulate dihydroxypyridine-sensitive calcium currents in clonal rat osteosarcoma cells. *J. Biol. Chem.* 264:20265.
- Caulfield, M.P., and Rosenblatt, M. 1990. Parathyroid hormone receptor interactions. *Tem.* Jan/Feb. 164.
- Caverzasio, J., Fleisch, G.H., and Bonjour, J.P. 1982. Parathyroid hormone-independent adaptation of the renal handling of phosphate in response to renal mass reduction. *Kidney Int.* 21:471.
- Chasson, A.L., Grady, H.J., and Stanley, M.A. 1961. Determination of creatinine by means of automatic chemical analysis. *Am. J. Clin. Pathol.* 35:83.
- Chen, T.L., Hauschka, P.V., Cabraless, S., and Feldman, D. 1986. The effects of $1,25$ -dihydroxyvitamin D₃ and dexamethasone on rat osteoblast-like primary cell cultures. Receptors occupancy and functional expression patterns for three different bioresponses. *Endocrinology* 118:250.
- Cheng, L., and Sacktor, B. 1981. Sodium gradient-dependent phosphate transport in renal brush border vesicles. *J. Biol. Chem.* 256:1556.

- Christakos, S., Gabrielideo, C., and Rhoten, W.B. 1989. Vitamin D-dependent calcium binding proteins: Chemistry, distribution, functional considerations, and molecular biology. *Endocr. Rev.* 10:3.
- Coburn, W.J., and Slatopolsky, E. 1991. Vitamin D, parathyroid hormone, and the renal osteodystrophies, in *The Kidney* (4thed), edited by Brenner, B.M., and Rector, F.C.Jr. pp.2036.
- Cross, H.S., and Peterlik, M. 1988. Calcium and inorganic phosphate transport in embryonic chick intestine: Triiodothyronine enhances the genomic actions of 1,25-dihydroxycholecalciferol. *J. Nutr.* 118:1529.
- de Boland, A.R., and Boland, R. 1985. In vitro cellular muscle calcium metabolism. Characterization of effects of 1,25-dihydroxyvitamin D₃ and 25-hydroxyvitamin D₃. *Z Naturforsch [C]* 40:102.
- de Boland, A.R., Nemere, I., and Norman, A.W. 1990. Ca²⁺-channel agonist bay k8644 mimics 1,25(OH)₂-vitamin D₃ rapid enhancement of Ca²⁺ transport in chick perfused duodenum. *Biochem. Biophys. Res. Commun.* 166:217.
- Delmez, J.T., Tindira, C., Grooms, P., Dusso, A., Windus, D.W., and Slatopolsky, E. 1989. Parathyroid hormone suppression by intravenous 1,25-dihydroxyvitamin D: A role for increased sensitivity to calcium. *J. Clin. Invest.* 83:1349.
- Demay, M.B., Gerardi, J.M., DeLuca, H.F. and Kronenberg, H.M. 1990. DNA sequences in the rat osteocalcin gene that bind the 1,25-dihydroxyvitamin D₃ receptor and confer responsiveness to 1,25-dihydroxyvitamin D₃. *Proc. Natl.Acad. Sci. USA.* 87:369.
- Dokoh, S., Donaldson, C.A., and Haussler, M.R. 1984. Influence of 1,25-dihydroxyvitamin D₃ on cultured osteogenic sarcoma cells: correlation with the 1,25-dihydroxyvitamin D₃ receptor. *Cancer Res.* 44:2103.
- Dorner, A., and Kemper, B. 1978. Conversion of preproparathyroid hormone to parathyroid hormone by dog pancreatic microsomes. *Biochemistry* 17:5550.

- Duda, R.J., O'Brien, J.F., Katzmann, J.A., Peterson, J.M., Mann, K.G., and Riggs, B.L. 1988. Concurrent assays of circulating bone gla-protein and bone phosphatase: effect of sex, age, and metabolic bone diseases. *J. Clin. Endocrinol. Metab.* 66:951.
- Dunlay, R., and Hruska, K., 1990. PTH receptor coupling to phospholipase C is an alternate pathway of signal transduction in bone and kidney. *Am. J. Physiol.* 258:F223.
- Edelstein, S., Noff, D., Sinai, L., Harell, A., Puschett, J.B., Golub, E.E., and Bronner, F., 1978. Vitamin D metabolism and expression in rats fed on low-calcium and low-phosphorus diets. *Biochem. J.* 170:227.
- Eliaschewitz, F.G., Goldberg, A.C. Borelli, A., and Quintao, E.R.C. 1982. Effect of 1,25-(OH)₂D₃ on P transport in rat seminiferous tubules. *Clin. Res.* 30:391.
- Evans, D.B., Thavatajah, M., Binderup, L., and Kanis, J.A. 1991. Actions of calcitriol (MC903) a novel vitamin D₃ analog on human bone derived cells: Comparison with 1,25-dihydroxyvitamin D₃. *J. Bone Miner. Res.* 12:1307.
- Fadda, G., Akmal, M., Lipson, L., and Massry, S.G. 1990. Direct effect of parathyroid hormone on insulin secretion from pancreatic islets. *Am. J. Physiol.* 258:E975.
- Faraco, J.H. Morrison, N.A., Baker, A., Shine, J., and Frossard, P.M. 1989. ApaI dimorphism at the human vitamin D receptor gene locus. *Nucleic Acids Res.* 17(5):2150.
- Fawthrop, D.J., Boobis, A.R., and Davies, D.S. 1991. Mechanisms of cell death. *Arch. Toxicol.* 65:437.
- Feher, J.J. 1983. Facilitated calcium diffusion by intestinal calcium-binding protein. *Am. J. Physiol.* 244:C303.
- Feinfeld, D.A., and Sherwood, L.M. 1988. Parathyroid hormone and 1,25-(OH)₂D₃ in chronic renal failure. *Kidney Int.* 33:1049.
- Finch, J., Brown, A.J., Kubodera, N., Nishii, Y., and Slatoplosky, E. 1993. Differential effects of 1,25-(OH)₂D₃ and 22-oxacalcitriol on phosphate and calcium metabolism. *Kidney Int.* 43(3):561.

- Fraser, D.R. 1980. Regulation of the metabolism of vitamin D. *Physiol. Rev.* 60:551.
- Fraser, D.R., and Kodicek, E. 1973. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature* 228:764.
- Fukagawa, M., Kaname, S-Y., Igarashi, T., Ogata, T., and Kurokawa, K. 1991. Regulation of parathyroid hormone synthesis in chronic renal failure in rats. *Kidney Int.* 39(5):874.
- Fukayama, S., and Tashjian, A.H. 1990. Stimulation of parathyroid hormone of $^{45}\text{Ca}^{2+}$ uptake in osteoblast-like cells: possible involvement of alkaline phosphatase. *Endocrinology* 126:1941.
- Galbraith, S.C., and Quarles, L.D. 1993. Tertiary hyperparathyroidism and refractory secondary hyperparathyroidism: In *Primer on the metabolic bone diseases and disorders of mineral metabolism*. An Official Publication of the American Society for Bone and Mineral Research. Raven Press, New York. pp.159.
- Garabedian, M., Holick, M.F., DeLuca, H.F., and Boyle, I.T. 1972. Control of 25-hydroxycholecalciferol metabolism by parathyroid glands. *Proc. Natl. Acad. Sci. USA* 69:1673.
- Ghijsen, W.E.J.M. and Van Os, C.H. 1982. 1 α ,25-dihydroxyvitamin D₃ regulates ATP-dependent calcium transport in basolateral plasma membranes of rat erythrocytes. *Biochem. Biophys. Acta* 689:170.
- Giuliano, A.R., and Wood, R.J. 1991. Vitamin D-regulated calcium transport in CaCO-2 cells: unique in vitro model. *Am. J. Physiol.* 260:G207.
- Goff, J.P., Horst, R.L., and Vskokovic, M. 1993. 1,25,28 trihydroxyvitamin D₂ up-regulates renal vitamin D receptor without causing hypercalcemia. *Bioorgan & Medic Chem Lett.* 3(9):1825.
- Goff, J.P., Reinhardt, T.A., Beckman, M.J., and Horst, R.L. 1990. Contrasting effects of exogenous 1,25-dihydroxyvitamin D [1,25-(OH)₂D] versus endogenous 1,25-(OH)₂D induced by dietary calcium restriction, on vitamin D receptors. *Endocrinology* 126(2):1031.

- Goff, J.P., Reinhardt, T.A., Engstrom, G.W., and Horst, R.L. 1992. Effect of dietary calcium or phosphorus restriction and 1,25-dihydroxyvitamin D administration on rat intestinal 24-hydroxylase. *Endocrinology* 131(1):101.
- Gordon, D.F., and Kemper, B. 1980. Synthesis, restriction analysis and molecular cloning of near full length DNA complementary to bovine parathyroid hormone mRNA. *Nucleic acid Res.* 8:5669.
- Gray, R.W., Wilz, D.R., Caldas, A.E., and Lemann, J.Jr. 1977. The importance of phosphate in regulating 1,25-(OH)₂-vitamin D levels in humans: Studies in healthy subjects, in calcium stone forms and in patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* 45:299.
- Gross, M., and Kumar, R., 1990. Physiology and biochemistry of vitamin D-dependent calcium binding proteins. *Am. J. Physiol.* 259:F195.
- Habener, J.F., and Potts, J.T. Jr. 1990. Fundamental considerations in the physiology, biology and biochemistry of parathyroid hormone. In Avioli L.V. and Krane, S.M. (eds): *Metabolic bone disease*. W.B.Saunders, Philadelphia, PA. pp.69.
- Hadley, M.E. 1992. *Endocrinology*. 3rd ed. Prentice Hall, Englewood Cliffs, NJ 07632. pp.225
- Haussler, M.R., 1986. Vitamin D receptors: Nature and function. *Annu. Rev. Nutr.* 6:527.
- Haussler, M.R., Mangelsdorf, D.J., Komm, B.S., Terpening, C.M., Yamaoka, K., Allegretto, E.A., Baker, A.R., Shine, J., McDonnell, D.P., Hughes, M., Weingel, N.L., O'Malley, B.W., and Pike, J.W. 1988. Molecular biology of the vitamin D hormone. *Rec. Progr. Horm. Res.* 44:263.
- Haussler, M.R., Manolagas, S.C., and Deftos, L.J. 1980. Evidence for a 1,25-dihydroxyvitamin D₃ receptor-like macromolecules in rat pituitary. *J. Biol. Chem.* 255:5007.
- Heinrich, G., Kronenberg, H.M., Potts, J.T.Jr., and Habener, J.F. 1984. Gene encoding parathyroid hormone. Nucleotide sequence of the rat gene and deduced aminoacid sequence of rat parathyroid hormone. *J. Biol. Chem.* 259:3320.

- Heizmann, C.W., and Hunziker, W. 1990. Intracellular calcium-binding molecules. In: Bronner, F. (ed). Intracellular calcium regulation. Alan, R. Liss Inc, New York. pp.211.
- Hendy, G.N., Kronenberg, H.M., Potts, J.J.Jr., and Rich, A. 1981. Nucleotide sequence of cloned cDNAs encoding human PreProParathyroid hormone. Proc. Natl. Acad. Sci. USA. 78:7365.
- Holick, M.F., Schnoes, H.K., DeLuca, H.F., Suda, T., and Cousins, R.J. 1971. Isolation and identification of 1,25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine. Biochemistry 10(14):2799.
- Horiuchi, N., Suda, T., Takahashi, H., Shimaqawa, E., and Ogata, E. 1977. In vivo evidence for the intermediary role of 3',5'-cyclic AMP in parathyroid hormone-induced stimulation of 1,25-dihydroxyvitamin D₃ synthesis in rats. Endocrinology 101:969.
- Horst, R.L. 1986. Regulation of calcium and phosphorus homeostasis in dairy cows. J. Dairy Sci. 69:604.
- Hughes, M.R., Brumbaugh, F.P., Haussler, M.R., Wergedal, E.J. and Baylink, J.D. 1975. Regulation of serum 1,25-dihydroxyvitamin D₃ by calcium and phosphate in the rat. Science 190:578.
- Insogna, K.L., Broadus, A.E., and Gertner, J.M. 1983. Impaired phosphorus conservation and 1,25-dihydroxyvitamin D generation during phosphorus deprivation in familial hypophosphatemic rickets. J. Clin. Invest. 71:1562.
- Ishikawa, T., Umesono, K., Mangelsdorf, D.J., Aburatani, H., Stanger, B.Z., Shibasaki, Y., Imawari, M., Evans, R.M., and Takaku, F. 1990. A functional retinoic acid receptor encoded by the gene on human chromosome 12. Mol. Endocrinol. 4(6):837.
- Johansson, H., Larsson, R., Nygren, P., Lindh, E., Rastad, J., Akerstrom, G., and Gylfe, E., 1987. Cytoplasmic Ca²⁺ concentration of single normal human and bovine parathyroid cells measured by dual wavelength microfluometry. Biosci. Rep. 7(9):705.
- Johnson, J.A., and Kumar, R. 1994. Renal and intestinal calcium transport: Role of vitamin D and vitamin D-dependent calcium binding proteins. Seminars in Nephrol. 14(2):119.

- Karsenty, G., Lacour, B., Ulmann, A., Pierandrei, E., and Druek, T. 1985. Early effects of vitamin D metabolites on phosphate fluxes in isolated rat erythrocytes. *Am. J. Physiol.* 248:G40.
- Kawashima, H. 1988. 1,25-dihydroxyvitamin D₃ stimulates Ca²⁺-ATPase in a vascular smooth muscle cell line. *Biochem. Biophys. Res. Commun.* 150:1138.
- Khosla, S., Demay, M., Pines, M., Hurwitz, S., Potts, J.T.Jr., and Kronenberg, H.M. 1988. Nucleotide sequence of cloned cDNAs encoding chicken preproparathyroid hormone. *J. Bone Miner. Res.* 3(6):689.
- Klinger, M., Alexiewicz, J., Linker-Israeli, M., Pitts, T.O., Gaciong, Z., Fadda, G.Z., and Massry, S.G. 1990. Effect of parathyroid hormone on human T-cell activation. *Kidney Int.* 37(6):1543.
- Kowarski, S., Cowen, L.A., Takahashi, M.T., and Schachter, D. 1987. Distribution and vitamin D dependence of IMCAL in the rat. *Am. J. Physiol.* 253:G411.
- Kronenberg, H.M. 1993. Parathyroid hormone: Mechanism of action. In *Primer on the metabolic bone diseases and disorders of mineral metabolism*. 2nd ed. An Official Publication of the American Society for Bone and Mineral Research. Raven Press, New York. pp.58.
- Kronenberg, H.M., Igarashi, T., Freeman, M.W., Okazaki, T., Brand, S.J., Wiren, K.M., and Potts, J.T.Jr. 1986. Structure and expression of the human parathyroid hormone gene. *Rec. Progr. Horm. Res.* 42:641.
- Kubrusly, M., Gagne, Eve-Reine., Urena, P., Hanrotel, C., Chabanis, S., Lacour, B., and Drueke, T.B. 1993. Effect of 22-oxa-calcitriol on calcium metabolism in rats with severe secondary hyperparathyroidism. *Kidney Int.* 44:551.
- Kumar, R. 1984. Metabolism of 1,25-dihydroxyvitamin D₃. *Physiol. Rev.* 64:478.
- Kumar, R. 1986. The regulation and mechanism of action of 1,25-dihydroxyvitamin D₃. *Kidney Int.* 30:793.

- Kurnik, B.R.C., Hurskey, M., and Hurska, K.A. 1987. 1,25-dihydroxycholecalciferol stimulates renal phosphate transport by directly altering membrane phosphatidylcholine composition. *Bioch. Biophys. Acta* 917:81.
- Labuda, M., Fujiwara, T.M., Ross, M.V., Morgan, K., Garcia-Heras, J., Ledbetter, D.H., Hughes, M.R., and Glorieux, F.H. 1992. Two hereditary defects related to vitamin D metabolism map to the same region of human chromosome 12q13-14. *J. Bone Min. Res.* 7(12):1447.
- Lau, K. 1989. Phosphate excess and progressive renal failure: The precipitation-calcification hypothesis. *Kidney Int.* 36:918.
- Lawson, D.E.M., Fraser, D.R., Kodicek, E., Morris, H.R., and Williams, D.H. 1971. Identification of 1,25-dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism. *Nature* 230(5291):228.
- Leathers, V.L., and Norman, A.W. 1985. Studies with a photoaffinity label for the vitamin D-dependent chick intestinal calcium binding protein: evidence for calcium dependent interactions. *Fed. Proc.* 44:754A.
- LeBoff, M.S., Rennke, H.G., and Brown, E.M. 1983. Abnormal regulation of parathyroid cell secretion and proliferation in primary cultures of bovine parathyroid cells. *Endocrinology* 113:277.
- Lee, W.T., Collins, J.F., Padayachi, K., and Cundy, T. 1994. A comparison of oral and intravenous Alfacalcidol in the treatment of uremic hyperparathyroidism. *J. Am. Soc. Nephrol.* 5(6):1344.
- Lidor, C., and Edelstein, S. 1987. Calciferol increases Ca^{2+} -ATPase in a vascular smooth muscle cell line. *Biochem. Biophys. Res. Commun.* 144:713.
- Lieberherr, M., Acker, G.M., Grosse, B., Pesty, A., and Balsan, S. 1984. Rat endometrial cells in primary culture: effects and interaction of sex hormones and vitamin D₃ metabolites on alkaline phosphatase. *Endocrinology* 115:824.
- Lieuallen, W.G., Weisbrode, S.E., Horst, R.L., and Nagade, L.A. 1990. The effect of uremia and dietary phosphorus on bone of rats. *Bone* 11:41.

- Liou, Hung-Hsiang., Chiang, ShooH-Shan., Huang, Tung-Po., Shieh, Shang-Der., and Akmal, M. 1994. Comparative effect of oral or intravenous calcitriol on secondary hyperparathyroidism in chronic hemodialysis patients. *Miner. Electr. Metab.* 20:97.
- Llach, F., and Massry, S.G. 1985. On the mechanism of secondary hyperparathyroidism in moderate renal insufficiency. *J. Clin. Invest.* 77:7.
- Llach, F., Massry, S.G., Singer, F.R., Kurokawa, K., Kay, J.M., and Coburn, J.W. 1975. Skeletal resistance to endogenous parathyroid hormone in patients with early renal failure: A possible cause of secondary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* 41:339.
- Lohnes, D., and Jones, G. 1987. Side chain metabolism of vitamin D₃ in osteosarcoma cell line UMR-106. *J. Biol. Chem.* 262:14394.
- Lopez-Hilker, S., Dusso, A.S., Rapp, N.S., Martin, K.J., and Slatopolsky, E. 1990. Phosphorus restriction reverses hyperparathyroidism in uremia independent of changes in calcium and calcitriol. *Am. J. Physiol.* 259:F432.
- Lopez-Hilker, S., Galceran, T., Chan, Y-L., Rapp, N., Martin, K.J., and Slatopolsky, E. 1986. Hypercalcemia may not be essential for the development of secondary hyperparathyroidism in chronic renal failure. *J. Clin. Invest.* 78:1097.
- Lowe, K.E., Maiyar, A.C., and Norman, A.W. 1992. Vitamin D-mediated gene expression. *Critical Reviews in Eukaryotic Gene Expression* 2(1):69.
- Lucas, P.A., Brown, R.C., and Woodhead, J.S. 1986. Acute responses of parathyroid hormone and 1,25-dihydroxyvitamin D₃ to unilateral nephrectomy in healthy donors. *Nephro. Dial. Transplant* 1(3):199.
- MacDonald, P.N., Dowd, D.R., and Haussler, M.R. 1994. New insight into the structure and functions of the vitamin D receptor. *Nephrol.* 14(2):101.
- MacGregor, R.R., Sarras, M.P.J., Houle, A., and Cohn, D.V. 1983. Primary monolayer cell culture of bovine parathyroids: Effects of calcium, Isoproterenol and growth factors. *Mol. Cell Endocrinol.* 30:313.

- Marx, S.J., Liberman, U.A., and Eil, C. 1983. Calciferols: Actions and deficiencies in actions. *Vit. Horm.* 40:235.
- Massry, S.G. 1983. Pathogenesis of the anemia of uremia: Role of secondary hyperparathyroidism. *Kidney Int.* 24:S204.
- Massry, S.G. 1985a. Assessment of 1,25 (OH)₂D₃ in the correction and prevention of renal osteodystrophy in patients with mild to moderate renal failure. In vitamin D, chemical, biochemical and clinical update. Walter de Gruyter, Berlin. pp.935.
- Massry, S.G. 1985b. Neurotoxicity of parathyroid hormone in uremia. *Kidney Int.* 17(suppl):S5. Massry, S.G. 1986. Prevention and treatment of the abnormalities in divalent ion metabolism in renal failure. *Nephrol.* 6:114.
- Massry, S.G. 1986. Prevention and treatment of the abnormalities in divalent ion metabolism in renal failure. *Nephrol.* 6:114.
- Massry, S.G., Goldstein, D.A., and Malluche, H.H. 1980. Current states of the use of 1,25-(OH)₂D₃ in the management of renal osteodystrophy. *Kidney Int.* 18:409.
- Mattei, M.G., Riviere, M., Krust, A., Ingvarsson, S., Vennstrom, B., Islam, M.Q., Levan, G., Kautner, P., Zelent, A., and Chambon, P. 1991. Chromosome assignment of retinoic acid receptor (RAR) genes in the human, mouse and rat genomes. *Genomics* 10(4):1061.
- Mayer, G.P., Habener, J.F., and Potts, J.T.Jr. 1976. Parathyroid hormone secretion in vivo: Demonstration of a calcium dependent, non-suppressible component of secretion. *J. Clin. Invest.* 57:679.
- Mayer, G.P., and Hurst, J.G. 1978. Sigmoidal relationship between parathyroid hormone secretion rate and plasma concentration in calves. *Endocrinology* 102:1036.
- McKenna, M.J., Hamilton, T.A., and Sussman, H.H. 1979. Comparison of human alkaline phosphatase isoenzymes. *Biochem. J.* 181:67.
- Meltzer, V., Weinreb, S., Bellorin-Font, E., and Hruska, K.A. 1982. Parathyroid hormone stimulation of renal phosphoinositide metabolism is a cyclic nucleotide-dependent effect. *Bioch. Biophys. Acta* 712:258.

- Mezzetti, G., Monti, M.G., Casolo, L.P., Piccinini, G., and Moruzzi, M.S. 1988. 1,25-dihydroxycholecalciferol-dependent calcium uptake by mouse mammary gland in culture. *Endocrinology* 122:389.
- Minghetti, P.P., and Norman A.W. 1988. 1,25 (OH)₂ vitamin D₃ receptors: Gene regulation and genetic circuitry. *FASEB J.* 2:3043.
- Morrissey, J.J., Hamilton, J.W., MacGregor, R.R., and Cohn, D.V. 1980. The secretion of parathormone fragments 34-84 and 37-84 by dispersed porcine parathyroid cells. *Endocrinology* 107:164.
- Mulkins, A.M., and Sussman, H.H. 1987. 1,25-dihydroxyvitamin D₃ exerts opposite effects on the regulation of human embryonic and non-embryonic alkaline phosphatase isoenzymes. *Endocrinology* 120:416.
- Nagata, A., Komoda, T., and Sakagishi, Y. 1989. Relationship between the uptake of calcium or phosphorus and alkaline phosphatase activity induced by certain modulators in rat organs. *Calcif. Tissue Int.* 45:173.
- Nagode, L.A., Chew, D.J., and Steinmeyer, C.L. 1992. The use of low doses of calcitriol in the treatment of renal secondary hyperparathyroidism. In: *Proceedings of the Waltham/OSU symposium for the treatment of small animal diseases.* *Endocrinology.* Kal Kan Foods Inc, Vernon, CA. pp.51.
- Napoli, J.L., and Horst, R.L. 1983. C(24) and C(23) oxidation, converging pathways of intestinal 1,25-dihydroxyvitamin D₃ metabolism: identification of 24-keto-1,23,25-trihydroxyvitamin D₃. *Biochem.* 22:5848.
- Napoli, J.L., Premanik, B.C., Royal, P.M., Reinhardt, T.A., and Horst, R.L. 1983. Intestinal synthesis of 24-keto 1,25-dihydroxy D₃. *J. Biol. Chem.* 258:2100.
- Naveh-Many, T., Friedlaender, M.M., Mayer, H., and Siver, J. 1989. Calcium regulates parathyroid hormone messenger ribonucleic acid (mRNA), but not calcitonin mRNA in vivo in rat. Dominant role of 1,25 dihydroxyvitamin D. *Endocrinology* 125:275.

- Naveh-Many, T., Marx, R., Keshet, E., Pike, J.W., and Silver, J. 1990. Regulation of 1,25-dihydroxyvitamin D₃ receptor gene expression by 1,25-dihydroxyvitamin D₃ in the parathyroid in vivo. *J. Clin. Invest.* 86:1968.
- Naveh-Many, T., and Silver, J. 1990. Regulation of parathyroid hormone gene expression by hypocalcemia, hypercalcemia, and vitamin D in the rat. *J. Clin. Invest.* 86:1313.
- Nemere, I., and Norman, A.W. 1991. Steroid hormone actions at the plasma membrane: Induced calcium uptake and exocytotic events. *Mol. Cell Endocrinol.* 80:C165.
- Nichols Institute Diagnostics. 1995. Immunoradiometric assay (IRMA) for the quantitative determination of parathyroid hormone levels in rat serum. Nichols Institute Diagnostics, Immutopics, Inc.
- Norman, A.W., and Henry, H.L. 1993. Vitamin D: Metabolism and mechanism of action. in *Primer on the metabolic bone diseases and disorders of mineral metabolism* (2nd ed). An Official Publication of the American Society for Bone and Mineral Research. Raven Press, New York. pp.63.
- Norman, A.W. 1979. Vitamin D: The calcium homeostatic steroid hormone. Academic Press, New York. pp.114.
- Nussbaum, S.R., Zahrdhnik, R.J., Lavigne, J.R., Brennan, G.L., Kimly, N-V.K., Keutmann, H.T., Wang, C., Potts, J.T.Jr., and Segre, G.V. 1987. Highly sensitive two-site immunoradiometric assay of parathyroid and the clinical utility in evaluating patients with hypercalcemia. *Clin. Chem.* 33:1364.
- Nygren, P., Larson, R., Johansson, H., Gylfe, E., Rastad, J., and Akerstrom, G. 1988. Inhibition of cell growth retains differentiated function of bovine parathyroid cells in monolayer culture. *Bone Miner.* 4(2):123.
- Olgaard, K., Arbeleaz, M., Schwartz, J., Klahr, S., and Slatopolsky, E. 1982. Abnormal skeletal response to parathyroid hormone in dogs with chronic uremia. *Calcif. Tissue Int.* 34(4):403.
- Omdahl, J.L., and DeLuca, H.F. 1977. Mediation of calcium adaptation by 1,25-dihydroxycholecalciferol. *J. Nutr.* 107:1975

- Omdahl, J.L., Hunsaker, L.A., Evan, A.P., and Torrez, P. 1980. In vitro regulation of kidney 25-hydroxyvitamin D₃-hydroxylase enzyme activities by vitamin D₃ metabolites. *J. Biol. Chem.* 255:7460.
- Parekh, A.C. and Jung, D.H. 1970. Serum inorganic phosphorus determination using p-phenylenediamine as a reducing agent. *Clin. Chem. Acta* 27:373.
- Perkin-Elmer Corp. 1965. Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer, Norwalk.
- Pike, J.W. 1991. Vitamin D₃ receptors: structure and function in transcription. *Annu. Rev. Nutr.* 11:189.
- Pike, J.W., Dokoh, S., Haussler, M.R., Liberman, U.A., Marx, S.J., and Eil, C.S. 1984. Vitamin D₃-resistant fibroblasts have immunoassayable 1,25-dihydroxyvitamin D₃ receptors. *Science* 224:879.
- Portale, A.A., Booth, B.E., Halloran, B.P., and Morris, R.C. Jr. 1984. Effects of dietary phosphorus on circulating concentrations of 1,25-dihydroxyvitamin D and immunoreactive parathyroid hormone in children with moderate renal insufficiency. *J. Clin. Invest.* 73:1580.
- Portale, A.A., Halloran, B.P., Murphy, M.M., and Morris, R.C. Jr. 1986. Oral intake of phosphorus can determine the serum concentration of 1,25-dihydroxyvitamin D by determining its production rate in human. *J. Clin. Invest.* 77:7.
- Reinhardt, T.A., and Horst, R.L. 1989. Self induction of 1,25-dihydroxyvitamin D metabolism limits receptor occupancy and target tissue responsiveness. *J. Biol. Chem.* 264(27):15917.
- Reinhardt, T.A., Horst, R.L., and Goff, J.P. 1988. *Vet. Clin. North Am.* 4:331.
- Reinhardt, T.A., Horst, R.L., Orf, J.W., and Hollis, B.W. 1984. A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: Application to clinical studies. *J. Clin. Endocr. Metab.* 58(1):91.
- Reiss, E., Canterbury, J., and Egdahl, R.H. 1968. Experience with a radioimmunoassay for parathyroid hormone in human sera. *Trans. Assoc. Am. Physiol.* 81:104.

- Ribovich, M.L., and DeLuca, H.F. 1978. 1,25-dihydroxyvitamin metabolism: The effect of dietary calcium and phosphorus. *Archiv. Biochem. Biophys.* 188:164.
- Rizk-Rabin, M., and Pavlovitch, J.H. 1988. Effect of vitamin D deficiency and 1,25-dihydroxycholecalciferol treatment on epidermal calcium-binding proteins (ECaBP) RNA activity. *Mol. Cell Endocrinol.* 60:145.
- Rodriguez, M., Felsenfeld, A.J., and Llach, F. 1991b. Calcemic response to parathyroid hormone in renal failure: Role of calciferol and the effect of parathyroidectomy. *Kidney Int.* 40:1063.
- Rodriguez, M., Martin-Malo, A., Martinez, M.E., Torres, A., Felsenfeld, A.J., and Llach, F. 1991a. Calcemic response to parathyroid hormone in renal failure: role of phosphorus and its effect on calcitriol. *Kidney Int.* 40:1055.
- Roth, S.I., and Raisz, L.G. 1966. The course and reversibility of the calcium effect on the ultrastructure of the parathyroid gland in organ culture. *Lab. Invest.* 15:1187.
- Rupp, G., Mayer, H., and Wingennder, E. 1990. The promotor of the human parathyroid hormone gene contains a functional cyclic AMP-responsive element. *Nucl. Acids Res.* 18:5677.
- Russell, J., Lettieri, D., Adler, J., and Sherwood, L.M. 1990. 1,25-dihydroxyvitamin D₃ has opposite effects on the expression of parathyroid secretory protein and parathyroid hormone genes. *Mol. Endocrinol.* 4:505.
- Russell, J., Lettieri, D., and Sherwood, L.M. 1983. Direct regulation of calcium of cytoplasmic messenger ribonucleic acid coding for pre-parathyroid hormone in isolated bovine parathyroid cells. *J. Clin. Invest.* 72:1851.
- Russell, J., Silver, J., and Sherwood, L.M. 1984. The effect of calcium and vitamin D metabolites on cytoplasmic mRNA coding for Pre-parathyroid hormone in isolated parathyroid cells. *Trans. Assoc. Am. Physicians* 97:296.
- Schelling, C.P., Didierjean, L., Rizk, M., Pavlovitch, J.H., Takagi, T., and Heinzmann, C.W. 1987. Calcium-binding proteins in rat skin. *FEBS Lett* 214:21.

- Schmeizer, H.J., Gross, G., Winder, G., and Mayer, H. 1987. Nucleotide sequence of a full length of cDNA clone encoding PreProParathyroid hormone from pig and rat. *Nucleic Acids Res.* 15:6740.
- Schwartz, Z., Knight, G., Swain, L.D., Boyan, B.D., 1988. Localization of vitamin D₃-responsive alkaline phosphatase in cultured chondrocytes. *J. Biol. Chem.* 263:6023.
- Sherwood, M., Mayer, G.P., Ramberg, C.F.Jr., Kronfeld, D.S., Aurbach, G.D., and Potts, J.T.Jr. 1968. Regulation of parathyroid hormone secretion: Proportional control by calcium, lack of effect of phosphate. *Endocrinology* 83:1043.
- Sigma Diagnostics. 1992. Creatinine quantitative, colorimetric determination in serum, plasma or urine at 500 nm. Sigma Diagnostics, St. Louis, MO 63178.
- Silver, J., and Naveh-Many, T. 1994. Regulation of parathyroid hormone synthesis and secretion. *Seminars in Nephrology* 14(2):175.
- Silver, J., Russell, J., and Sherwood, L.M. 1985. Regulation by vitamin D metabolites of messenger ribonucleic acid for preproparathyroid hormone in isolated bovine parathyroid cells. *Proc. Natl. Acad. Sci. USA.* 82:4270.
- Slatopolsky, E., Berkoben, M., Kelber, J., Brown, A., and Delmez, J. 1992. Effects of calcitriol and calcemic vitamin D analogues on secondary hyperparathyroidism. *Kidney Int.* 40 suppl. 38:S43.
- Slatopolsky, E., Gradowska, L., Kashemsant, C., Keltner, R., Manley, C., and Bricker, N.S. 1966. The control of phosphate excretion in uremia. *J. Clin. Invest.* 45(5):672.
- Slatopolsky, E., Robson, A.M., Elkan, J., and Bricker, N.S. 1968. Control of phosphate excretion in uremic man. *J. Clin. Invest.* 47:1865.
- Slatopolsky, E., and Bricker, N.S. 1973. The role of phosphorus restriction in the prevention of secondary hyperparathyroidism in chronic renal disease. *Kidney Int.* 4:142.

- Slatopolsky, E., Caglar, S., Gradowska, L., Canterbury, J., Reiss, E., and Bricker, N.S. 1972. On the prevention of secondary hyperparathyroidism in experimental chronic renal disease using "proportional reduction" of dietary phosphorus intake. *Kidney Int.* 2:147.
- Slatopolsky, E., Weerts, C., Thielan, J., Horst, R.L., Harter, H., and Martin, K.J. 1984. Marked suppression of secondary hyperparathyroidism by intravenous administration of 1,25-dihydroxycholecalciferol in uremic patients. *J. Clin. Invest.* 74:2136.
- Snedecor, G.W., and Cochran, W.G. 1980. *Statistical methods.* 7th ed. The Iowa State University Press. Ames, Iowa USA.
- Steele, T.H., Engle, J.E., Tanaka, Y., Lorenc, R.S., Dudgeon, K.L. and DeLuca, H.F. 1975. Phosphatemic actions of 1,25-dihydroxyvitamin D₃. *Am. J. Physiol.* 227:489.
- Summerville, B.A., Swaminathan, R., and Care, A.D. 1978. A comparison of the effect dietary calcium and phosphorus deficiency on the in vitro and in vivo metabolism of 25-hydroxycholecalciferol in the chick. *Br. J. Nutr.* 39:411.
- Svensson, O., Wernerson, A., and Reinholt, F.P. 1988. Effect of calcium depletion on the rat parathyroids. *Bone Miner* 3:259.
- Szpirer, J., Szpirer, C., Riviere, M., Levan, G., Marynen, P., Cassiman, J.J., Wiess, R., and DeLuca, H.F. 1991. The SP1 transcription factor gene (SP1) and the 1,25-dihydroxyvitamin D₃ receptor gene (VDR) are colocalized on human chromosome arm 12q and rat chromosome 7. *Genomics* 11(1):168.
- Tan, S., Pesigan, M. Lorr, E., Crockett, R., Ondrizek, T., and Lau, K. 1989. Pathogenetic role of increased tubule cytosolic free Ca concentration ($[Ca^{2+}]_i$) in progressive renal insufficiency: Amelioration by a low PO₄ diet. *Kidney Int.* 350:438A.
- Tanaka, Y., and DeLuca, H.F. 1973. The control of 25-hydroxyvitamin D metabolism by inorganic phosphorus. *Arch. Bioch. Biophys.* 154:566.
- Tanaka, Y., Frank, H., and DeLuca, H.F. 1973. Intestinal calcium transport: Stimulation by low phosphorus diets. *Science* 181:564.

- Taylor, C.M., Caverzasio, J., Jung, A., Trechsel, U., Fleisch, H., and Bonjour, J.P. 1983. Unilateral nephrectomy and 1,25-dihydroxyvitamin D₃. *Kidney Int.* 24(1):37.
- Teresita, B., and Ricardo, B. 1991. Effect of 1,25-dihydroxyvitamin D₃ on phosphate accumulation by myoblasts. *Horm. Metab. Res.* 23:113.
- Varghese, S., Lee, S., Huang, Y-C., and Christakos, S. 1988. Analysis of vitamin D-dependent calbindin-D_{28K} gene expression. *J. Biol. Chem.* 263:9776.
- Von Heijine, G. 1983. Patterns of amino acids near signal sequence cleavage sites. *Eur. J. Biochem.* 133:17.
- Walters, M.R. 1992. Newly identified actions of the vitamin D endocrine system. *Endocrine Reviews* 13(4):719.
- Walters, M.R., Bruns, M.E., Carter, R.M., and Riggle, P.C. 1991. Vitamin D-independence of small calcium binding proteins in nonclassical target tissues. *Am. J. Physiol.* 260:E794.
- Walters, M.R., Hebert, G., Ilenchuk, T.T., Riggle, P.C., and Claycomb, W.C. 1987. Further evidence for 1,25-dihydroxyvitamin D effects in new targets: Heart and Lung. *Excerpta Med. Int.* 735:479.
- Waynforth, H.B. 1980. Experimental and surgical technique in the rat. Academic Press, New York. pp.159.
- Wernerson, A., Svensson, O., and Reinholt, F.P. 1989. Parathyroid cell number and size in hypercalcemic rats: A stereologic study employing modern unbiased estimators. *J. Bone Miner Res* 4:705.
- Wilson, L., Felsenfeld, A., Drezner, M.K., and Llach, F. 1985. Altered divalent ion metabolism in early renal failure: Role of 1,25-(OH)₂D₃. *Kidney Int.* 27:565.
- Yamaguchi, D.T., Hahn, T.J., Ilda-Klein, A., Kleeman, C.R., and Muallem, S. 1987. Parathyroid hormone-activated calcium channels in an osteoblast-like clonal osteosarcoma cell line. cAMP-dependent and cAMP-independent calcium channels. *J. Biol. Chem.* 262:7711.

- Yamamoto, M., Igarashi, T., Muramatsu, M., Fukagawa, M., Motokura, T., and Ogata, E. 1989. Hypocalcemia increases and hypercalcemia decreases the steady-state level of parathyroid hormone messenger RNA in the rat. *J. Clin. Invest.* 83(3):1053.
- Zelinski, J.M., Sykes, D.E., and Weiser, M.M. 1991. The effect of vitamin D on rat intestinal plasma membrane Ca-pump mRNA. *Biochem. Biophys. Res. Commun.* 179:749.

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APPENDIX A

FEEDING HIGH PHOSPHORUS DIETS SUPPRESSES PLASMA
1,25-DIHYDROXYVITAMIN D₃ PRODUCTION VIA RETENTION
OF PLASMA PHOSPHATE IN UNILATERALLY NEPHRECTOMIZED RATS

ABSTRACT

Secondary hyperparathyroidism (SHPT) is a complication of chronic renal failure. The two major pathogenic mechanisms responsible for the development of SHPT in advanced renal insufficiency are phosphorus retention and low levels of 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃]. Therefore, feeding of nephrectomized rats (reduced renal cell mass) with increasing levels of dietary phosphorus subsequently enhances plasma parathyroid hormone (PTH) and depresses plasma 1,25-(OH)₂D₃. In this study, 40 rats (20 normal and 20 nephrectomized) were fed increasing levels of phosphorus in the diet. The Ca/P % in the diets were 0.47/0.68, 0.36/2.55, 0.35/2.99, and 0.47/4.00 on an as fed basis. Blood samples were collected after 1st, 2nd, and 3rd months of feeding the diets for the control rats, whereas it was only after the 1st and 2nd months feeding for the nephrectomized rats. Plasma phosphate increased for both groups. But declined after the 2nd month for the nephrectomized rats. Plasma calcium also increased for both groups for the 1st month but declined the 2nd month for the nephrectomized rats. However, the 3rd month's plasma calcium was found to be lower than the 2nd month's plasma calcium for the control rats. Plasma 1,25-(OH)₂D₃ was found to be declining all the way through the months for both groups. However, the rate of

decline was higher in the nephrectomized rats. Plasma creatinine was found to be higher for the nephrectomized rats as compared to the control rats.

Although a uniform pattern of the data did not occur, increasing phosphorus in the diet depressed the plasma 1,25-(OH)₂D₃. Furthermore, increasing phosphorus in the diet elevated plasma phosphate significantly. However, the declining trend of plasma calcium and phosphate, particularly in the nephrectomized rats after the 1st month, must be verified by further research. Equally important is the duration of the study. The longer the nephrectomized rats stayed on the diets, the less appetite they had for the diets and hence the more body weight they lost.

INTRODUCTION

Renal insufficiency is characterized by several alterations in mineral homeostasis (Lopez-Hilker et al., 1990). Secondary hyperparathyroidism (SHPT) is present even in the early stages of renal insufficiency and leads to the development of renal osteodystrophy (Slatopolsky and Bricker, 1973; Wilson et al., 1985; Tessitore et al., 1987; Pitts et al., 1988; Kubrusly et al., 1993).

The two major pathogenic mechanisms responsible for the development of SHPT in advanced renal insufficiency are

phosphorus retention and low levels of 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃] (Lopez-Hilker et al., 1990).

Hyperphosphatemia results in hypocalcemia by several possible mechanisms, including inhibition of the activity of the renal enzyme 1 α -hydroxylase (1 α -OHase) which is already limited by the decrease in renal mass (Lopez-Hilker et al., 1990). Therefore, low levels of 1,25-(OH)₂D₃ lead to decreased calcium transport by the gastrointestinal tract (Coburn et al., 1973), altered synthesis and secretion (Slatopolsky et al., 1984; Silver et al., 1985; Chan et al., 1986) of parathyroid hormone (PTH) and possibly skeletal resistance to the calcemic actions of PTH (Lopez-Hilker et al., 1990).

1,25-(OH)₂D₃ is the metabolite of vitamin D currently considered to be the most biologically active with respect to bone resorption and intestinal absorption of calcium and phosphorus (Portale et al., 1984).

The synthesis of 1,25-(OH)₂D₃ from its endogenous precursor, 25-hydroxyvitamin D₃ (25-OHD₃), occurs in mitochondria of the proximal renal tubule and is catalyzed by the enzyme 1 α -OHase (Kawashima et al., 1981). In patients with severe (Chesney et al., 1982; Cheung et al., 1983) and moderate (Portale et al., 1982) reductions in the plasma concentrations of 1,25-(OH)₂D₃ presumably reflect a reduction in the renal synthesis of 1,25-(OH)₂D₃ in the proximal tubule of the kidney (Lopez-Hilker et al., 1990).

In patients with renal insufficiency, reduction in 1,25-(OH)₂D₃ reflects a reduced amount of 1 α -OHase, but could also reflect a reversible suppression of the activity of the remaining enzyme (Hausler and McCain, 1977; Van Stone et al., 1977) even though the activity of this enzyme is normally stimulated by PTH (Norman et al., 1980; Lopez-Hilker, 1984).

Reports indicate that the activity of 1 α -OHase and apparent production of 1,25-(OH)₂D₃ are not principally determined by PTH, but rather by a suppressive effect of inorganic phosphorus (Broadus et al., 1983).

Support for the major role of phosphorus retention has been provided by several investigators who have shown that decreasing the amount of phosphorus in the diet in proportion to the fall in glomerular filtration rate (GFR) prevents SHPT (Slatopolsky et al., 1972) or ameliorates (Kaplan et al., 1979; Portale et al., 1984; Llach and Massry, 1985). The mechanism of this effect remains unclear (Lopez-Hilker et al., 1990).

In normal humans (Portale et al., 1986) and patients with moderate renal insufficiency (Portale et al., 1984; Llach and Massry, 1985), restriction of dietary phosphorus increases the production of 1,25-(OH)₂D₃, thus, decreasing the levels of immunoreactive PTH (iPTH). However, this mechanism may not be operative in advanced renal insufficiency, because the decrease in renal mass may severely limit the production of

1,25-(OH)₂D₃ (Lopez-Hilker et al., 1990).

Therefore, the present study was designed to investigate if plasma phosphate retention could be associated with reduced plasma 1,25-(OH)₂D₃ production in unilaterally nephrectomized rats used as SHPT model.

Materials and Methods

Animals

Forty male Sprague-Dawley-Harlan 60-day-old rats weighing 450 ± 50 gm were used in this study. All rats were housed individually and fed a standard laboratory rat chow until the beginning of the experiment.

When the adaptation period was over the rats were randomly divided into two groups, control (20 rats) and treatment (20 rats) and each group again divided into four subgroups. Then, they were assigned to synthetic diets having different combinations of calcium and phosphorus as indicated below. Water was always offered ad libitum.

The treatment rats were ventrodorsally shaved and anesthetized with intraperitoneal injection of 60 mg/kg Ketamine and their entire left kidney was removed (Lieuallen et al., 1990). Following the removal of the kidneys, the rats were maintained on standard laboratory rat chow for seven days of recovery. They were housed and maintained according to

Federal regulation regarding animal care.

Diets

A low calcium diet (0.02% calcium) was purchased from Teklad, Madison, WI and fortified with different levels of calcium and phosphorus using either calcium phosphate (CaHPO_4), calcium carbonate (CaCO_3) or sodium phosphate (Na_2HPO_4) (laboratory grade, Fisher Scientific, Fairlawn, NJ) as source materials. The levels of vitamin D remained unaltered. Thus, there were four diets (D1, D2, D3 and D4) assigned to each control and treatment subgroups as below. Calcium and phosphorus levels were expressed on an as fed basis.

Diet	Ca (%)	P (%)	Ca:P Ratio
D1	0.47	0.68	1:1.45
D2	0.36	2.55	1:7.08
D3	0.35	2.92	1:8.34
D4	0.46	4.00	1:8.70

Body weight measurements

Body weight measurements (gm) were made for each rat at the beginning and at the end of the experiment. Body weight changes (%) were calculated from the differences between the beginning and end body weights expressed as a percentage.

Feed intake

Daily feed intake (gm/day) was measured by offering the rats known amounts of the diets in aluminium dishes (purchased from Wal-Mart store). Every day the left overs were weighed and subtracted the diets offered upon which the daily intakes were found by differences.

Urine collection

Daily urine was collected from each rat in a metabolic cage. Then, urine calcium (mg/day), phosphorus (mg/day) and creatinine (mg/day) were determined according to Perkin-Elmer Corp (1965), Parekh and Jung (1970), and Chasson et al. (1961), respectively.

Blood sample collection and plasma analyses

Blood samples (1.5 - 2.0 ml) were collected from the external jugular vein except the final sampling.

At the end of third and second months of feeding (control and treatment, respectively), all rats were anesthetized under CO₂-O₂ (50%:50% vol/vol) and decapitated. Blood sample was collected in heparinized tubes and centrifuged for 5 minutes at 2300 rpm. Plasma was aspirated and refrigerated in the deep freezer until analysis.

Plasma analyses were made for calcium (Perkin-Elmer corp., 1965), phosphorus (Parekh and Jung, 1970), 1,25-(OH)₂D₃

(Reinhardt et al., 1984) and creatinine (Chasson et al., 1961).

Statistical analysis

One way analysis of variance (ANOVA) was used to test the hypothesis that the means difference between the control and unilaterally nephrectomized rats were the same. Results were considered significant when probability was less than 0.05 (Snedecor and Cochran, 1980).

RESULTS

The mean of feed intake (gm/day) of control and nephrectomized rats is presented in Table 3. Feed intake was decreased as the level of phosphorus increased in the diets. But there was no significant change among the subgroups except the nephrectomized subgroup on 0.46/4.00 Ca/P percentage which showed to consume more feed (18.47 ± 0.71) than the other subgroups. This higher value was attributed to wastages and the real consumption was much less than that.

The mean plasma phosphate concentration (mg/dl) for the control and nephrectomized rats for the 1st, 2nd, and 3rd months is shown in Figure 13. The mean plasma phosphate of nephrectomized rats was higher than the control rats during the 1st month, however, it declined during the 2nd month. As the

level of phosphorus increased in the diets, the plasma phosphate increased.

The mean plasma calcium concentration (mg/dl) for the control and nephrectomized rats fed increasing levels of phosphorus in the diet for 3 and 2 consecutive months, respectively, is presented in Figure 14. During the 1st month there was no difference between the control and nephrectomized rats, although, a trend has been observed that plasma calcium declined as diet phosphorus level increased in the diets. In the second month, plasma calcium was depressed in the nephrectomized rats, although there was a slight increase in the control rats. In the third month, plasma calcium in nephrectomized rats was found to be significantly lower than the control rats.

The mean plasma 1,25-(OH)₂D₃ (pg/ml) for the control and nephrectomized rats fed increasing levels of phosphorus in diets is presented in Figure 15. There was a progressive decline in plasma 1,25-(OH)₂D₃ concentration in both the control and the nephrectomized rats throughout the study period. However, the values observed for the nephrectomized rats were much lower than the control rats throughout the study period. Therefore, increasing levels of phosphorus in diets, together with reduced renal cell mass, reduced plasma 1,25-(OH)₂D₃ in the nephrectomized rats.

The mean plasma creatinine (mg/dl) of control and

nephrectomized rats fed increasing levels of phosphorus in diets is presented in Figure 16. Plasma creatinine of nephrectomized rats was higher than the control rats during the first and second months of the study period.

The mean urine volume (ml/day), urine phosphorus (mg/day), urine calcium (mg/day), and urine creatinine (mg/day) are presented in Figures 17, 18, 19, and 20, respectively. The urine volume of nephrectomized rats was found to be much higher than the control rats and increased as the level of phosphorus increased in the diets. Urine phosphorus also showed an increasing trend as the level of phosphorus increased in the diets. Urine calcium did not show much difference between the control and nephrectomized rats. But, urine creatinine of nephrectomized rats was increased as the level of phosphorus increased in diets.

Table 2. Mean body weight changes (%) of two groups of rats
(control and nephrectomized) fed increasing
levels of phosphorus in the diets

Ca/P (%)	Control*	Nephrectomized*	P value
0.47/0.68	89.22 ± 6.24	28.62 ± 4.20	P<0.001
0.36/2.55	31.00 ± 5.32	- 5.77 ± 8.36	P<0.05
0.35/2.92	22.73 ± 4.42	- 1.68 ± 7.69	ns
0.46/4.00	24.57 ± 14.09	- 20.44 ± 0.46	P<0.05

+ = gain in body weight.

- = loss in body weight.

ns = not significant.

* = mean ± SEM (standard error of mean).

Table 3. Mean feed intake (gm/day) of two groups of rats
(control and nephrectomized) fed increasing levels of
phosphorus in the diets.

Ca/P (%)	Control*	Nephrectomized*	P value
0.47/0.68	22.93 ± 0.65	22.22 ± 3.12	ns
0.36/2.55	17.10 ± 3.31	17.17 ± 2.56	ns
0.35/2.92	14.27 ± 2.00	8.82 ± 1.30	ns
0.46/4.00	13.47 ± 2.70	18.47 ± 0.71	ns

* = mean ± SEM.

ns = not significant

Figure 13. Mean plasma phosphate (mg/dl) of two groups of rats control and nephrectomized) fed increasing levels of phosphorus in the diets. Plasma phosphate was determined at the 1st, 2nd, and 3rd months of feeding the diets. The calcium/phosphorus percentages were 0.47/0.68, 0.36/2.55, 0.35/2.92, and 0.46/4.00 on an as fed basis. Results are expressed as mean \pm SEM (standard error of mean). Insert is plasma phosphate from normal rats fed laboratory rat chow for three months.

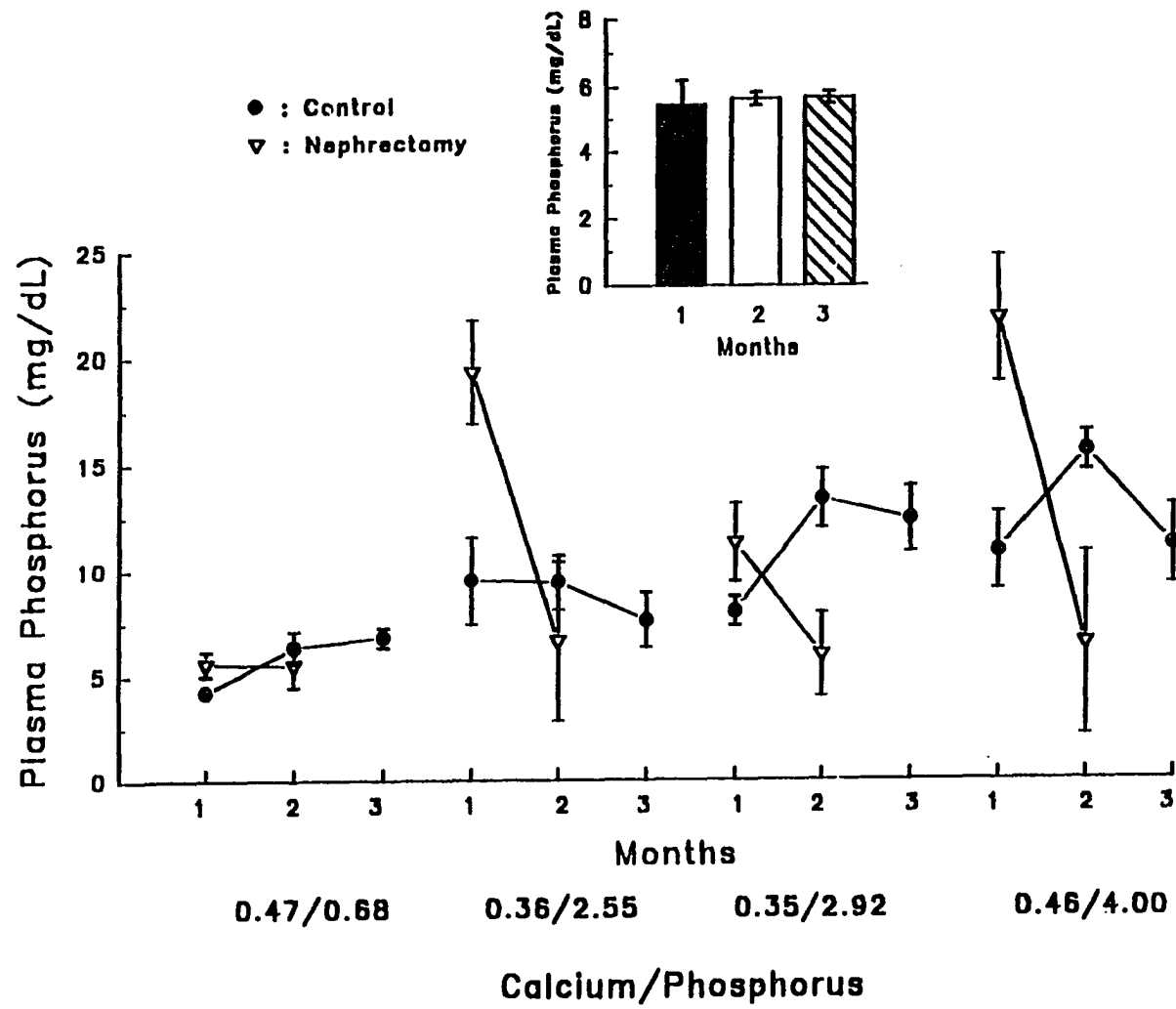


Figure 14. Mean plasma calcium (mg/dl) of two groups (control and nephrectomized) of rats fed increasing levels of phosphorus in the diets. Plasma calcium was determined at 1st, 2nd, and 3rd months of feeding the diets. The calcium/phosphorus percentages were 0.47/0.68, 0.36/2.55, 0.35/2.92, and 0.46/4.00 on an as fed basis. Results are expressed as mean \pm SEM. Insert is plasma calcium from normal rats fed laboratory rat chow for three months.

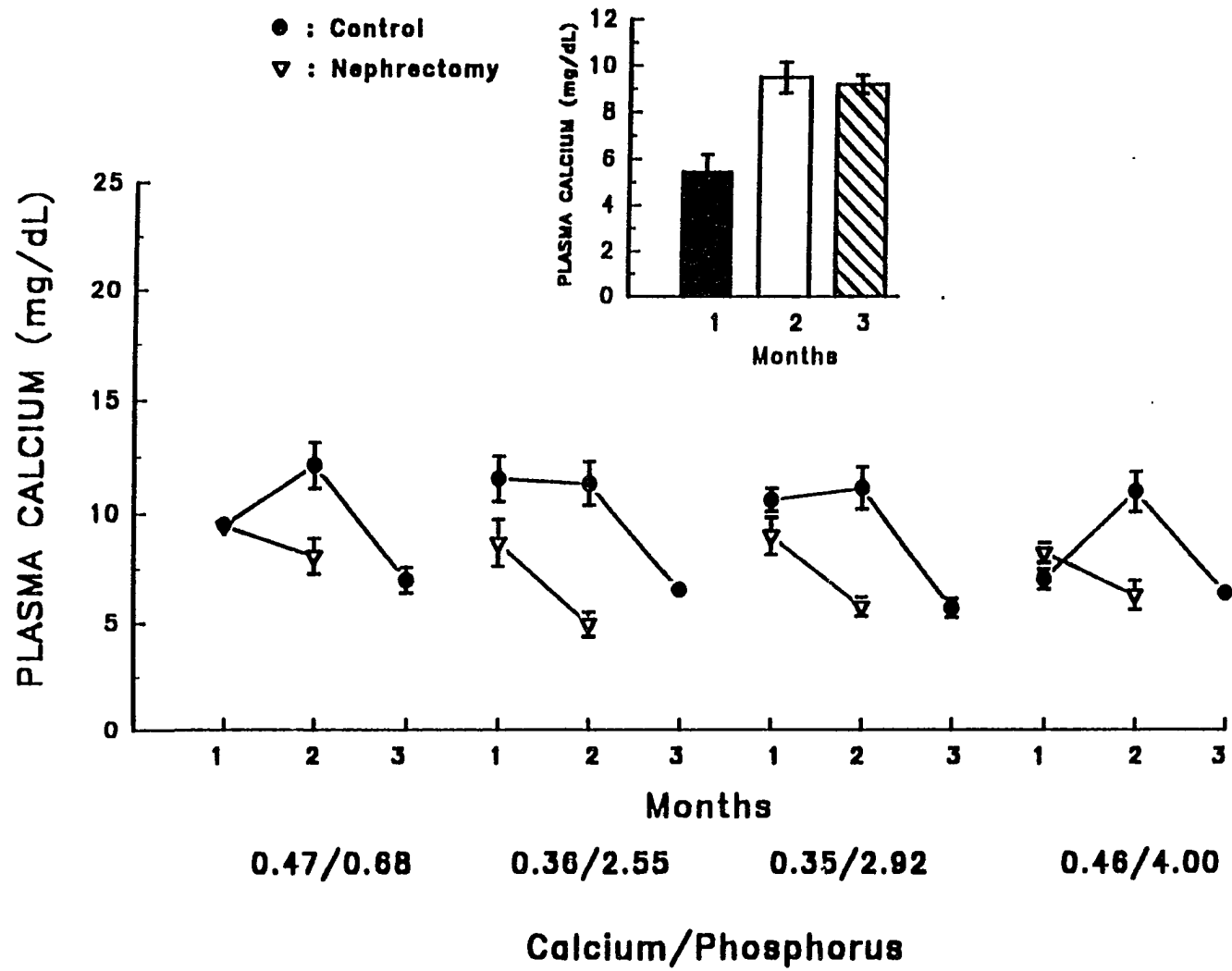


Figure 15. Mean plasma 1,25-(OH)₂D₃ (pg/ml) of two groups (control and nephrectomized) of rats fed increasing levels of phosphorus in the diets. Plasma 1,25-(OH)₂D₃ was determined at 1st, 2nd, and 3rd months of feeding the diets. The calcium/phosphorus percentages were 0.47/0.68, 0.36/2.55, 0.35/2.92, and 0.46/4.00 on an as fed basis. Results are expressed as mean ± SEM. Insert is plasma 1,25-(OH)₂D₃ from normal rats fed laboratory rat chow for three months.

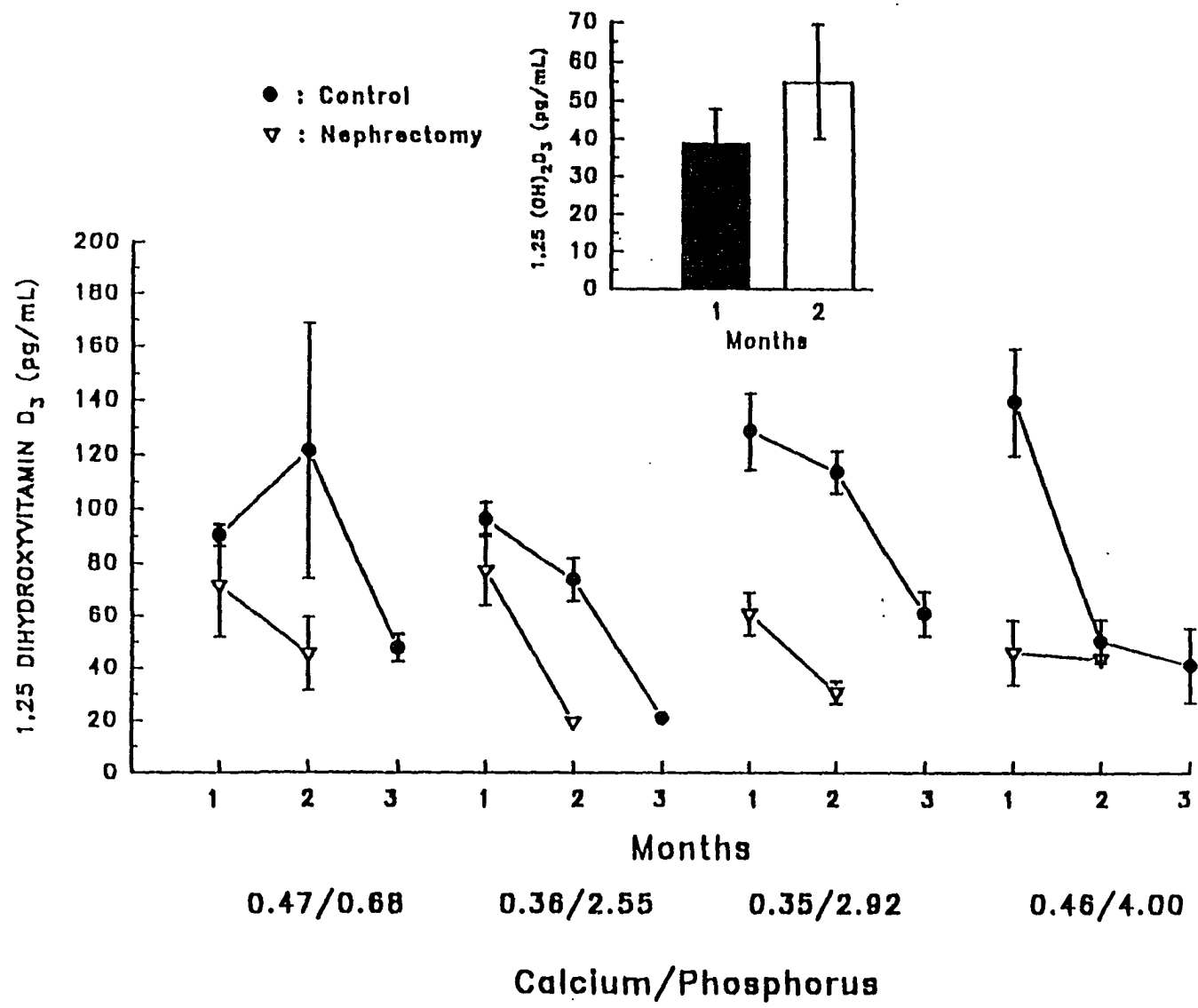


Figure 16. Mean plasma creatinine (mg/dl) of two groups (control and nephrectomized) of rats fed increasing levels of phosphorus in the diets. Plasma creatinine was determined at 1st, 2nd, and 3rd months of feeding the diets. The calcium/phosphorus percentages were 0.47/0.68, 0.36/2.55, 0.35/2.92, and 0.46/4.00 on an as fed basis. Results are expressed as mean \pm SEM. Insert is plasma creatinine from normal rats fed laboratory rat chow for three months.

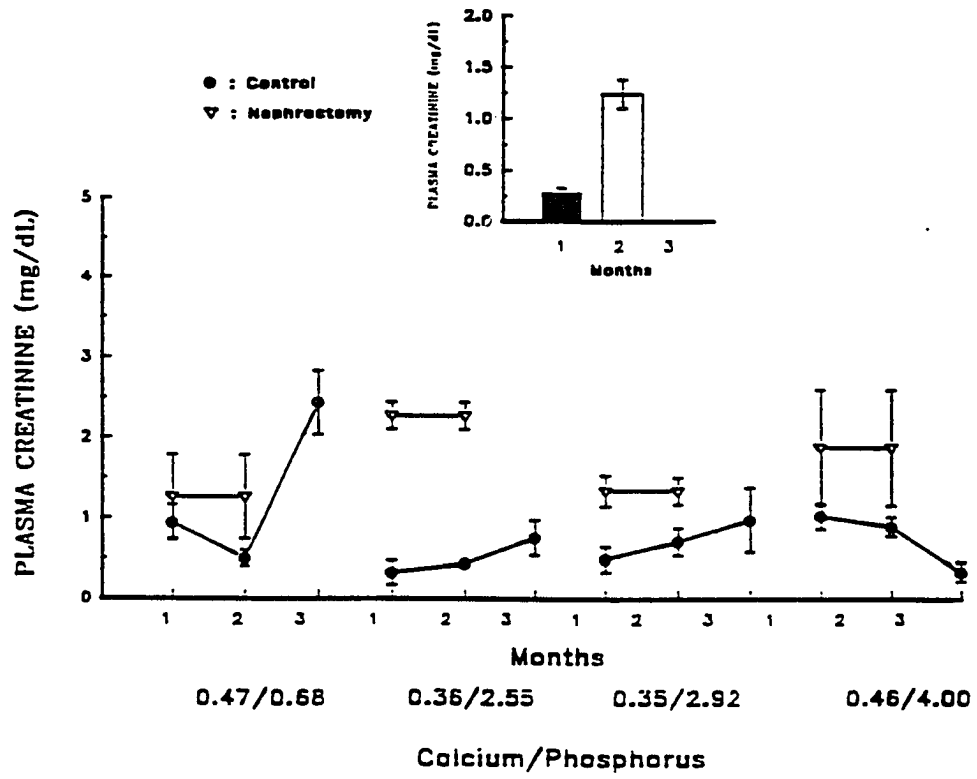


Figure 17. Mean urine volume (ml/day) of two groups (control and nephrectomized) rat fed increasing levels of phosphorus in the diets. Urine volume was collected from two rats from each groups for seven days. The calcium/phosphorus percentages were 0.47/0.68, 0.36/2.55, 0.35/2.92, and 0.46/4.00 on an as fed basis.

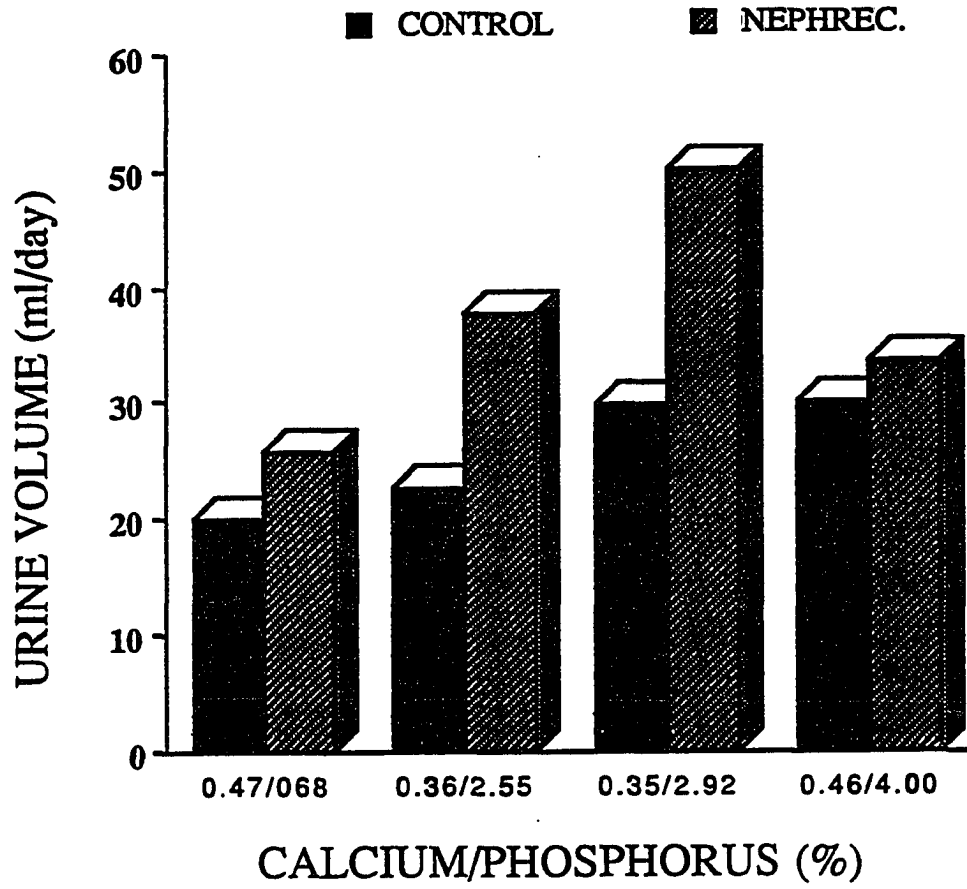


Figure 18. Mean urine phosphorus (mg/day) of two groups (control and nephrectomized) rats fed increasing levels of phosphorus in the diets. Urine phosphorus was determined from urine of two rats from each groups collected for seven days. The calcium/phosphorus percentages were 0.47/0.68, 0.36/2.55, 0.35/2.92, and 0.46/4.00 on an as fed basis.

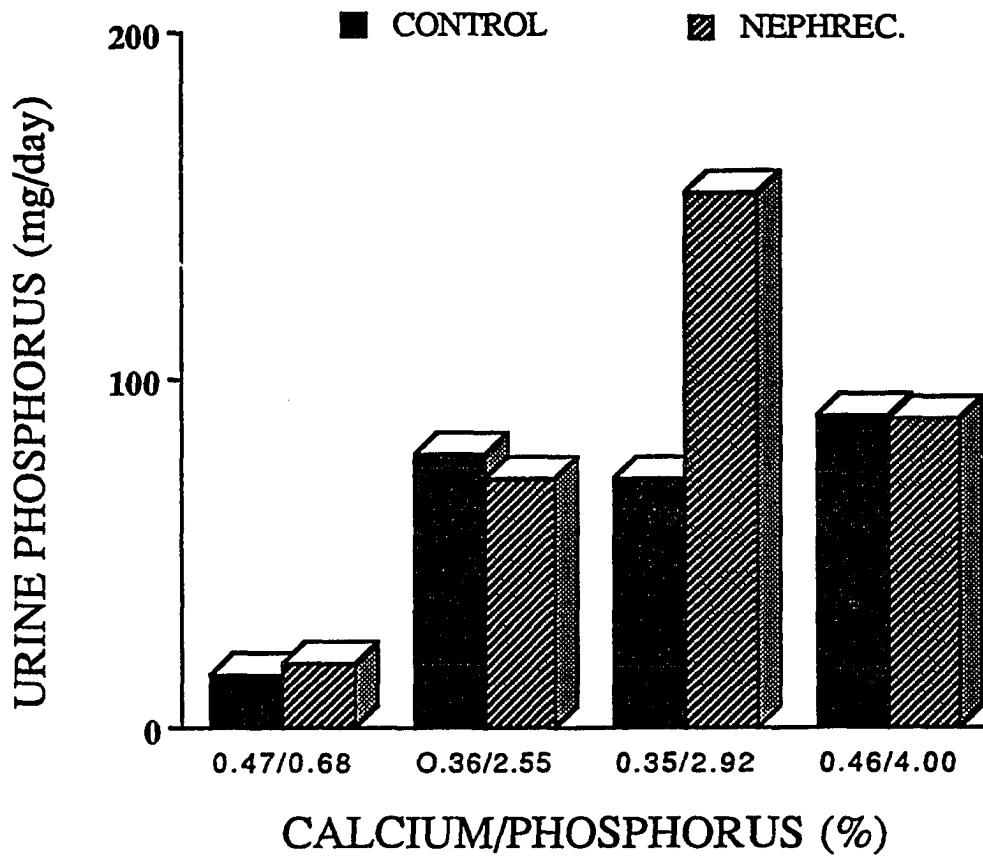


Figure 19. Mean urine calcium (mg/day) of two groups (control and nephrectomized) of rats fed increasing levels of phosphorus in the diets. Urine calcium was determined from urine of two rats from each groups collected for seven days. The calcium/phosphorus percentages were 0.47/0.68, 0.36/2.55, 0.35/2.92, and 0.46/4.00 on an as fed basis.

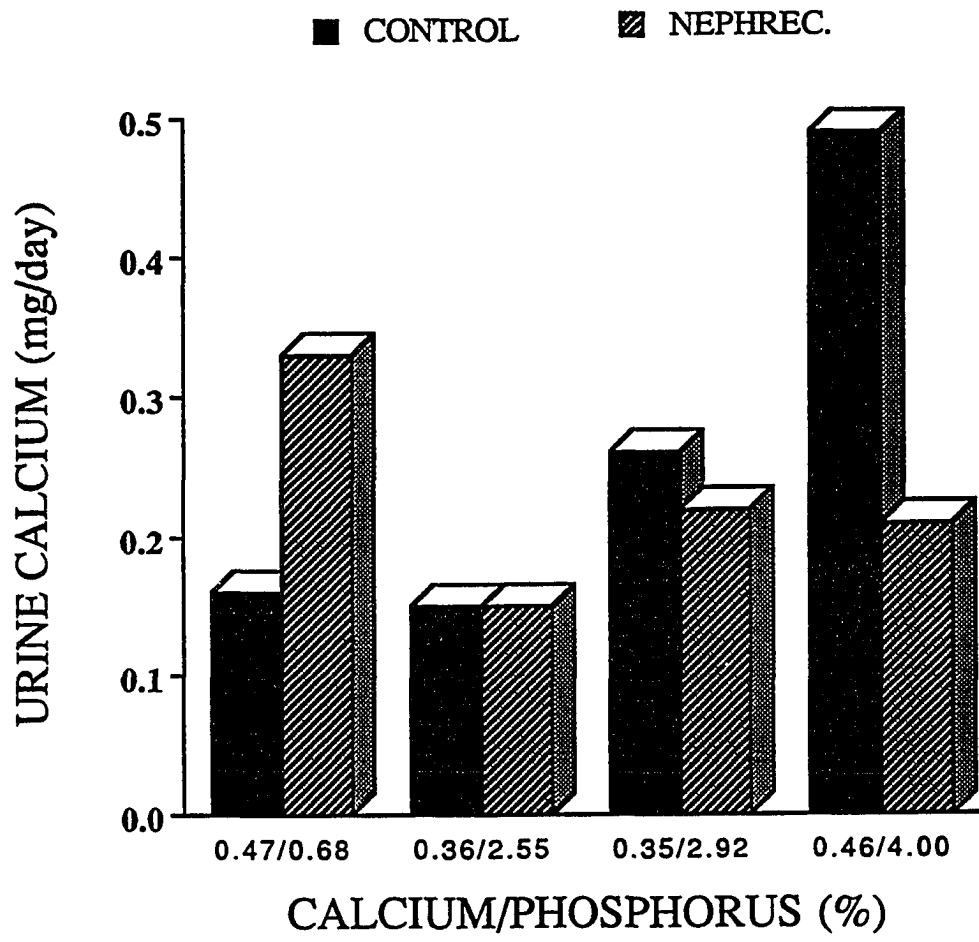
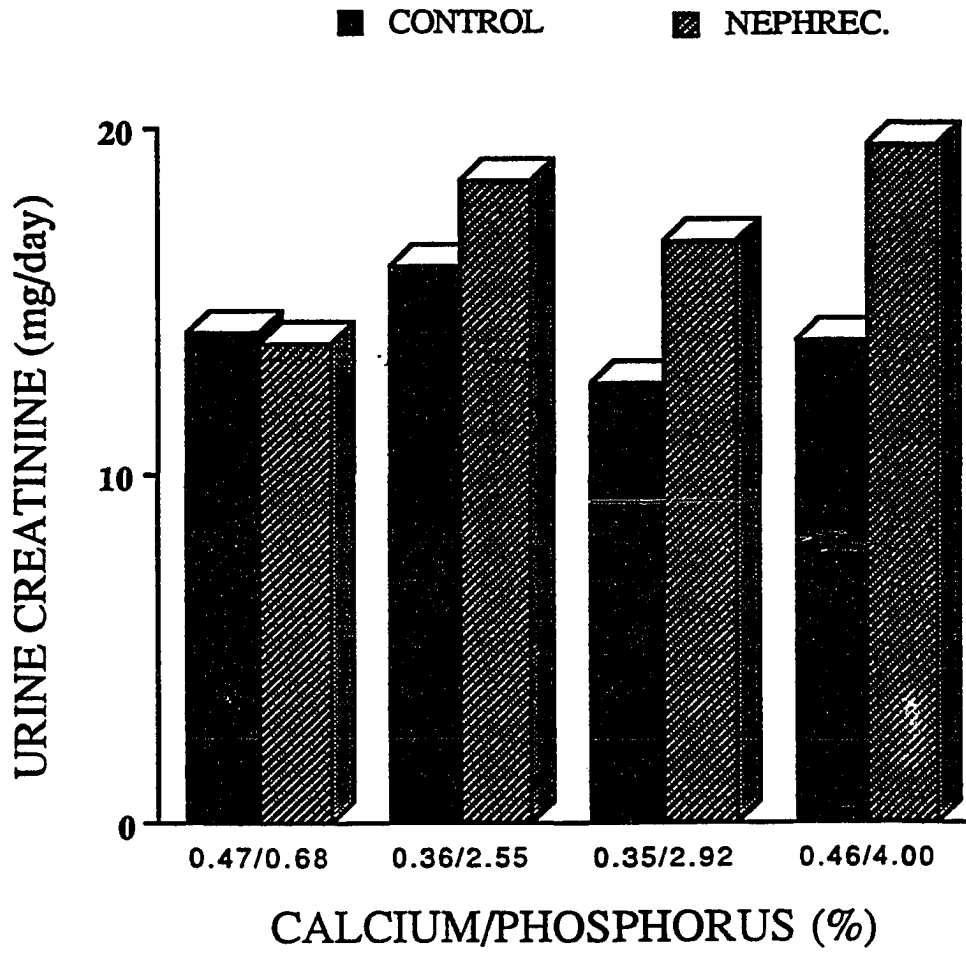


Figure 20. Mean urine creatinine (mg/day) of two groups (control and nephrectomized) of rats fed increasing levels of phosphorus in the diets. Urine creatinine was determined from urine of two rats from each group collected for seven days. The calcium/phosphorus percentages were 0.47/0.68, 0.36/2.55, 0.35/2.92, and 0.46/4.00 on an as fed basis.



DISCUSSION

Feeding increasing levels of phosphorus in the diets to control (normal) and nephrectomized rats showed that the feed intake was progressively decreased as the dietary phosphorus level increased. But it seems likely that the control rats ate more than the nephrectomized rats. There was a wastage of diets on both sides, however, the degree of wastage was higher in the nephrectomized rats. Nephrectomized rats showed to tear the aluminium dishes (used for feeding) and did not have appetite for the diets. Moreover, they had a greater tendency to drink more water as compared to the control rats. Because of these factors and others, nephrectomized rats were emaciated and lost much of their body weights (as much as 60% in some cases) during the study. Consequently, most of them died and the study was terminated at the end of the second month. Therefore, the study must have been limited to a duration of one month where data should have been collected either every 5 or 10 days apart. But this was not done in this study. Thus, keeping the rats on the diets for the duration of 3 months did not contribute much either in information collection nor improvement of the data.

Feeding increasing levels of phosphorus in the diets to control and nephrectomized rats progressively decreased feed intake (data not shown) as the phosphorus levels in the diets

increased. Particularly, nephrectomized rats did not have appetite for the diets and they spent most of their time around waterers. Because of these factors and others, they were emaciated and lost much of their body weight (as much as 60%) (data not shown) during the study. Consequently, most of them died and the study was terminated at the end of the second month. Therefore, the study should have been limited to a duration of one month where data should have been collected either every 5 or 10 days apart. But this was not done in this study. Thus, keeping the rats on the diets for 3 months did not contribute much either in information collection nor improvement of the data.

The present study demonstrated that feeding increasing levels of phosphorus in the diets reduced the plasma 1,25-(OH)₂D₃ both in the control and nephrectomized rats. Several reports indicate that restriction of dietary phosphorus can induce an increase in the serum concentration of 1,25-(OH)₂D in normal men and women (Gray et al., 1977; Insogna et al., 1983; Lufkin et al., 1983; Maierhofer et al., 1984) and in children with moderate renal insufficiency (Portale et al., 1986). Conversely, supplementation decreases serum concentration of 1,25-(OH)₂D from supernormal to normal levels (Portale et al., 1986). It has been inferred that these phosphorus-induced changes in serum concentration of 1,25-(OH)₂D reflect changes in the rate of its renal production,

because in the chick and rat, restriction of dietary phosphorus can increase both the activity of renal 1α -OHase (Baxter and DeLuca, 1976) and the synthetic rate of $1,25-(\text{OH})_2\text{D}_3$ (Gray and Napoli, 1983; Lobaugh and Drezner, 1983) when measured in vitro (Portale *et al.*, 1986). Furthermore, when phosphorus was restricted and then supplemented, the observed increase and decrease in serum concentration of $1,25-(\text{OH})_2\text{D}$ was associated with an increase and decrease, respectively in the production rate of the hormone, but not with a significant change in its metabolic clearance rate (Portale *et al.*, 1986). Thus, the findings of Portale *et al.* (1986) demonstrated that in healthy men, changes in production rate of $1,25-(\text{OH})_2\text{D}$ account entirely for the phosphorus-induced changes in serum concentration of this hormone as opposed to increased turnover of hormone.

During chronic renal failure, the number of functioning renal tubules progressively decreases (Nagode *et al.*, 1992). Because of the loss of the tubular cells that produce $1,25-(\text{OH})_2\text{D}$, its synthesis becomes limited.

$1,25-(\text{OH})_2\text{D}$ formation is also affected by the powerful inhibition of the 1α -hydroxylation of 25-hydroxyvitamin D (25-OHD) by high levels of blood phosphorus (Slatopolsky *et al.*, 1972; Slatopolsky and Bricker, 1973; Feinfeld and Sherwood, 1988).

As serum phosphorus levels increase following reduced

glomerular filtration rates (GFRs), serum phosphorus concentrations are achieved that block synthesis of 1,25-(OH)₂D₃ (Nagode et al., 1992).

The importance of the inhibitory effect of phosphorus on canine 1,25-(OH)₂D has been shown previously by Nagode and Steinmeyer (1979). It is revealed by the more regular drop in 1,25-(OH)₂D with increasing levels of serum phosphorus. Although, high levels of inorganic phosphorus (P_i) inhibit 1,25-(OH)₂D, lowering serum P_i below normal does not raise serum 1,25-(OH)₂D above normal in the dog (Nagode and Steinmeyer, 1982).

The following statements could be summarized from this study:

1. Feed intake declined in both control and nephrectomized rats as the level of phosphorus increased in the diets.
2. Increasing dietary phosphorus increased plasma phosphate both in the control and nephrectomized rats.
3. Plasma calcium of control rats was higher than in the nephrectomized rats.
4. Plasma 1,25-(OH)₂D₃ progressively declined both in the control and nephrectomized rats as the level of phosphorus increased in the diets.
5. Plasma creatinine of nephrectomized rats was higher than the control rats.
6. Urine volume was higher in the nephrectomized rats and

increased as the level of phosphorus increased in the diets. Thus, failure to concentrate urine is the first sign of renal failure.

References

- Baxter, L.A., and DeLuca, H.F. 1976. Stimulation of 25-hydroxyvitamin D₃-1 α -hydroxylase by phosphate depletion. *J. Biol. Chem.* 251:3158.
- Broadus, A.E., Magee, J.S., Mallette, L.E., Horst, R.L., Lang, R., Jensen, P.S., Gertner, J.M., and Baron, R. 1983. A detailed evaluation of oral phosphate therapy in selected patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* 56:953.
- Chan, Y.L., McKay, C., Dye, E., and Slatopolsky, E. 1986. The effect of 1,25-dihydroxyvitamin D₃ suppresses parathyroid hormone secretion from bovine parathyroid cells in tissue culture. *Endocrinology* 117:2114.
- Chasson, A.L., Grady, H.J., and Stanley, M.A. 1961. Determination of creatinine by means of automatic chemical analysis. *Am. J. Clin. Pathol.* 35:83.
- Chesney, A.K., Manolagas, S.C., Catherwood, B.D., Mosely, C.A.Jr., Mitas, J.A.II., Blantz, R.C., and Deftos, L.J. 1983. Determinants of serum 1,25-(OH)₂D levels in renal disease. *Kidney Int.* 24:104.
- Coburn, J.W., Hartenbower, D.L., and Massry, S.G. 1973. Intestinal absorption of calcium and the effect of renal insufficiency. *Kidney Int.* 4:96.
- Feinfeld, D.A., and Sherwood, L.M. 1988. Editorial Review: Parathyroid hormone and 1,25-dihydroxyvitamin D, intestinal calcium transport, and bone turnover in infant dogs fed diets varying in calcium and phosphorus, in Norman, A.W., Schaefer, K., Herrath, D.V., and Grigoleit, H-G. (eds): *Vitamin D: Chemical, Biochemical, and Clinical Endocrinology of calcium metabolism.* W. de Gruyter, New York. pp.487.

- Gray, R.W., and Napoli, J.L. 1983. Dietary phosphate deprivation increases 1,25-dihydroxyvitamin D₃ synthesis in rat kidney in vitro. *J. Biol. Chem.* 258:1152.
- Gray, R.W., Wilz, D.R., Caldas, A.E., and Lemann, J.Jr. 1977. The importance of phosphate in regulating plasma 1,25-(OH)₂D-vitamin D levels in human: studies in healthy subjects, in calcium-stone forms and in patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* 45:299.
- Haussler, M.R., and McCain, T.A. 1977. Basic and clinical concepts related to vitamin D metabolism and action. *N. Engl. J. Med.* 297:974; 1041.
- Insogna, K.L., Broadus, A.E., and Gertner, J.M. 1983. Impaired phosphorus conservation and 1,25-dihydroxyvitamin D generation during phosphorus deprivation in familial hypophosphatemic rickets. *J. Clin. Invest.* 71:1562.
- Kaplan, M.A., Canterbury, J.M., Bourgoignie, J.J., Veliz, G., Gavellas, G., Reiss, E., and Bricker, N.S. 1979. Reversal of hyperparathyroidism in response to dietary phosphorus restriction in the uremic dog. *Kidney Int.* 15:43.
- Kawashima, H., Torikai, S., and Kurokawa, K. 1981. Localization of 1,25-hydroxyvitamin D₃ 1 α -hydroxylase and 24-hydroxylase along the rat nephron. *Proc. Natl. Acad. Sci. USA.* 78:1199.
- Kubrusly, M., Gagne, Eve-Reine., Urena, P., Hanrotel, C., Chabanis, S., Lacour, B., and Drueke, T.B. with technical assistance of Jehenne, G., Duchambon, P., Banide, H., and Pacher, N. 1993. Effect of 22-oxa-calcitriol on calcium metabolism in rats with severe secondary hyperparathyroidism. *Kidney Int.* 44:551.
- Llach, F., and Massry, S.G. 1985. On the mechanism of secondary hyperparathyroidism in moderate renal insufficiency. *J. Clin. Endocrinol. Metab.* 61:601.
- Lobaugh, B., and Drezner, M.K. 1983. Abnormal regulation of renal 25-hydroxyvitamin D-1 α -hydroxylase activity in the X-linked hypophosphatemic mouse. *J. Clin. Invest.* 71:400.
- Lopez-Hilker, S., Dusso, A.S., Rapp, N.S., Martin, K.J., and Slatopolsky, E. 1990. Phosphorus restriction reverses hyperparathyroidism in uremia independent of changes in calcium and calcitriol. *Am. J. Physiol.* 259:F432.

- Lufkin, E.G., Kumar, R., and Heath, III. H. 1983.
Hyperphosphatemic tumoral calcinosis: effect of phosphate depletion on vitamin D metabolism, and of acute hypocalcemia on parathyroid hormone secretion and action. *J. Clin. Endocrinol.* 56:1319.
- Maierhofer, W.J., Gray, R.W., and Lemann, J.Jr. 1984.
Phosphate deprivation increases serum 1,25-(OH)₂-vitamin D concentration in healthy men. *Kidney Int.* 25:571.
- Nagode, L.A., Chew, D.J., and Steinmeyer, C.L. 1992. The use of low doses of calcitriol in the treatment of renal secondary hyperparathyroidism. In: Proceedings of the Waltham/OSU symposium for the treatment of small animal diseases. *Endocrinology*. Kal Kan Foods Inc, Vernon, CA. pp.49.
- Nagode, L.A., and Steinmeyer, C.L. 1979. Regulation of plasma levels of 1,25-dihydroxyvitamin D in growing dogs with thyroparathyroidectomy and varying amounts of parathyroid hormone, in Norman, A.W. Shaefer, K., Herrath, D.V. and Grigoleit, H-G. (eds): *Vitamin D, Basic research and its clinical application*. W. de Gruyter, New York. pp.567.
- Nagode, L.A., and Steinmeyer, C.L. 1982. Relationship between serum 1,25-dihydroxyvitamin D, intestinal calcium transport, and bone turnover in infant dogs fed diets varying in calcium and phosphorus. In Norman, A.W., Schaefer, K., Herrath, D.V., and Grigoleit, H-G (eds): *Vitamin D: chemical, biochemical and clinical endocrinology of calcium metabolism*. W. de Gruyter, New York. pp.487
- Norman, M.E., Mazur, A.T., Borden, S. IV., Gruskin, A., Anast, C., Baron, R., and Rasmussen, H. 1980. Early diagnosis of juvenile renal osteodystrophy. *J. Pediatr.* 97:226.
- Parekh, A.C. and Jung, D.H. 1970. Serum inorganic phosphorus determination using p-phenylenediamine as a reducing agent. *Clin. Chem. Acta.* 27:373.
- Perkin-Elmer Corp. 1965. Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer, Norwalk.
- Pitts, T.O., Piraino, B.H., Mitro, R., Chen, T.C., Segre, G.V., Greenberg, A., and Puschett, J.B. 1988.
Hyperparathyroidism 1,25-dihydroxyvitamin D deficiency in mild, moderate and severe renal failure. *J. Clin. Endocrinol. Metab.* 67:876.

- Portale, A.A., Booth, B.E., Halloran, B.P., and Morris, R.C.Jr. 1984. Effect of dietary phosphorus on circulating concentrations of 1,25-dihydroxyvitamin D and immunoreactive parathyroid hormone in children with moderate renal insufficiency. *J. Clin. Invest.* 73:1580.
- Portale, A.A., Booth, B.E., Tsai, H.C., and Morris, R.C.Jr. 1982. Reduced plasma concentration of 1,25-dihydroxyvitamin D in children with moderate renal insufficiency. *Kidney Int.* 21:627.
- Portale, A.A., Halloran, B.P., Murphy, M.M., and Morris, R.C. 1986. Oral intake of phosphorus can determine the serum concentration of 1,25-dihydroxyvitamin D by determining its production rate in humans. *J. Clin. Invest.* 77:7.
- Silver, J., Russell, J., and Sherwood, L.M. 1985. Regulation by vitamin D metabolites of messenger ribonucleic acid for preproparathyroid hormone in isolated bovine parathyroid cells. *Proc. Natl. Acad. Sci. USA.* 82:4270.
- Slatopolsky, E., and Bricker, N.S. 1973. The role of phosphorus restriction in the prevention of secondary hyperparathyroidism in chronic renal disease. *Kidney Int.* 141.
- Slatopolsky, E., Caglar, S., Gradowska, L., Canterbury, J., Reiss, E., and Bricker, N.S. 1972. On the prevention of secondary hyperparathyroidism in experimental chronic renal disease using "proportional reduction" of dietary phosphorus intake. *Kidney Int.* 2:147.
- Slatopolsky, E., Weerter, H., Thielan, J., Horst, R.L., Harter, H., and Martin, K.J. 1984. Marked suppression of secondary hyperparathyroidism by intravenous administration of 1,25-dihydroxycholecalciferol in uremic patients. *J. Clin. Invest.* 74:2136.
- Snedecor, G.W. and Cochran, W.G. 1980. *Statistical methods* (7thed). Iowa State University Press, Ames, Iowa USA.
- Tessitore, N., Venturi, A., Adami, S., Roncari, C., Rugiu, C., Corgnati, A., Bonucci, E., and Maschio, G. 1987. Relationship between plasma vitamin D metabolites and dietary intake of phosphate in patients with early renal failure. *Miner. Electr. Metab.* 13:38.

- Van Stone, J.C., Frank, D.E., and Bradford, W.R. 1977. The effect of decreased renal function with and without reduction in renal mass on 1,25-dihydroxycholecalciferol production in rats. *J. Lab. Clin. Med.* 89:1168.
- Wilson, L., Felsenfeld, A., Drezner, M.K., and Llach, F.D. 1985. Altered divalent ion metabolism in early renal failure: Role of 1,25-(OH)₂D. *Kidney Int.* 27:565.

APPENDIX B

ASSAYS

Extraction and Binding Assay of 1,25-(OH)₂D₃Extraction of 1,25-dihydroxyvitamin D₃

1. Pipette 1 ml of sample into 12 x 75 test tube. If using less than 1 ml bring volume to 1 ml with saline.
2. Add 50 μ l of radioactivity (1000 cpm/50 ml) to each tube and to a vial for total count (TCT). Vortex and let sit for 5 minutes.
3. Add 1 ml of acetonitrile (ACN) (with the drop of NH₄OH/liter) to each tube and vortex for 30 seconds on the multi-tube vortexer.
4. Centrifuge tubes for 15 minutes at 2300 rpm .
5. While tubes are centrifuging, add 500 μ l 0.4 M K₂HPO₄ buffer to another set of 12 x 75 tubes. Set up (Incstar) C18OH cartridges in the black rack. Label 13 x 100 test tubes to collect into.
6. Wash the cartridges with the usual order of solvents:
 - a. 5 ml Hexane and suction.
 - b. 5 ml Chloroform and suction.
 - c. 5 ml of Methanol and suction.
7. After washing add 5 ml of distilled water (dd H₂O) to each cartridge and leave on until the samples are out of centrifuge.
8. When samples are finished centrifuging, transfer the supernatant into the appropriate tube containing the

- phosphate buffer and vortex.
9. Apply sample to cartridge with a pasteur pipette after suctioning off the dd H₂O and then wash as follows:
 - a. 5 ml of dd H₂O and suction
 - b. 5 ml of 70:30 (MeOH/H₂O) and suction
 - c. 5 ml of 90:10 (Hex/MeCl₂) and suction
 - d. 5 ml of 99:1 (Hex/Isop) and then suction
 - e. 5 ml of 95:5 (Hex/Isop) and collect**
 10. Samples are now ready for binding assay. When ready to collect move the top of the rack from waste to collect and place your tubes under the appropriate spouts.

Binding assay of 1,25-(OH)₂D₃

1. Dry down 95:5 Hex/Isop fraction from silica Sep-pak.
2. Reconstitute a frozen thymus pellet in 7 ml of thymus buffer and then add the specified amount of buffer for that batch of thymus.
3. Add 100 μl reagent alcohol (Fisher brand) to each tube. Cork tightly. Vortex. Let sit 30 minutes in ethyl alcohol (EtOH) bath.
4. Label duplicate 12 x 75 tubes for each sample and standards. Also label a set of scintillation vials for the recoveries of the samples (r₁, r₂, ...) and label duplicate scintillation vials for samples and standards.
5. Add 500 μl of thymus mixture to each tube.

6. Add the standards (0,1,2,4,8,16,32,64 pg and NSB) to the appropriate tubes.
7. After the samples have set for 30 minutes in ethanol bath, pipette two 25 μ l aliquots, for each sample, into the two tubes in the ice water bath and one 25 μ l aliquot into a scintillation vial for recovery.
8. Vortex, keeping on ice.
9. Incubate in shaking water bath at 25° C for 45 minutes. At this time you can get the magic charcoal stirring on the cold stir plate.
10. Place samples in ice water bath for 5 minutes and then add 5000 cpm [³H] 1,25 (OH)₂D₃/20 μ l.
11. Vortex and put back in shaking water bath at 25°C for 30 minutes.
12. Place samples in ice water bath and add 200 μ l of charcoal. Charcoal should be stirring for at least one hour before use and should be kept cold.
13. Vortex and let tubes sit in ice water for 15 minutes (or let them sit in cooler). Vortex once more and centrifuge at 2300 rpm for 10 minutes.
14. Pipette 500 μ l from each sample into scintillation vials. Add 5 ml cocktail.
15. Count radioactivity in the scintillation counter by putting the right program.

Standard curve of 1,25-(OH)₂D₃

1. Check 1,25 (OH)₂D₃ cold standard on the spectrophotometer.
2. Make 4 ml of the NSB tube which is at a concentration of 800 pg/25 μl (32 pg/μl).

$$32 \text{ pg}/\mu\text{l} \times 4000 \mu\text{l} = 128,000 \text{ pg} = 128 \text{ ng}$$

$$128 \text{ ng}/\text{concentration of standard} = \mu\text{l of standard}$$

that needed and bring the volume up to 4 ml with reagent alcohol.

3. Make 4 ml of stock standard at a concentration of 640 pg/25 μl (25.6 pg/μl).

$$25.6 \text{ pg}/\mu\text{l} \times 4000 \mu\text{l} = 102,400 \text{ pg} = 102.4 \text{ ng}$$

$$102.4 \text{ ng}/\text{concentration of standard} = \mu\text{l of standard}$$

that needed and bring the volume up to 4 ml with reagent alcohol.

4. Make a 1:10 dilution of this 640 pg/25 μl standard (400 μl in 3600 μl of reagent alcohol) to get to the 64 pg/25 μl standard that would be the final standard of the curve.

5. Make 2:1 serial dilution down from this 64 pg/25 μl standard for the rest of the curve (i.e., 32,16,8,4, 2&1 pg/25 μl) so that it could be ended up with 4 ml in the 1 pg/25 μl standard and 2 ml in all the rest.

6. Use the Rainin pipette to do the serial dilutions.

7. Blow nitrogen over all the standards when finished and cork wrap parafilm around the cork and store in the

freezer.

Phosphorus Assay

Reagents

All reagents are prepared from analytical grade chemicals using deionized distilled water.

1. TCA: 10%
2. Sulfuric acid solution: Dilute 30 ml of concentrated sulfuric acid up to 100 ml with water. Add acid to water.
3. Molybdic acid solution: Dissolve 5 g of ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$) in 100 ml of sulfuric acid solution.
4. Molybdic-TCA reagent: Mix 1 volume of molybdic acid solution with 2 volumes of 10% TCA solution.
5. p-Phenylenediamine reagent: Dissolve 0.5 g of p-phenylenediamine dihydrochloride in 100 ml of 5% sodium meta bisulfite ($\text{Na}_2\text{S}_2\text{O}_5$) solution.
6. Phosphorus standard: (a) stock solution: Dissolve 0.6589 g of pure, well dried mono potassium phosphate (KH_2PO_4) in 10 ml of 10 N sulfuric acid. Q.S. to 1 liter in volumetric flask. Yields 15 mg inorganic phosphorus/100 ml. Standards used are 15, 10, 7, 5, 3, & 1.

Phosphorus assay in plasma and bone**Precipitation**

1. Pipette 25 μ l serum (avoid hemolysis) sample into a microtiter plate. NB: for bone sample use a 1:21 dilution with water.
2. Add 200 μ l of molybdic-TCA reagent. Do the same for standards and blank using standard and water respectively in place of serum.
3. Vortex and let stand for 5 minutes. Centrifuge for 5 minutes for 5 minutes.

Color Development

1. Transfer 50 μ l supernatant into a new microtiter plate and add 200 μ l p-phenylenediamine reagent. Vortex.
2. Let stand for 20 minutes.
3. Read microplate reader using program "Phosphorus Assay".
4. For bone phosphorus, spin at end of 20 minutes and transfer to new plate before reading.

Phosphorus assay in the urine

Do 1/5 and 1/25 dilutions of urine in water (usually one of these dilutions will be on the standard curve).

1. Pipette 25 μ l of standard or sample into microtiter plate well and add 200 μ l of molybdic acid-TCA reagent.
2. Shake for 5 minutes then centrifuge for 5 minutes at

- 2300 rpm.
3. Transfer 50 μ l supernatant from plate 1 into corresponding well of plate 2.
 4. Add 200 μ l p-phenylenediamine reagent. Shake plate.
 5. Incubate for 20 minutes at room temperature.
 6. Read on microplate reader using "Phosphorus Assay".

Calcium Assay

Machine set-up

1. Turn on compressed air tank 10 minutes before wish to read samples.
2. Enter the settings on the machine as follows:
setting for the AA: Lamp = # 1 at 15 ma program # 1.
Setting for the P.E. 5000 Automatic Burner Control:
Fuel = 49, Air = 59. To enter the settings, enter
The number and then press the appropriate button.
3. Allow the machine to warm up for at least 5 minutes.
4. After machine warmed up for at least 5 minutes, turn the acetylene tank on and then push the flame on/off button on the burner control. It may be necessary to push this button 2 or 3 times before the flame actually ignites.
5. Allow the machine to warm up for an additional 5 minutes with the flame on. Make sure there is sufficient

distilled water in the container with the sipper tube.

6. After the additional five minutes check to see that the energy level is in the 70's before an attempt to read samples.
7. Check the printer to make sure there is adequate paper in there to complete the run.

Preparations of samples

1. Plasma samples are usually diluted 1 to 41. Pipette 100 μ l of sample into 17 x 100 mm falcon tube and dilute with 4 ml of 0.1% lanthanum oxide working solution and vortex. Urine samples are usually diluted 1 to 41 first using 0.5% lanthanum oxide working solution and then diluted additionally from this point if they need to be.
2. Standards are pipetted just like the samples (1/41). The 0 standard is just straight La_2O_3 . Other standards include 5, 10 and 15 mg%.
3. Samples and standards are usually run in duplicate.
4. Stock lanthanum oxide solution:
 - 58.64 gm/liter La_2O_3 (Sigma brand).
 - 250 ml Nitric acid (HNO_3).
 - Bring up to 1 liter with deionized water.
5. Working lanthanum oxide solution:
 - a. for plasma: 20 ml stock solution/liter (0.1%).
 - b. for urine: 100 ml stock solution/liter (0.5%)

6. Standard Preparation:

Use Fisher brand (Certified Atomic Absorption Standard)

Calcium at a concentration of 1 ml = 1 mg Ca.

5 mg% = 50 ml/liter

10 mg% = 100 ml/liter

15 mg% = 150 ml/liter

Dilute up to 1 liter with 0.1 N HNO₃.

Running standards and samples

1. Zero the machine by pressing all the buttons on the left of the calibration set (AZ, S1, S2, S3) once going and down once coming back up the line.
2. Place sipper tube into the 0 standard and press the AZ button twice.
3. Place sipper tube into the 5 mg% standard and punch in the numerical key pad and then punch the S1 button twice. Do this step for the 2 standards punching in the appropriate number (10.00 or 15.00) and the S2 and S3 buttons.
4. Now go back and read the 5 and 10 standards by placing the sipper tube into the standard and pressing the read button. If the standards come out to be within 0.2 mg% of the reading it should be then ready to continue with samples. If not then see below under troubles shooting.
5. To get values to print out push the print button to the

left of the machine (on the top). The printer will start at number 1 and number each sample read in order from this point. To number each sample differently, punch in the number first on the numeric key pad and then press before reading sample.

6. Place sipper tube into the sample and punch the read button.
7. In doing several samples at once it is important to make up a sample that can be run as an internal standard after every 10 samples to make sure that the machine is not drifting. If drift is encountered (ie., the value of this sample changes by more than 0.2 mg% over the course of the reading), then it is necessary to go back and rezero the machine and start over again.

Creatinine Assay in Plasma and Urine

Reagents

Creatinine kit: Sigma catalog # 55-A or Picric acid

$[(O_2N)_3C_6H_5OH$ or 2,4,6 trinitrophenol] 0.04M

(9.16 gm anhydrous or 10.17 gm reagent grade with water)

NaOH 3 N.

Stock Creatine 160 mg/ml in 0.1 N HCl.

Protocol

1. Dilute urine samples 1:10 with dd water.
2. Prepare a standard curve 0, 2.5, 5, 7.5 and 10 mg/ml
3. Pipette 10 ul of standard in duplicate or triplicate into titer plate. Use dd water for blank and 0 standards respectively.
4. Pipette diluted samples into the titer plate in duplicates/triplicates.
At this point if using Creatinine kit from Sigma go to step 10 now.
5. For every 20 ml of 0.04 picric acid to be used add 1 ml of 3N NaOH and mix. Mix in separate beaker.
6. Add 200 ml of Alkaline/Picrate mixture to each standard and sample well. Vortex.
7. Let sit at room temperature for 30 minutes.
8. Read absorbance between 490 and 520 nm.
9. For plasma creatinine use the same procedure but do not dilute.
10. For every 5 ml of 0.04 M picric acid to be used add 1 ml of 1N NaOH from kit and mix. Or add 5 ml of 0.6% creatinine reagent for every 1 ml of 1 N NaOH.
11. Add 200 ul the alkaline /picrate solution to each standard and sample well. Vortex.
12. Let sit at room temperature for 30 minutes.
13. Read absorbance between 490 and 520 nm.

Assay of PTH Levels in Rat SerumReagents preparation and storage

Store the kit at 2-8°C upon receipt. Store the standards and controls at -20°C after reconstitution. Prior to use allow all reagents to come to room temperature and mix by gentle swirling and inversion.

1. Rat PTH antibody coated beads: 100 polystyrene beads (64. diameter) coated with antibody to rat PTH plus desiccant.
2. ¹²⁵I labeled rat PTH antibody: two vials each containing 5 ml of ¹²⁵I labeled anti-rat PTH in 0.6M phosphate buffered saline with protein stabilizers and 0.1% sodium azide. Each vial contains less than 10 μCi (370 kBq) of radioactivity.
3. Rat PTH standards: six vials containing rat PTH (1-34) lyophilized in a protein matrix with 0.1% sodium azide. Before use reconstitute the vials with the rat PTH concentration of 0pg/ml with 4.0 ml of deionized water. Before use reconstitute the other five vials of standards with 2.0 ml of deionized water. Allow the vials to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

4. Rat PTH controls I&II: two vials each containing rat PTH (1-34) lyophilized in a protein matrix with 0.1% sodium azide. Before use reconstitute the controls with 2.0 ml of deionized water. Allow the vials to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.
5. Wash concentrate: one bottle containing 30 ml of a 30 fold concentrate. Before use dilute the contents to 900 ml deionized water and mix well. Upon dilution this yields a working solution containing a surfactant in 0.01M phosphate buffered saline with 0.05% sodium azide. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit box.

Other Materials Required

1. Polystyrene or polypropylene tubes, 12 x 75 mm.
2. Test tube rack.
3. Marking pen for labeling tubes.
4. 2.0 ml and 4.0 ml volumetric pipettes for reconstituting standards and controls.
5. Precision pipettes capable of delivering 100 μ l and 200 μ l.
6. Forceps or suitable bead dispenser.
7. Parafilm or equivalent for covering tubes.

8. Repeating dispenser suitable for delivering 2.0 ml.
9. Aspiration device or suitable bead washer.
10. Container for storage of of wash solution.
11. Gamma counter.
12. Deionized water.
13. Vortex mixer.
14. Timer.

Protocol

1. Pipette 200 μ l of standard, control or sample into appropriately labeled tubes.
2. Pipette 100 μ l of ^{125}I labeled rat PTH antibody into all tubes.
3. Vortex all tubes.
4. Using forcepsor appropriate bead dispenser, and one bead to each tube. If using forceps, tilt tube rack to approximately a 30 degree angle to prevent splashing. Cover tube rack with parafilm or equivalent.
5. Incubate tubes at room temperature for 18 to 24 hours.
6. Aspirate the contents of each tube. Wash beads three times by dispencing 2.0 ml of wash solution into each tube then completely aspirating the contents.
7. Count each tube in a Gamma counter for one minute and record the counts.

Calculation of results

The standard curve is generated using the rat PTH standards contained in the kit. Generate the curve as follows:

1. Calculate the average cpm for each pair of duplicate tubes.
2. Subtract the average cpm of the 0 pg/ml standard from all other average cpms to obtain corrected cpm.
3. The standard curve is generated by plotting the corrected cpm of each standard level on the ordinate against the standard concentration on the abscissa using log-log paper. Appropriate computer assisted data reduction programs may also be used for calculation of rat PTH results.
4. The rat PTH concentrations of the controls and samples are read directly from the curve using their respective corrected cpm. Samples having corrected cpms between the 0 pg/ml standard and next highest standard should be calculated by dividing the corrected cpm (unknown) by corrected cpm (2nd std.) and multiplying by the value of the 2nd std.