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(71)(72) Applicant and Inventor: NAGODE, Larry, A. [US/US]; 2837 Helston Road, Columbus, OH 43220 (US).

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(54) Title: METHOD OF CONTROLLING RENAL SECONDARY HYPERPARATHYROIDISM

(57) Abstract

The present invention relates to a method of ameliorating the symptoms associated with the disease of renal secondary hyperparathyroidism and the pathophysiological responses to renal disease by orally administering to an animal manifesting this disease an amount of 1,25 dihydroxyvitamin D_3 effective to ameliorate such symptoms and pathophysiological responses.

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DESCRIPTION

METHOD OF CONTROLLING RENAL SECONDARY HYPERPARATHYROIDISM

TECHNICAL FIELD

The present invention relates to a method of controlling renal secondary hyperparathyroidism in animals by use of daily calcitriol (1,25 dihydroxyvitamin D_3) doses which allow animals that have kidney disease to live longer, more comfortable lives.

BACKGROUND ART

It is known that kidneys are not the only organs affected by kidney disease. When damaged kidneys do not function properly many body control systems are impaired. One of the most important of these is the production of calcitriol, the active hormonal form of vitamin D made in the kidneys where its synthesis can be stimulated by parathyroid hormone (PTH). If there is too little calcitriol in the animal's blood, as happens in kidney disease, the body asks for more. A too low level of calcitriol acts as a signal to the parathyroid gland to produce more parathyroid hormone regardless of whether blood calcium is normal or even markedly elevated.

It is still widely believed throughout the veterinary and much of the medical profession that parathyroid hormone production and secretion is controlled exclusively by levels of blood calcium. This belief is now shown to be incorrect, as the inventor shows herein. In addition, there is now new information relating to parathyroid organ

culture studies and in vivo studies in rats showing that calcitriol is the dominant factor controlling production of parathyroid hormone within its gland of origin. Calcitriol inhibits formation of parathyroid hormone at normal levels of blood calcium. The parathyroid hormone works together with calcitriol to maintain adequately high levels of blood calcium. When calcitriol levels are normal, intestinal calcium absorption (which depends only upon calcitriol and not upon PTH) is very efficient and there is no need for PTH to take calcium from bone to the blood or to recapture it to blood from the forming urine in the kidney tubules.

At increased levels, parathyroid hormone acts as a trophic hormone to stimulate formation of calcitriol in the kidney. The inhibition of parathyroid hormone synthesis by calcitriol forms a feedback loop for effective interactive relations of these two hormones which work together to control calcium levels in the blood of animals. Calcitriol increases calcium absorption from food and also helps parathyroid hormone to provide blood with calcium from both the bones and the urine. Although the bone synergism of the two hormones is more important, reabsorption of calcium from urine forming in the kidney is also accomplished by parathyroid hormone and calcitriol working together, thus preventing loss of blood calcium via that route.

An important effect of kidney disease is that the diseased kidneys cannot properly filter phosphorus out of the blood. As the level of blood phosphorus increases, the phosphorus inhibits the formation of calcitriol. Since there are fewer working kidney cells in a diseased kidney to make calcitriol, these two major factors, too much phosphorus and too few kidney cells mitigate against adequate production of calcitriol. The present invention shows herein that, in diseased dogs, the afflicted kidney is less able to maintain normal blood levels of calcitriol.

Because of calcitriol deficit and the failure of its normal inhibition of parathyroid hormone synthesis, the parathyroid gland continues to produce high levels of parathyroid hormone effectively calling for more calcitriol production from fewer kidney cells. More and more parathyroid hormone is secreted into the blood. Unfortunately for animals in this condition, parathyroid hormone in such high levels is damaging to many body systems. It has been well-established in recent years that at high levels, the parathyroid hormone is toxic, so much so that it is considered one of the most important toxic factors increased in blood of man and animals with kidney failure. (S.G. Massry, Chapt 65, part 1 in Textbook of Nephrology, 1989, [S.G. Massry & R.J. Glassock eds]).

Long known to weaken bone, parathyroid hormone also inhibits the heart's ability to function and affects blood pressure. In high levels parathyroid hormone damages both brain and peripheral nerves, slowing brain waves and conduction velocity. It injures metabolism in the muscles interfering on more than one level with utilization of energy from food. High levels of PTH cause anemia by several mechanisms, cause pruritis and impotence but perhaps most importantly further reduce the function of the already damaged kidneys.

A statement made by an acknowledged leader in the field of vitamin D research (A.W. Norman, chpt. 15, part 6 in Textbook of Nephrology 1989, [S.G. Massry & R.J. Glassock eds.]) indicated that "long term treatment of patients with chronic renal failure with $1,25(OH)_2D_3$ is usually associated with suppression of parathyroid gland activity" but goes on "This effect is due to the rise in the concentration of blood calcium induced by the treatment with $1,25(OH)_2D_3$, and to the restoration of the set point of calcium toward normal". It has never been reported that a low daily oral dose of calcitriol given dogs would shut down the synthesis of parathyroid hormone within the gland resulting in lowering of elevated serum

PTH levels and doing so without appreciably affecting levels of blood calcium. An intravenous administration weekly during dialysis at about 10 times the daily oral dose claimed herein achieved this objective in human patients for at least 3 hours after infusion. (J.A. Delmez et al., J. Clin. Invest. 83:1355, 1989). Due to rapid catabolism of calcitriol, such approaches suppress PTH only temporarily each week and so are both impractical and of no value in uremic canine patients.

The acute effects of 1,25-dihydroxycholecalciferol on serum immunoreactive parathyroid hormone in 10 kg puppies was discussed by Oldham et al., in Endocrinology, 104:248-254 (1979). The dosage of approximately 100 ng/kg, which is about 40 times higher than the dosage of the present invention, had no direct inhibitory effect on parathyroid hormone secretion either in hypocalcemic vitamin D-deficient or normal puppies.

Likewise in a study of experimentally uremic dogs, Lopez-Hilker et al., Amer. Soc. for Clin. Invest., 78:1097-1102 (1986) stated that hypocalcemia may not be essential for the development of secondary hyperparathyroidism in chronic renal failure. In this study the two calcitriol doses used were about 3 and 15 times higher than the dosage of the present invention, assuming 40 lb dogs. Lopez-Hilker et al. sought to prevent but did not try to reverse hyperparathyroidism. Lopez-Hilker et al. were consistently able to prevent PTH increases with the higher dose levels, but only inconsistently with lower dose levels.

Although calcitriol is understood to lower the "set point" for PTH suppression by calcium, it has not previously been understood that calcitriol is the dominant controller of PTH synthesis. (T. Naveh-Many et al., Endocrinology, 125:275-280, 1989). A normal level of calcium is only necessary for the calcitriol receptor-calcitriol complex to bind and function with regulatory elements at parathyroid cells DNA to shut down synthesis of PTH in those cells.

Early attempts by veterinarians who are knowledgeable specialists in nephrology to use calcitriol in the high dosages presently available and existing as capsules made for humans led to abandonment of its use due to experiences of hypercalcemic toxicity. Experiences repeated throughout the profession have led to a present general opinion that the use of calcitriol is too dangerous and so without value in treatment of canine renal disease. The only published doses of calcitriol recommended for uremic dogs (in micrograms/kg body wt.) prior to 1989 is found in Small Animal Clinical Nutrition, Chapter 8, pp. 4-5 & 38, published by Mark Morris Associates, Topeka Kansas (1987), which discloses a dosage which the present invention shows is nearly 7 times too high. This dose of 0.02 micrograms calcitriol/kg.body wt./day) has been shown by Dzanis et al., Vitamin D Molecular, Cellular and Clinical Endocrinology (1988) 787-798, Walter de Gryter & Co., Berlin, New York, to be extremely toxic. The Dzanis et al. reference discloses a study of experimentally uremic approximately 20 kg Beagles which used a 0.50 microgram dosage in the uremic Beagle dogs for 14 weeks. This dosage caused the subject dogs to lose 52% of their body weight. The same dosage given to normal dogs for 6-8 weeks, removed completely for 8 weeks, then followed by 6-8 weeks of 0.25 micrograms/dog/day resulted in anorexia and weight loss analogous to that seen in the uremic dogs. Dzanis et al., concluded that calcitriol exacerbated the progression of the dogs renal disease, actually worsening the disease rather than helping treat it.

The 0.25 microgram human dose Rocaltrol® capsule given 3 times/week, (available from Hoffman LaRoche) has been recently recommended without references or data for all dogs regardless of size in the 1989 version of the most widely consulted therapeutic reference book in veterinary medicine, Current Veterinary Therapy (1989) 1195-1198, published by Saunders. The inventor shows herein that this decrease in weekly dosage is inadequate and will lead to

many more calcitriol intoxicated dogs, most dramatically the smaller ones. These recommendations were made without supporting data and were possibly felt to be safe because they were 1/500th the weekly dosage of the next most potent form of vitamin D (Dihydrotachysterol) which has been recommended for many years for use in hypocalcemia occurring in canine renal failure. As an example, a small breed dog weighing 5.5 lbs. would be getting 13 times as much calcitriol per week with this dosage as with the median dosage claimed in the present invention. These levels are clearly toxic, as reported by Dzanis et al. Because calcitriol is cleared from the body in about 24 hours, such intermittent dosage as 3 times/week produces unacceptable peaks and valleys of blood levels throughout the week with peaks at toxic levels and valleys at ineffective levels.

Previously it has been believed that the use of any form of vitamin D in dogs with chronic renal failure was dangerous, should be used with great caution, and then only in dogs which are hypocalcemic (total calcium below 10 mg/dl). The negative experience using the 0.02 micrograms/kg body wt. dose in dogs has supported this body of opinion that this compound is too dangerous for veterinary use. It is current general veterinary practice now that calcitriol therapy is contraindicated in normocalcemic dogs even if uremic.

DISCLOSURE OF INVENTION

The present invention thus relates to a method for controlling the disease of renal secondary hyperparathyroidism by using calcitriol to control parathyroid hormone directly rather than only by increasing blood calcium as has been the previously accepted belief.

By measuring the levels of parathyroid hormone and calcitriol in the blood on a regular basis the animal's hormonal balance can be monitored with great accuracy. Reducing dietary phosphorus is

necessary to relieve inhibition of remaining calcitriol synthesizing systems. If this is not sufficient to lower the serum phosphorus, a compound is administered to the animal which binds phosphorus in the intestine, preventing its absorption from food. These actions often reduce serum phosphorus but when too little kidney tissue remains, as commonly occurs, levels of calcitriol in blood cannot be maintained normal.

An important consequence of the invention described herein is that calcitriol in appropriately low dosages is of great benefit in the vast majority of uremic animals which do have normal blood levels of calcium. Hypocalcemia, a necessary precondition for all previously recommended uses of any form of vitamin D in canine or feline uremia almost never occurs in uremia as actually seen in dogs and cats.

The present invention relates to a method of controlling in animals and dogs in particular, the amount of parathyroid hormone produced by the parathyroid gland which method comprises daily administering an appropriate level of calcitriol.

According to the present invention, animals who are unable to produce enough calcitriol of their own are supplemented directly with calcitriol. The animals are given an appropriate dosage of calcitriol which corresponds with the animal's weight. This calcitriol dosage does not depend upon increased absorption of calcium from the intestines to shut down parathyroid hormone production which commonly has an altered and higher "set point" for calcium inhibition of parathyroid hormone synthesis and secretion. The calcitriol dosage does not depend upon lowering the "set point" for calcium below a normal level of blood calcium. The "calcitriol affecting calcium set point" argument treats calcium as the primary controller which has historically been the accepted belief. Since it is the calcitriol receptor which directly interacts with parathyroid cell DNA, this interaction (requiring a normal level of blood calcium) is

the primary one. A certain normal level of calcium should be considered to be required for the "set point" of calcitriol bound to calcitriol receptor-DNA interaction in order to successfully block formation of the messenger RNA for PTH. The administration of a low calcitriol dose, according to the method of the present invention, acts then as a direct effect upon the parathyroid gland cells' DNA through its receptor. This novel low dosage of calcitriol acts in both normocalcemic and hypercalcemic states and is lower than a dose that would significantly increase blood calcium. The calcitriol therapy is so effective that within a short time as 12 hours to a few days, there is a dramatic drop of parathyroid hormone levels in the treated animal.

In a method of use aspect, the present invention relates to a method of ameliorating the symptoms associated with the disease of renal secondary hyperparathyroidism and the pathophysiological responses to renal disease which method comprises orally administering to an animal manifesting such symptoms and pathophysiological responses an amount of 1,25 dihydroxyvitamin D_3 effective to ameliorate such symptoms and pathophysiological responses. In preferred embodiments, the 1,25 dihydroxyvitamin D_3 is administered in a daily dosage and the amount of 1,25 dihydroxyvitamin D_3 administered per dosage is from about 1.5 to about 3.5 nanograms/kg body wt. of the animal.

In another aspect, the present invention relates to a method for treatment of an animal suffering from kidney disease due to in part hyperparathyroidism. Hyperparathyroidism is an established cause of renal disease in patients with parathyroid tumors and a cause obvious to renal specialists, even when the parathyroid hyperactivity was itself caused by an earlier and lesser extent of renal disease. The elevated PTH caused by renal disease is a very significant toxin contributing to the progressive worsening of the renal disease. The method of treatment comprises administering to the animal an

effective amount of 1,25 dihydroxyvitamin D_3 together with a physiologically acceptable carrier or excipient.

In a composition aspect, the present invention relates to pharmaceutical compositions for use in veterinary medicine for treating kidney disease containing as the active principle 1,25 dihydroxyvitamin D_3 with suitable pharmaceutical diluents or carriers, optionally in association with other active principles, in an amount of about 1.5-3.5 nanograms/kg. body wt. in a unitary dose.

The present invention also relates to a dietary supplement for reducing the animal's level of parathyroid hormone comprising an effective amount of 1,25 dihydroxyvitamin D_3 for lowering production of parathyroid hormone.

BRIEF DESCRIPTION OF FIGURES

Fig. 1 is a graph depicting the mean and standard deviation in about 170 dogs with varying degrees of uremia of levels of serum calcitriol versus serum parathyroid hormone (PTH).

Fig. 2 is a graph depicting normals and the levels of serum calcitriol versus creatinine in about 170 uremic dogs indicating irregularity in the lowering of calcitriol associated with increasing serum creatinine (loss of nephrons) in chronic renal failure.

Fig. 3 is a graph depicting normals and the levels of serum parathyroid hormone versus creatinine in about 170 uremic dogs indicating increase of PTH associated with increasing serum creatinine (loss of nephrons) in chronic renal failure.

Fig. 4 is a graph depicting the levels of serum calcitriol versus serum phosphorus in about 170 uremic dogs indicating decreased calcitriol with increasing serum phosphorus.

Fig. 5 is a graph depicting the levels of serum parathyroid hormone versus serum phosphorus in about 170 uremic dogs indicating increase of PTH associated with increased serum phosphorus.

Fig. 6 is a graph depicting the rapidly changing levels of serum parathyroid hormone and calcitriol in a dog with acute tubular nephrosis produced by administration of Gentamycin.

Fig. 7 is a graph depicting the rapidly changing levels of serum parathyroid hormone and calcitriol in a second dog with acute tubular nephrosis produced by administration of Gentamycin.

Fig. 8 is a graph depicting the changing relative levels in serum parathyroid hormone (PTH), creatinine (Creat), phosphorus (Pi) and calcitriol (1,25 D) for a young congenitally uremic dog over a 25 day period.

Fig. 9 is a graph depicting the changing levels in serum parathyroid hormone (PTH), calcitriol, phosphorus (Pi) and creatinine (Creat) for a uremic dog over a 3.5 year period.

Fig. 10 is a graph depicting the levels of serum parathyroid hormone (PTH), calcitriol, phosphorus and creatinine for a uremic dog over a 4 month period.

Fig. 11 is a graph depicting the mean and standard deviation (indicated only above the mean but extending equally below it) of levels of serum phosphorus (Pi) and parathyroid hormone (PTH) comparing normal (N) levels to before (B) and after (A) phosphorus manipulations in 5 uremic dogs where the PTH values returned to normal or near normal for dogs given dietary phosphorus restriction and an intestinal phosphate binder.

Fig. 12 is a graph depicting the mean and standard deviation (indicated only above the mean but extending equally below it) of levels of serum phosphorus (Pi) and parathyroid hormone (PTH) comparing normal (N) levels to before (B) and after (A) phosphorus manipulations in 7 uremic dogs where the PTH values were only partially normalized for dogs given dietary phosphorus restriction and an intestinal phosphate binder.

Fig. 13 is a graph depicting the decline of serum PTH to normal within 12 hours of initiating daily dosing with 2.3 ng/kg calcitriol with serum calcium levels unchanging.

Figs. 14A and 14B are graphs depicting the serum PTH, calcitriol and calcium responses for a dog given daily doses of 2.8 ng/kg calcitriol during earlier (14A) and later (14B) time periods on calcitriol.

Figs. 15A and 15B are graphs depicting the serum PTH, calcitriol and calcium responses for a dog given daily doses of 3.4 ng/kg calcitriol during earlier (15A) and later (15B) time periods on calcitriol.

Figs. 16A and 16B are graphs depicting the serum PTH, calcitriol and calcium responses for a dog given daily doses of 1.8 ng/kg calcitriol during earlier (16A) and (16B) later time periods on calcitriol; at days 85 and 220 the patients owner had run out of medication prior to clinic visits.

Figs. 17A and 17B are graphs depicting the serum PTH, calcitriol and calcium responses for a dog's first period on 2.1 ng/kg calcitriol/day (17A) with consequences for serum PTH levels of stopping calcitriol dosing (17B) done at the end of the dog's life.

Figs. 18A and 18B are graphs depicting the serum PTH, calcitriol and calcium responses for a dog given daily doses of 2.6 ng/kg calcitriol during earlier (18A) and later (18B) time periods on the trial.

Fig. 19 is a graph depicting the serum PTH, calcitriol and calcium responses for a moderately uremic and hyperparathyroid dog given daily doses of 2.1 ng/kg calcitriol during the 190 day trial.

BEST MODE OF CARRYING OUT INVENTION

The present invention is especially useful after determination of the earliest stage of hyperphosphatemia and uremia (as measured by serum phosphorus and creatinine) at which derangements occur of either calcitriol levels which are more than 1 s.d. below mean of calcitriol of normal dogs, or parathyroid hormone (PTH) levels which are more than 1 s.d. above the mean of PTH of normal dogs. This

stage of hyperphosphatemia and uremia indicates the need for initiation of corrective steps to reverse or prevent parathyroid hyperactivity. The stage of hyperphosphatemia indicates when rigorous dietary phosphorus restriction coupled sometimes with intestinal phosphorus binding agents should be initiated. Determination of the stage of uremia where such derangements of PTH and for calcitriol begin helps to indicate when, together with phosphorus restriction, the direct use of calcitriol is indicated.

Using Gentamycin to provide a controlled rapid onset deficit of renal function, the present invention demonstrates herein the rapidity of development of alterations in levels of both parathyroid hormone and calcitriol and shows the correlation between the levels of both within a very short time frame. With the demonstration of the rapidity of onset and dynamicity of these hormonal changes the inventor now shows that effective calcitriol therapy can correct these changes with a similar rapidity. According to the method of the present invention, there is no need to face a prolonged trial of a particular therapeutic approach in order to determine its success or failure in correcting these abnormalities of serum hormone levels. Changes occur very rapidly when the calcitriol therapy is appropriate.

The present invention thus relates to a method of treatment of animals with appropriately low and safe oral doses (1.5 to about 3.5 nanograms/kg. body wt. of the animal) of 1,25 dihydroxyvitamin D_3 (calcitriol) to effectively and dramatically lower serum levels of parathyroid hormone in animals with renal secondary hyperparathyroidism. Although not previously understood by the veterinary profession to be the case, this use is strongly indicated without regard to whether or not the afflicted animals are hypocalcemic. Findings in experimental medicine during the past few years have clearly shown that elevated levels of parathyroid hormone have marked deleterious affects upon functioning of many organs and contribute to progression of chronic renal disease.

The present invention shows that the dose effective for lowering blood parathyroid levels to normal is about 1/3000th the dihydrotachysterol recommended dose. It is shown herein that although long term dosage near the upper end of the dose range disclosed may help produce mild hypercalcemia in some dogs, the dosage of the present invention has been used without significant hypercalcemic toxicity.

The inventor proposes that secondary hyperparathyroidism caused by canine renal disease requires treatment because of the toxicity of high levels of circulating parathyroid hormone regardless of the presence or absence of bone disease. Also, the low dose of calcitriol used in the present invention is both highly effective and safe and can be used in hyperparathyroid normocalcemic as well as hypocalcemic patients with renal disease. If an occasional patient should be hypocalcemic, this dosage of calcitriol may return blood calcium to normal but it does not need to increase it above normal to stop synthesis of PTH. This is because although calcitriol suppression of PTH synthesis requires a certain level of blood calcium to be present synergistically to accomplish calcitriol suppression of PTH synthesis, this level of blood calcium can be normal to low normal. Prior to administering the dosage of the present invention of calcitriol, it is desirable that serum phosphorus be lowered to no higher than 5.5-6.0 mg/dl depending upon the serum calcium X phosphorus product, which should not exceed 66.

Although cats have a somewhat lesser incidence of chronic renal disease compared to dogs, it is significant that the present invention is also useful in uremic cats. The principles guiding development of dosage requirements of calcitriol useful for dogs can also be applied to cats and are being so applied currently.

The dosage system of the present invention is such that the level of calcitriol ingested corresponds to the size of the dog. Capsule dosing with capsules produced in appropriately varying sizes

for different sized dogs is the most convenient method of calcitriol administration. Alternatively, appropriate doses of calcitriol can be incorporated into one of the specialized forms of dog food now marketed for use in dogs which have kidney disease. A number of these specialized forms of dog food exist, the most prominent of which are K/D (kidney diet) and U/D (urinary diet). Dogs eat amounts of food which correspond well with their body size allowing fairly accurate dose adjustment. This approach may have greatest application early in renal disease before interference with appetite occurs after which capsule dosage would be required in most cases.

The initiating dosages depend on the weight of the dog. In particular, a different and increasing dosage is given for dogs in the 0-10, 10-20, 20-30 and 30-40 pounds weight ranges. That is, the dosage increases for every 10 pounds of dog weight. While for the dogs whose weight ranges from about 40 to about 100 pounds, the increase in dosage generally is given for every 20 pounds of dog weight. Thus, a different and increasing dosage is given for dogs in the 40-60, 60-80, and 80-100 pound weight ranges. The amount of 1,25 dihydroxyvitamin D_3 administered per dosage ranges from about 1.5 to about 3.5 nanograms/kg of body weight of the animal.

These dosages are initiating doses and refinements in dosage are individualized to the uremic patient dogs as therapy progresses. For each dose range, the midpoint weight for that range was selected and using the two concentrations of Rocaltrol® in capsules commercially available [1.56 nanograms/microliter (from 0.25 microgram Rocaltrol® capsules which contain 160 microliters of content) and 3.12 nanograms/microliter (from 0.5 microgram Rocaltrol® capsules which also contain 160 microliters of content)], specific volumes were devised to dispense into either #4 or #5 gelatin capsules marketed by Eli Lilly.

For dogs between 0-10 lbs the 1.56 nanogram/microliter fluid was diluted 1:1 with olive oil and mixed thoroughly before placing 10

microliters of fluid in each capsule. This produced a dose of 3.4 nanograms/kg for a 5 lb recipient, and a dose of 1.7 nanograms/kg for a 10 lb patient.

For 10-20 lb dogs, 10 microliters of the undiluted 1.56 nanograms/microliter fluid was used. For the midpoint 15 lb dog the dose would be 2.3 nanograms/kg whereas for a 10 lb dog it would be 3.4 nanograms/kg and for a 20 lb dog it would be 1.7 nanograms/kg.

For 20-30 lb dogs, 20 microliters of 1.56 nanograms/microliter fluid produced a dose for a 25 lb dog of 2.7 nanograms/kg, for a 20 lb dog it was 3.4 nanograms/kg and for a 30 lb dog it was 2.3 nanograms/kg.

For 30-40 lb dogs, 30 microliters of the 1.56 nanograms/microliter fluid was placed in each capsule. For 30, 35 and 40 lb dogs, the effective doses are approximately 3.4 ng/kg, 2.9 ng/kg, and 2.6 ng/kg, respectively.

For 40-60 lb dogs, 40 microliters of the 1.56 nanogram/microliter fluid were used. For 40, 50 and 60 lb dogs, the effective doses are approximately 3.4 ng/kg, 2.7 ng/kg, and 2.3 ng/kg, respectively.

For 60-80 lb dogs the more concentrated 3.12 nanograms/microliter fluid was used and 30 microliter of fluid was placed in each capsule. For 60, 70 and 80 lb dogs the effective doses are approximately 3.4 ng/kg, 2.9 ng/kg and 2.6 ng/kg, respectively. A dog with weight at one of the division points, for example, 20 lbs, can be given either the 10-20 lb capsule size or the 20-30 lb capsule size with a difference of doubling the 1.7 ng/kg dose of the smaller capsule to 3.4 ng/kg if given the larger one. The severity of hyperparathyroidism and achieved levels of lowering of serum phosphorus is taken into account to aid dose selection with the overriding goal of avoiding calcitriol toxicity as the most important consideration in determining the appropriate dosage. During the testing period described below in the examples, although it ultimately

proved of little significance, avoidance of calcitriol toxicity was one of the considerations in determining the appropriate dosage which were made during the initial treatment of the clinical patients. As a result, some patients had slower lowerings of PTH toward normal than they could have had because of the dose of calcitriol initially administered was near the low end of the tested range.

The mean and standard deviation (s.d.) of 25 normal dogs was determined for dogs in a clinic environment (in contrast to normals for single breed dogs in a research environment) to be for calcitriol [34 +/- 10] and for PTH [24 +/- 10]. Because in uremia, an initial deficit of calcitriol is expected to be masked by the stimulatory effects of an increased PTH, the entry level PTH and calcitriol were analyzed and considered abnormal if PTH was more than one s.d. increased from the normal mean, or alternatively, if calcitriol was decreased more than one s.d. from its normal mean. As will be seen from Fig. 1, high levels in uremic dogs of serum PTH at the graphs top are associated with low levels of calcitriol. The results shown in Fig. 1 are consistent with the fact that the immediate cause of the increase of PTH is the antecedent decline of calcitriol levels with hyperphosphatemia and nephron loss exerting their direct effects upon calcitriol levels. For a decision regarding need for corrective therapy, correction is indicated if either of the two hormones is abnormal as a high PTH may be returning an earlier lowered calcitriol toward normal.

INDUSTRIAL APPLICABILITY

The following examples illustrate the present invention.

EXAMPLE 1

A study was conducted on 25 normal and about 170 established renal uremic dogs which were selected from a total population of over 220 uremic dogs collected as possible renal uremic patients. Of

150 dogs with entry serum creatinine levels 1.5 mg/dl or higher, 135 dogs had the above defined derangement in one or the other hormone. Figs. 2 and 3 show the detail on the extent of derangement at different degrees of severity of uremia. When analyzed with respect to serum phosphorus on entry into the study, it was noted that of 83 dogs with serum phosphorus levels above 7.1 mg/dl, all had derangement. Of 43 dogs with serum phosphorus levels between 5 and 7 mg/dl, 30 dogs had derangements and 13 dogs had both hormones within 1 s.d. of the normal mean. Of 46 dogs with serum phosphorus between 2.5 and 5 mg/dl, 24 were deranged and 22 had both hormones within the described limits. Therefore, correction of hyperphosphatemia by dietary restrictions to achieve serum levels below 7 mg/dl is important in certain cases and further restriction to below 5 mg/dl (which may require addition of intestinal phosphorus binders to a restricted phosphorus diet) may be important in efforts to normalize this hormonal system.

Evaluation of Figs. 4 and 5 demonstrates the closer association of both PTH increases and calcitriol deficits with serum phosphorus level than is apparent for their association with loss of nephrons (as measured in Figs. 2 and 3 by serum creatinine levels). Because of the compensatory stimulation of serum calcitriol levels at each stage of nephron loss by the increased PTH present, calcitriol levels have forces driving them both down (nephron loss and hyperphosphatemia) and up (increased serum PTH) and are as expected quite variable when considered versus degrees of uremia, as can be determined by reviewing Fig. 2. The fact that PTH cannot usually return calcitriol levels fully to normal is supported by the result that of 170 dogs tested, 115 dogs were more than 1 s.d. below the normal calcitriol mean, (of which 78 dogs were below 15 pg/ml, 51 dogs below 10 pg/ml, and 30 dogs had undetectable serum calcitriol). The fact that serum calcitriol deficits are responsible for serum PTH elevations is difficult to effectively demonstrate due, again, to the compensatory

effects exerted by PTH which (when most elevated) exerts maximal stimulation upon calcitriol synthesis. Nevertheless, it is demonstrated in Fig. 1 that this system cannot completely compensate since it is clear that the highest levels of PTH (the groups toward the top of the graph) are associated with progressively lower calcitriol levels.

The nephrosis generated by administration of Gentamycin demonstrates the correlation between deficits of calcitriol and increases in the serum PTH. The dogs were administered Gentamycin (30 mg/kg total daily dose) for 7 days after which an onset of nephrosis developed. Creatinine clearances were used as index of glomerular filtration rate. Marked declines in the glomerular filtration rate were first noted at 7 days which correspond very well, as shown in Figs. 6 and 7, to the sudden and dramatic alterations of levels of calcitriol and PTH in serum. While all dogs in the study show a similar pattern, the Figs. 6 and 7 best illustrate the fact that changes of these hormones are very rapid and follow virtually immediately upon the causal changes in the renal tubules. The close correlation of calcitriol declines with increase of PTH supports the causal role proposed for the calcitriol deficits engendering the hyperparathyroidism.

In addition, the sequential changes in the hormonal levels in dogs treated for naturally occurring uremia have been analyzed. As a model for a therapy for use in naturally occurring uremia, naturally uremic dogs have many advantages over dogs with artificially induced uremic states, which have been studied by others. Oldham et al., supra, using a dosage at least 40 times the median dose of the present invention could find no direct inhibitory effect of calcitriol on PTH secretion. Lopez-Hilker et al., supra, using experimentally uremic dogs and calcitriol doses about 3 and 15 times the levels of the present invention assuming they used about 40 lb dogs, did not try to reverse hyperparathyroidism but were able to prevent PTH increases

easily with the higher (15 times the present invention) dose level but inconsistently with the lower (3 times the present invention) dose level. Naturally uremic dogs apparently behave differently than dogs with either nephrectomy and/or ligation of renal arterial supply. Such dogs in the study of Lopez-Hilker et al., used doses which would be toxic in clinical patients to achieve PTH suppression in those model animal systems. In addition, other partially related studies have also used experimentally uremic animals and higher doses of calcitriol than used in the present inventive method for treating naturally occurring diseases in clinical patients. Work done with such artificial renal disease models in rats needed higher doses than used in the present invention for treating naturally occurring canine renal disease in order to have effects by calcitriol to slow PTH formation. Such artificially perturbed experimentally uremic dogs possibly cannot effectively model all aspects of naturally occurring renal disease with the slow inexorably progressive character of renal disease that occurs spontaneously. The slow progression characteristic of the natural disease has time for development of many homeostatic adjustments which cannot be fully mimicked by artifical models. The sequential changes of hormones and calcium, as shown herein, in naturally occurring uremic patients over significant time periods (approaching two years in longest studied patient) can be interpreted without the biologic variability encountered in making comparisons from different experimental animals studied in short term experiments. Many dogs with naturally occurring renal disease in addition to the 15 calcitriol treated uremic patients have been analyzed in this manner. Such analysis has proven very helpful in clarifying events during the improvement, worsening or stabilization of a given clinical patient. The first case presented in Fig. 8 represents a young dog with a congenital renal disease not treated with calcitriol which, toward the end of the time frame represented, responded very well to marked dietary phosphorus restriction

combined with intestinal phosphorus binders. During the last three samplings it can be seen that although serum creatinine remained constantly elevated at about 3.5-4 mg/dl, serum phosphorus progressively declined, from initial very high levels to about 7.5 mg/dl. This can be seen to correspond to stepwise increases in the serum calcitriol (designated 1,25 D) in Fig. 8. In turn, one can note that serum PTH levels underwent a stepwise decline corresponding to both the decline of phosphorus and the increase of calcitriol.

Another case is shown in Fig. 9 which is a dog studied over a one and one-half year period. The dog had been maintained in moderate clinical remission but during the period depicted the dog underwent a progressive worsening shortly after which it died. One can see the increases in creatinine and phosphorus in the last two samplings which correspond to depressions of calcitriol to unmeasureable levels and to increases of PTH to 240 pg/ml.

Another case is shown in Fig. 10 which is a dog which remains alive and is existing in a stabilized state of a moderate degree of hyperparathyroidism of 80-90 pg/ml PTH. Although creatinine has not declined below 3.5 mg/dl during the depicted period, serum phosphorus has been carefully maintained by a diligent owner to below 4.5 mg/dl throughout. The calcitriol levels which were at 17 pg/ml early on have slowly increased to 33 pg/ml as serum phosphorus on last two samplings fell below 3.5 mg/dl. It seems that given proper dietary phosphorus management, a level of hyperparathyroidism of 80-90 pg/ml does not appear to be leading to worsening of renal functioning and the dog is alert and in good general health. Levels of PTH above 100 pg/ml (which is 4 times the normal mean PTH value) are of most concern to the veterinary care givers.

Of the more than 170 renal uremic dogs tested during the past 5 years, 57 dogs have had PTH levels above 100 pg/ml, 35 dogs had levels about 200 pg/ml, 23 dogs had levels over 300 pg/ml, and 9 dogs

had levels over 500 pg/ml. These results make clear that many uremic dogs have a very marked hyperparathyroidism with all its deleterious consequences. They will benefit in several ways from reduction of their PTH.

EXAMPLE 2

A three level approach to control of renal secondary hyperparathyroidism was used to evaluate 14 dogs with dietary (only) restriction of phosphorus (Pi), and 26 dogs with combined dietary Pi restriction and intestinal Pi binder. A group of 15 dogs in which Pi restriction even with binder failed to lower serum PTH into the normal range was given calcitriol orally in order to determine appropriate dosages and demonstrate efficacy of this therapy. The results from a few of these dogs are presented herein.

Referring now to the Figs. 11 and 12 in particular, results from 12 additional dogs given both dietary and binder Pi restrictions are presented. The (N), (B), and (A) designations related to both Pi and PTH on the graphs refer to (N) Normal, (B) Before, and (A) After, phosphorus manipulations. After serum Pi normalization, 5 dogs returned PTH to the normal range (Fig. 11) and 7 dogs failed to normalize PTH (Fig. 12). In 5 other dogs studied, Pi could not be normalized but was lowered from 18 +/- 4 to 10 +/- 1 mg/dl. The PTH in these dogs decreased from 428 +/- 76 to 198 +/- 73 pg/ml, but because Pi remained high, calcitriol was not used to lower PTH further into a nontoxic range.

Results from various dogs entered into the calcitriol therapy trial are depicted in Figs. 13 through 19.

In Fig. 13 a uremic dog with a serum creatinine of 3.8 mg/dl, and phosphorus of 5.1 mg/dl was given a midrange calcitriol dose of 2.3 ng/kg. The sharp drop of PTH to normal levels at 12 hours was accompanied by a slight drop in serum calcium rather than an increase suggesting that it was not by increasing serum calcium that calcitriol brought about its PTH suppression.

In Figs. 14A and 14B are depicted changes in a dog started on calcitriol in late July, 1988 and which remains alive and doing well on daily calcitriol treatment at present. It had a serum creatinine of 5.1 twenty days prior to starting on calcitriol with serum phosphorus of 10.3. By starting calcitriol treatment, serum phosphorus was lowered to 6.9 and creatinine had also lowered. To best depict changes in serum calcitriol and PTH responses, most dogs including this one are graphed in two separate time frames, i.e., "early on" and "later on." In each instance the first values in the "later on" graph are identical to the last values given in the "early on" graph. The dramatic drop in serum PTH first checked at 7 days in this patient was not associated with an appreciable increase in levels of serum calcium. Again PTH suppression by calcitriol was independent of and did not need any increase in serum calcium.

In Figs. 15A and 15B, the dog entered the study with serum creatinine level of 4.1 mg/dl and it remained between 4 and 5 mg/dl throughout the nearly 2 years this dog remained on a daily dose near the top of our range at 3.4 ng/kg calcitriol. The dog responded rapidly to decrease PTH to normal and remained in excellent control for over 350 days. Although mildly hypercalcemic before and throughout the calcitriol dosing study, the dog did not increase serum calcium in association with the PTH drop to normal within the first week of calcitriol dosing.

In Figs. 16A and 16B the dog had a dose of calcitriol in the lower portion of the range (1.8 ng/kg) and so took a longer time to decrease the PTH. for the last 230 days of this 333 day long therapy trial, PTH was well controlled except on a couple occasions when the owner ran out of calcitriol capsules a day or two prior to coming in for checkup. This dog's creatinine remained between 2 and 3 mg/dl throughout most of the year of study showing that the present inventive method successfully interupted the progression of the renal disease.

In Fig 17A a dog given 2.1 ng/kg calcitriol daily is depicted. It had serum PTH decreased progressively to normal in 12 days. A slight increase in blood calcium accompanied the first detected drop in PTH but the further drop to normal between days 6 and 14 had no associated change in serum calcium. During early stages of this trial serum creatinine levels were between 4-6 mg/dl but in the last week of life (Fig. 17B) they were increased to between 9-12 mg/dl. Fig. 17B illustrates the consequence of cessation of calcitriol dosing which was done in this dog during the last 5 days it was alive in intensive care in the clinic. Blood levels of calcitriol did not fall despite cessation of dosing most likely because the increased PTH levels were stimulating renal production of calcitriol to substitute for exogenous calcitriol.

In Figs. 18A and 18B, the dog was maintained on 2.6 ng/kg calcitriol. This dog was the only entering patient of the 15 tested which was hypocalcemic on entry. For this reason the initial calcitriol doses which dropped PTH markedly were associated with an increase in serum calcium because these low doses of calcitriol will correct a hypocalcemic condition. Were one to have only studied this one patient, the existing dogma that PTH drops in response to calcitriol require an increase in blood calcium would have been supported. This dog became moderately hypercalcemic throughout the remainder of the study. Between 40 and 100 days on study calcitriol dosing was stopped because of hypercalcemia but at day 100 was reinstituted. Calcium again rose. The dog remained in good general health although moderately hypercalcemic and after 360 days on trial died of an unrelated accident. This dog was unusual in being a small dog that was extremely fat (26 lb. Dachshundt) so that if calcitriol did not partition well into fat (being not nearly as fat soluble as the parental vitamin D molecule) the dose level at other tissues could have run higher than the nominal 2.6 ng/kg.

In Fig. 19 is depicted a mild to moderately uremic dog with serum creatinine levels throughout the 190 day calcitriol dosing trial ranging between 2 and 2.5 mg/dl. Calcitriol dose was 2.1 ng/kg. Although not dramatically increased at the trial's beginning, the serum PTH continued to fall progressively after the quick drop in the first two weeks. Serum calcium does not change appreciably and the dog was eventually euthanatized for reasons unrelated to its stage of renal disease as it was doing well at the time of its death.

The amount of active ingredient in these illustrative examples may be varied to achieve the dosage unit range set forth above, and the amounts and nature of the inert pharmaceutical carrier ingredients may be varied to meet particular animal requirements. The pharmaceutical composition can be administered in the form of capsules, tablets, dragees, syrups, solutions, vials and the like suitable for oral administration.

While the present invention has been illustrated with the aid of certain specific embodiments thereof, it will be readily apparent to others skilled in the art that the invention is not limited to these particular embodiments, and that various changes and modifications may be made without departing from the spirit of the invention or the scope of the appended claims.

CLAIMS:

1. A method of ameliorating the symptoms associated with the disease of renal secondary hyperparathyroidism and the pathophysiological responses to renal disease, which responses are at least one of elevated levels of parathyroid hormone and/or lowered calcitriol levels, which method comprises orally administering to a canine or feline animal manifesting such symptoms and pathophysiological responses an amount of 1,25 dihydroxyvitamin D_3 effective to ameliorate such symptoms and pathophysiological responses without need for elevating levels of blood calcium in its mechanism of action wherein the amount of 1,25 dihydroxyvitamin D_3 administered per dosage is from about 1.5 to about 3.5 nanograms/kg body wt. of the animal.

2. The method according to claim 1, wherein the 1,25 dihydroxyvitamin D_3 is administered in a daily dosage.

3. The method of claim 1, in which prior to the administration of 1,25 dihydroxyvitamin D_3, the animal's serum phosphorus level is lowered to or maintained at about 5.0 - 7.0 mg/dl.

4. A pharmaceutical composition for use in veterinary medicine for treating kidney disease containing as the active principle 1,25 dihydroxyvitamin D_3 with suitable pharmaceutical diluents or carriers, optionally in association with other active principles, in an amount of about 1.5-3.5 nanograms/kg. body wt. in a unitary dose.

5. The pharmaceutical composition of claim 4 in the form of capsules, tablets, dragees, syrups, solutions, or vials suitable for oral administration.

6. A dietary supplement comprising an orally administrable, effective amount of 1,25 dihydroxyvitamin D_3 for increasing a canine or feline animal's serum calcitriol and reducing the animal's level of parathyroid hormone.

7. The dietary supplement of claim 6 administered with a phosphorus binder substance for controlling the animal's phosphorus levels.

8. A method of ameliorating the pathophysiological response of elevated levels of parathyroid hormone associated with the disease of renal secondary hyperparathyroidism comprising orally administering to a canine or feline animal manifesting such symptoms and pathophysiological responses an amount of 1,25-dihydroxyvitamin D_3 effective to ameliorate such symptoms and pathophysiological responses without elevating levels of blood calcium wherein the amount of 1,25-dihydroxyvitamin D_3 administered per dosage is from about 1.5 to about 3.5 nanograms/kg body wt. of the animal.

9. The method according to claim 8 in which the dosage is from about 1.5 to about 2.5 nanograms/kg body wt. of the animal.

10. The method according to claim 1, wherein the 1,25-dihydroxyvitamin D_3 is administered in a daily dosage.

11. The method according to claim 1 in which prior to the administration of 1,25-dihydroxyvitamin D_3, the animal's serum phosphorus level is lowered to or maintained at about 5.0-7.0 mg/dl.

12. The method according to claim 1 in which the 1,25 dihydroxyvitamin D_3 is incorporated into a food product.

13. The dietary supplement of claim 6, wherein the 1,25 dihydroxyvitamin D_3 is incorporated into a food product.

14. The dietary supplement of claim 7, wherein the 1,25 dihydroxyvitamin D_3 is incorporated into a food product.

15. The method according to claim 8, in which the 1,25 dihydroxyvitamin D_3 is incorporated into a food product.

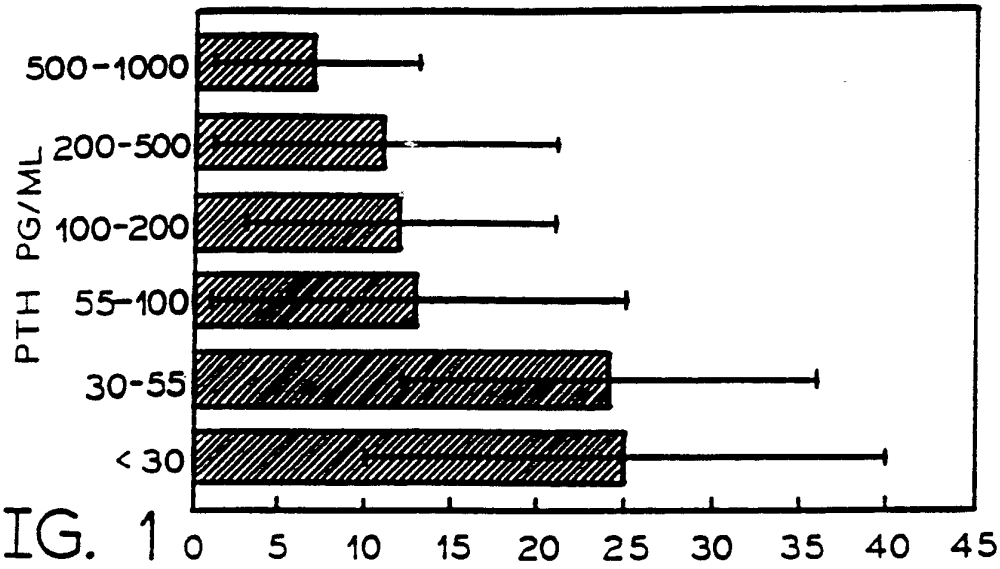


FIG. 1

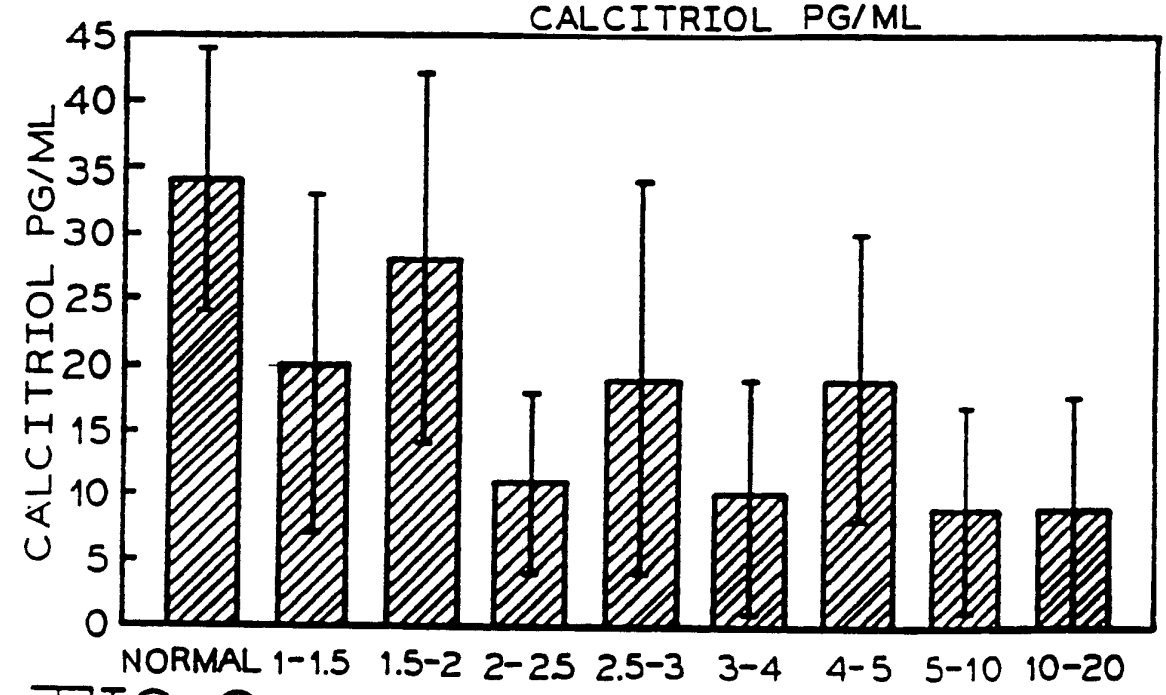


FIG. 2

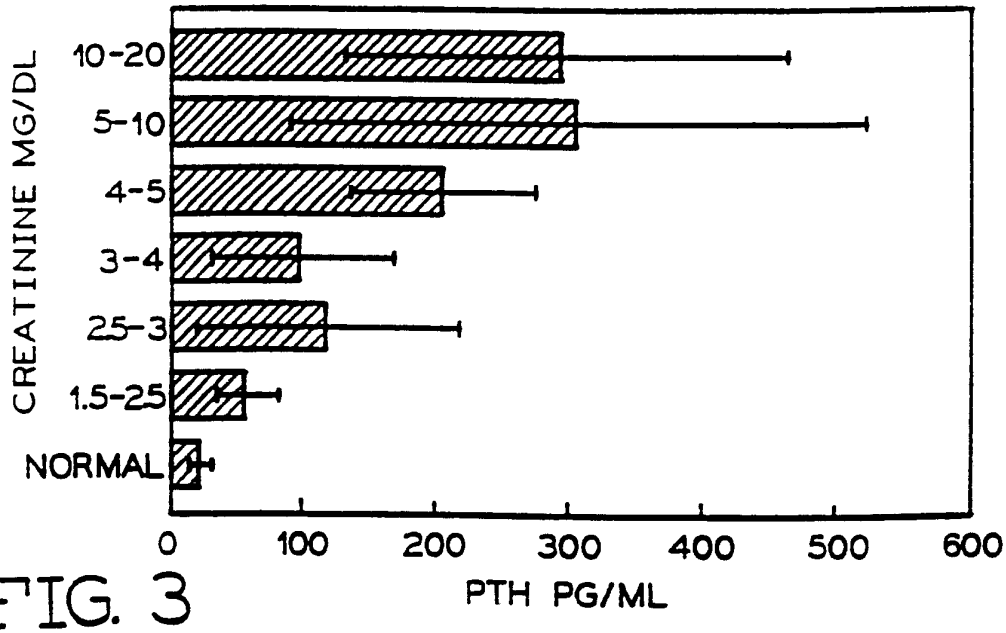


FIG. 3

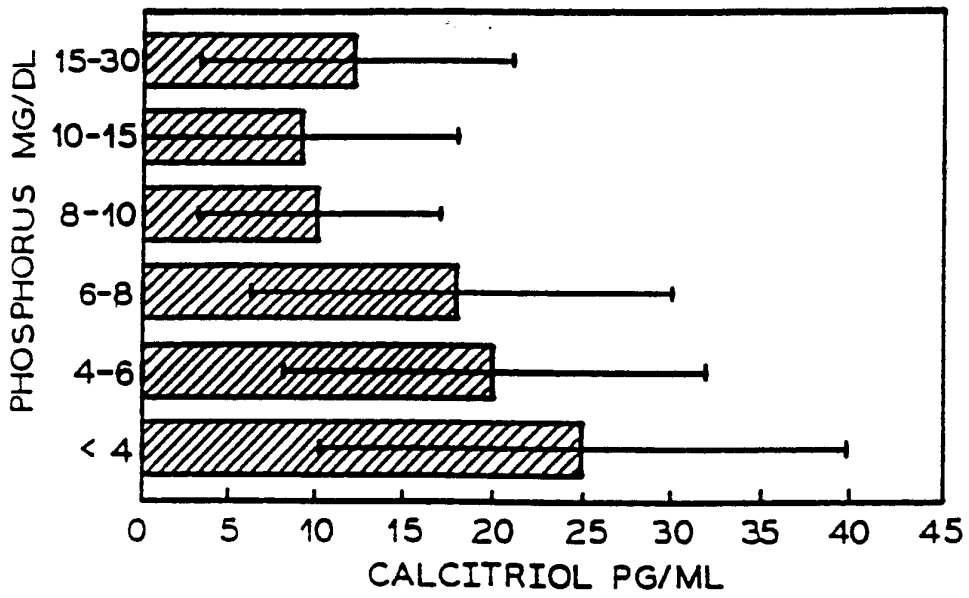

PHOSPHORUS MG/DL
15-30
10-15
8-10
6-8
4-6
< 4
0
5
10
15
20
25
30
35
40
45
CALCITRIOL PG/ML

FIG. 4

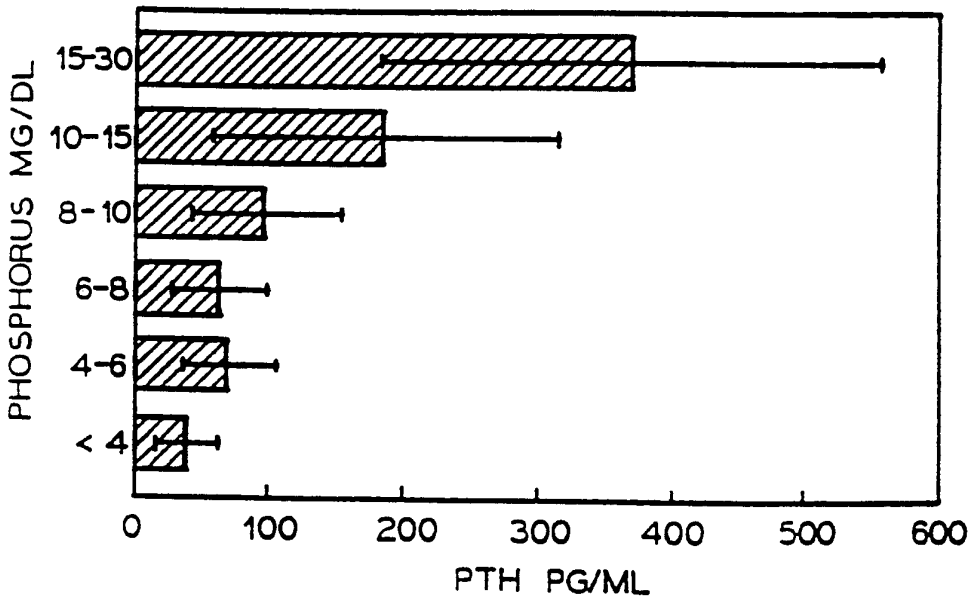

PHOSPHORUS MG/DL
15-30
10-15
8-10
6-8
4-6
< 4
0
100
200
300
400
500
600
PTH PG/ML

FIG. 5

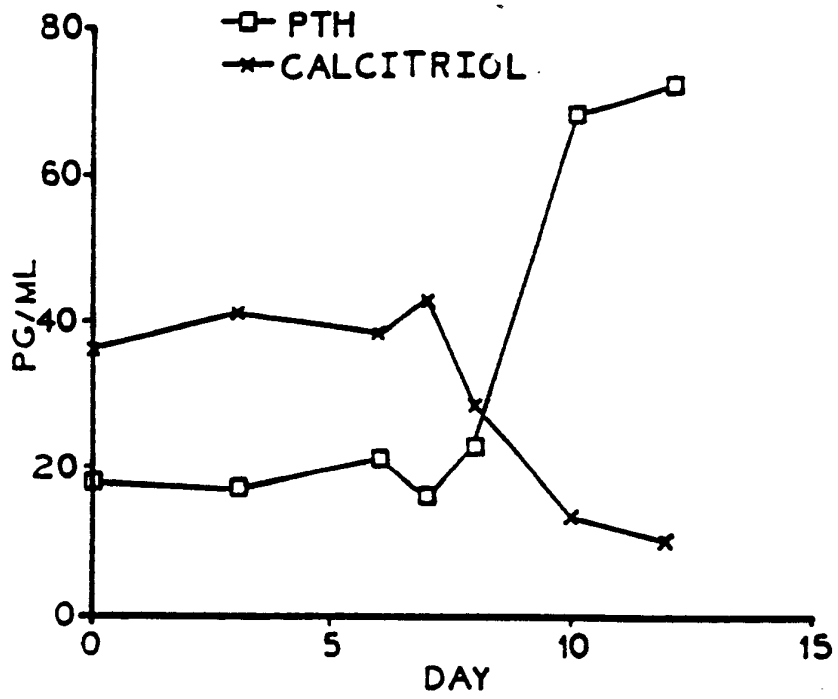

PTH
CALCITRIOL
80
60
40
20
0
PG/ML
0
5
10
15
DAY

FIG. 6

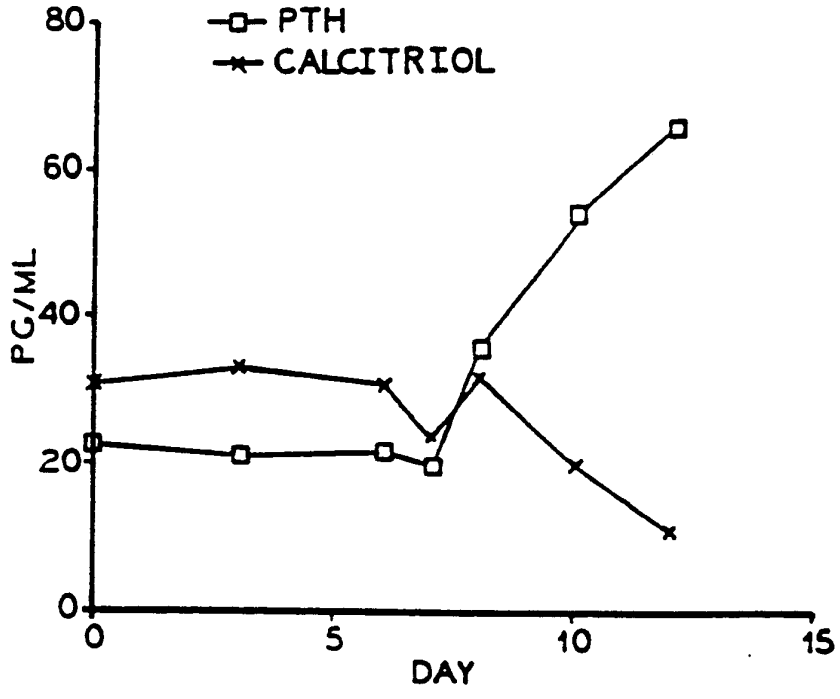

PTH
CALCITRIOL
80
60
40
20
0
PG/ML
0
5
10
15
DAY

FIG. 7

FIG. 8

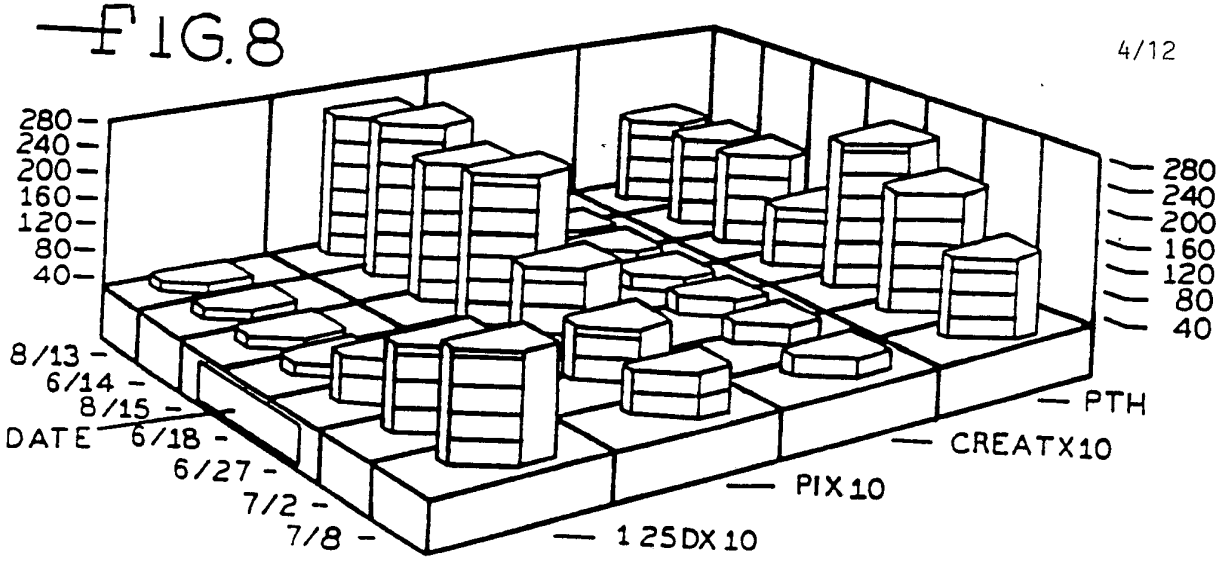


FIG. 9

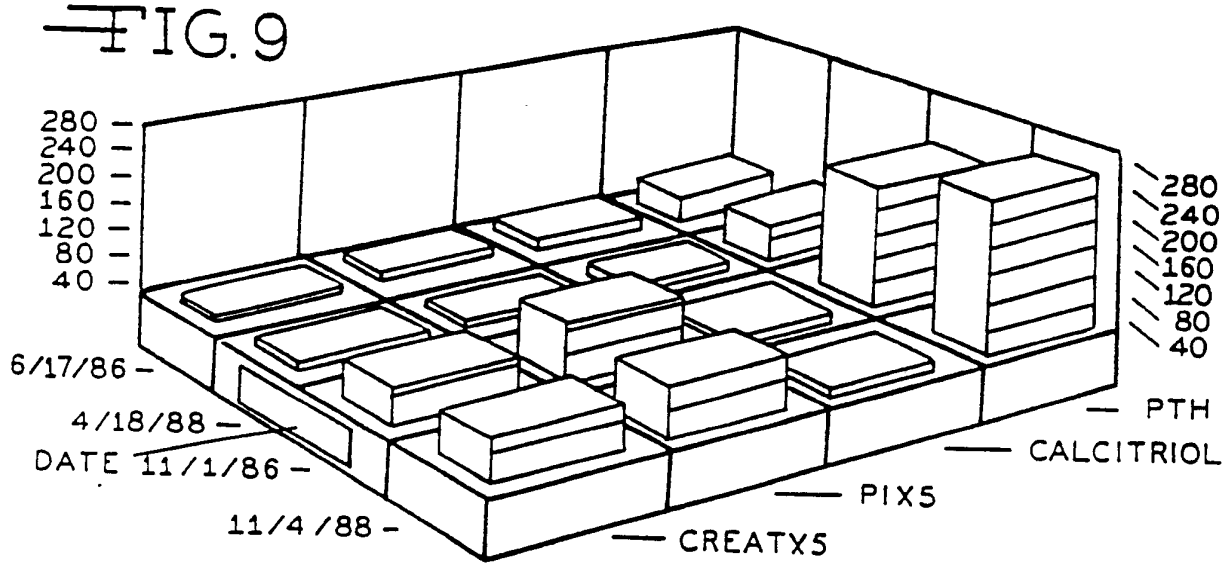


FIG. 10

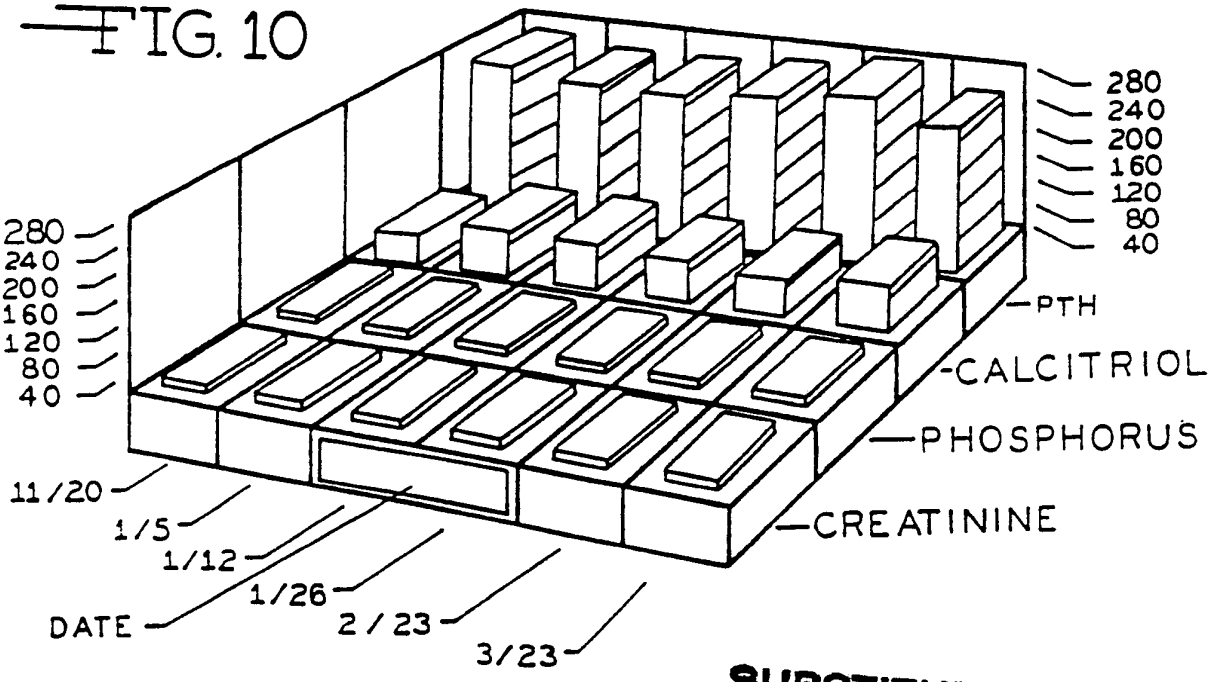


FIG. 11

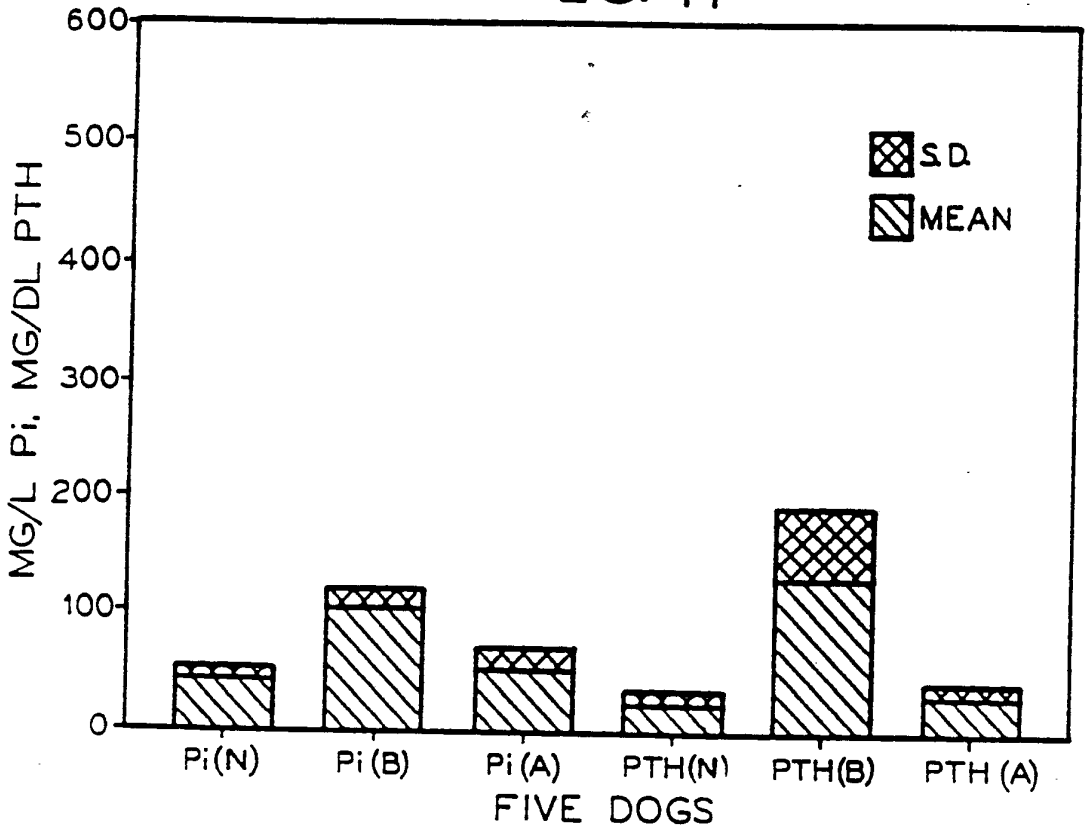
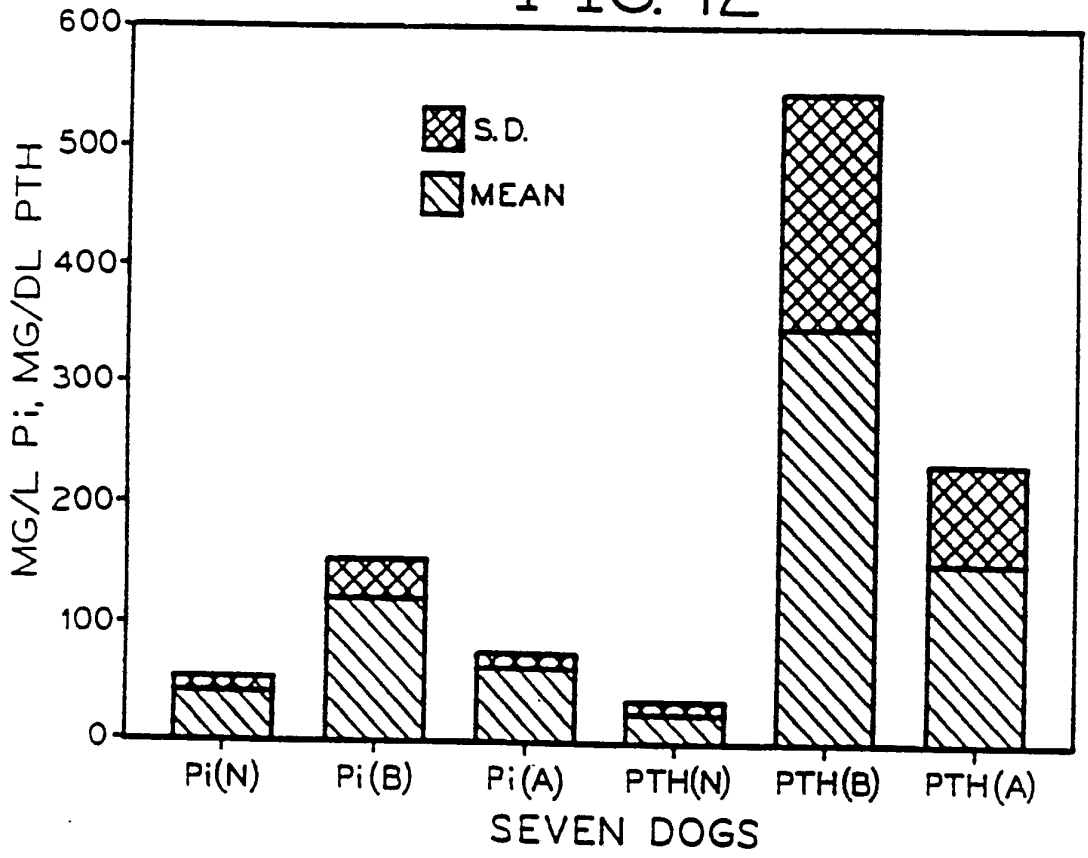

600
500
400
300
200
100
0
MG/L Pi, MG/DL PTH
S.D.
MEAN
Pi(N)
Pi(B)
Pi(A)
PTH(N)
PTH(B)
PTH(A)
FIVE DOGS

FIG. 12


600
500
400
300
200
100
0
MG/L Pi, MG/DL PTH
S.D.
MEAN
Pi(N)
Pi(B)
Pi(A)
PTH(N)
PTH(B)
PTH(A)
SEVEN DOGS

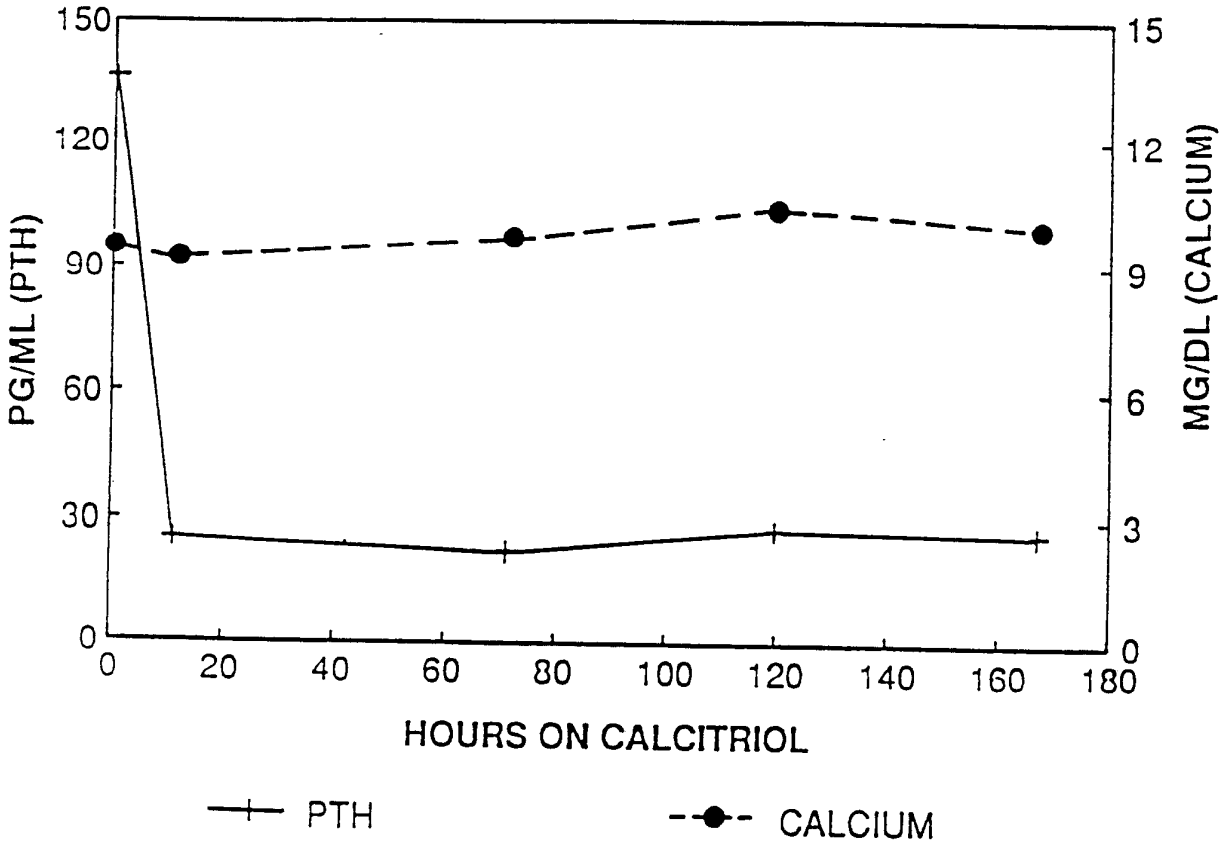

150
120
90
60
30
0
PG/ML (PTH)
15
12
9
6
3
0
MG/DL (CALCIUM)
0
20
40
60
80
100
120
140
160
180
HOURS ON CALCITRIOL
PTH
CALCIUM

FIG. 13

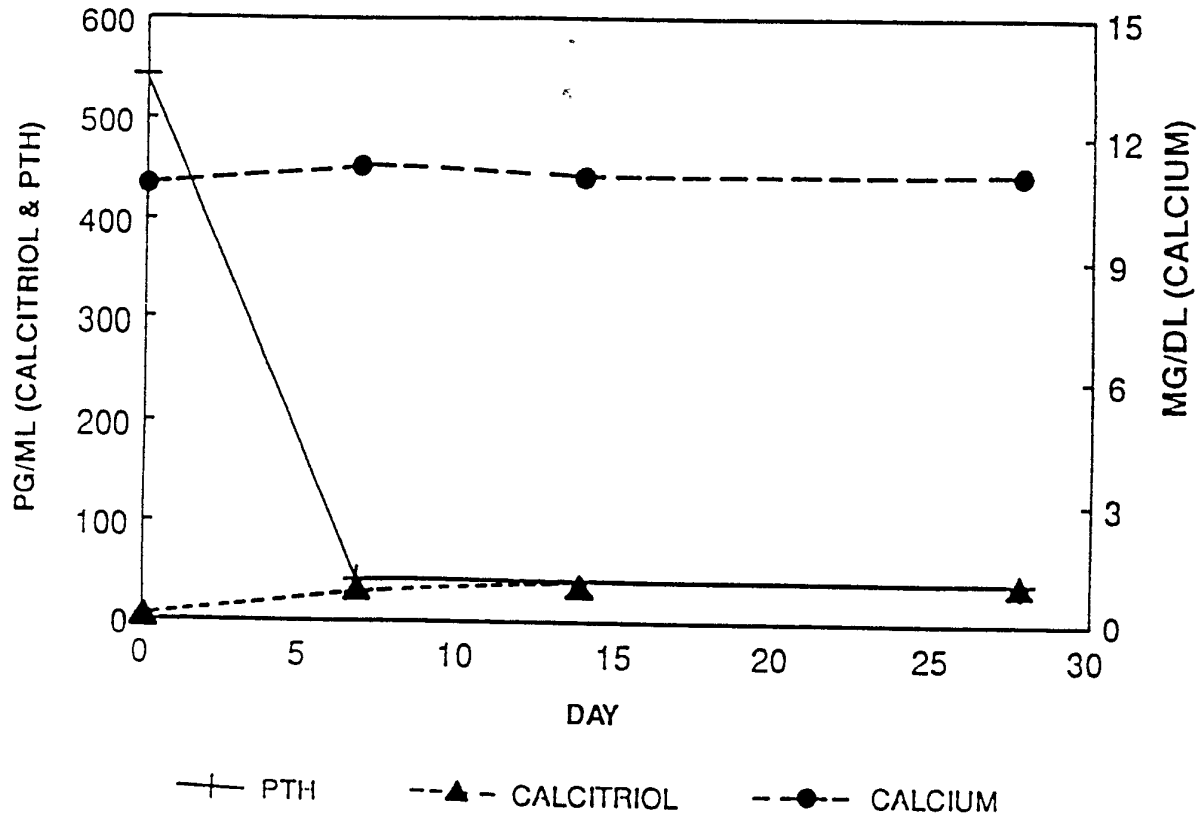


FIG. 14A

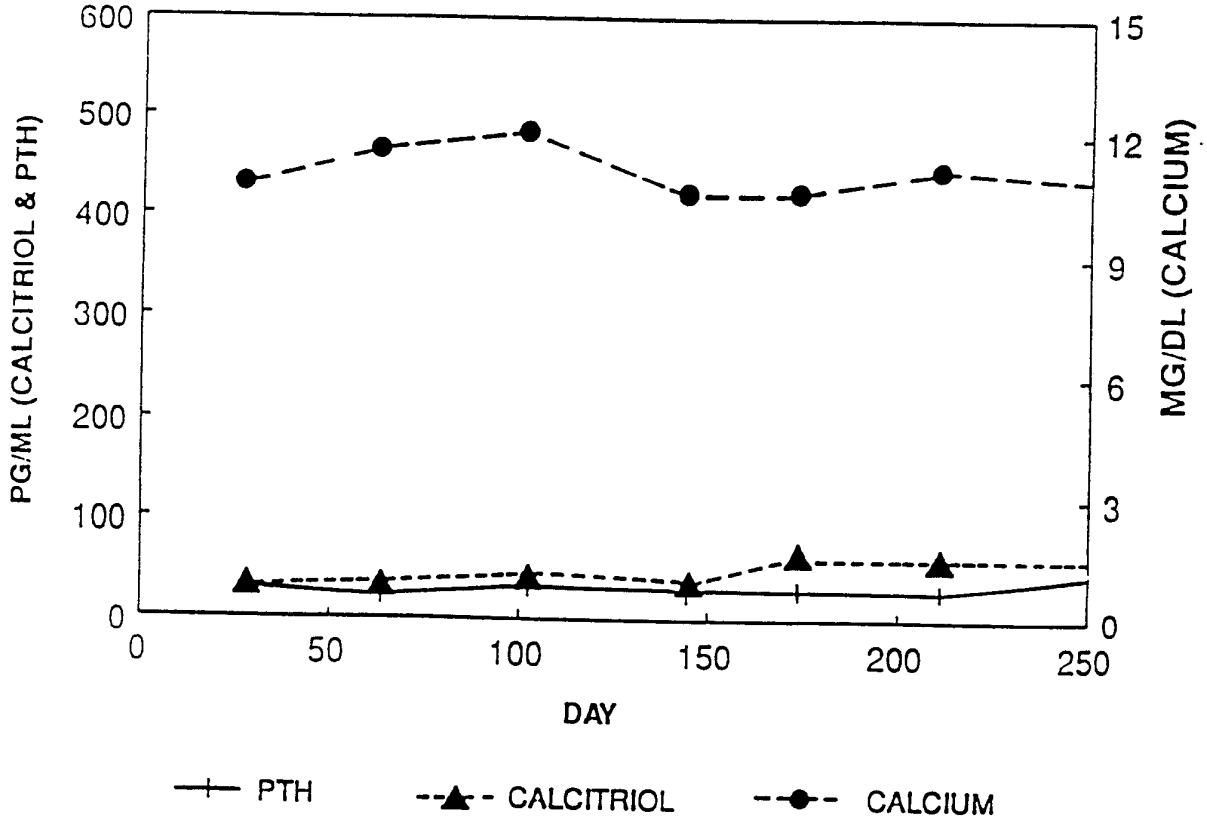


FIG. 14B

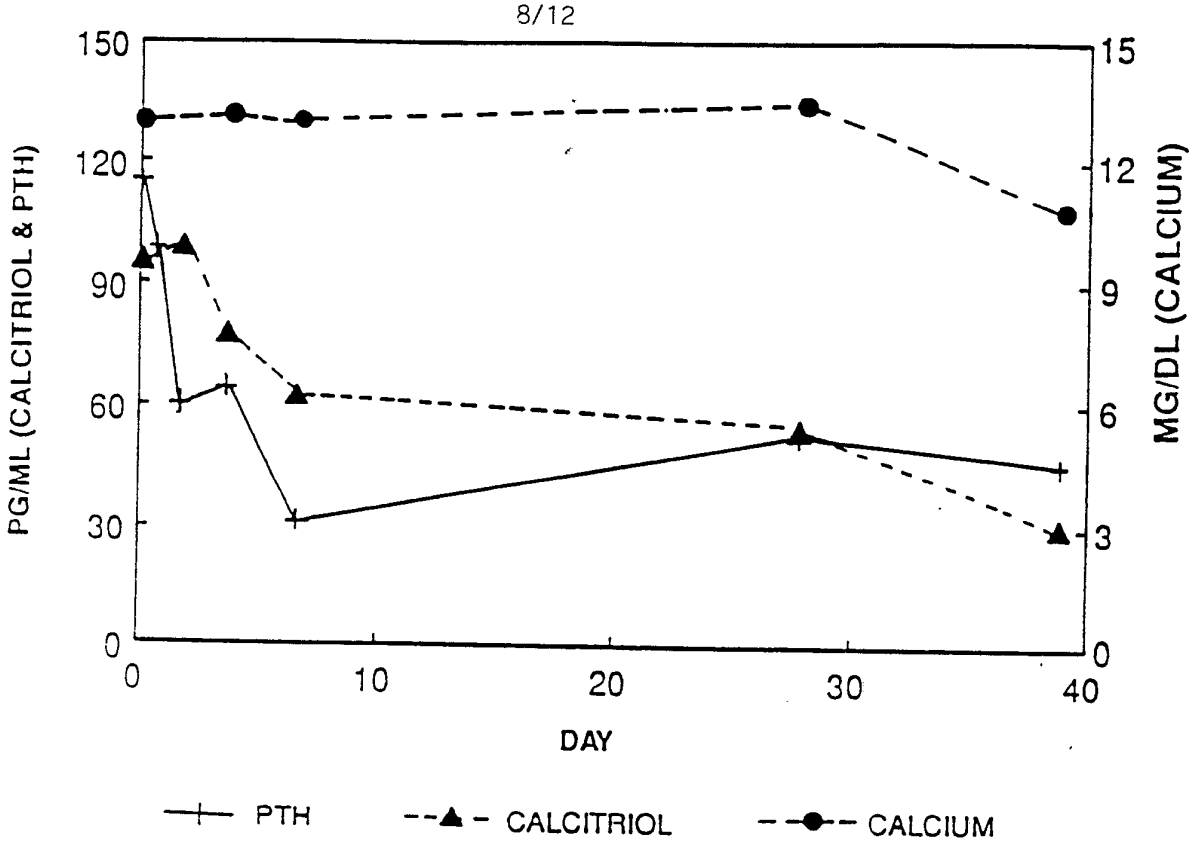


FIG. 15A

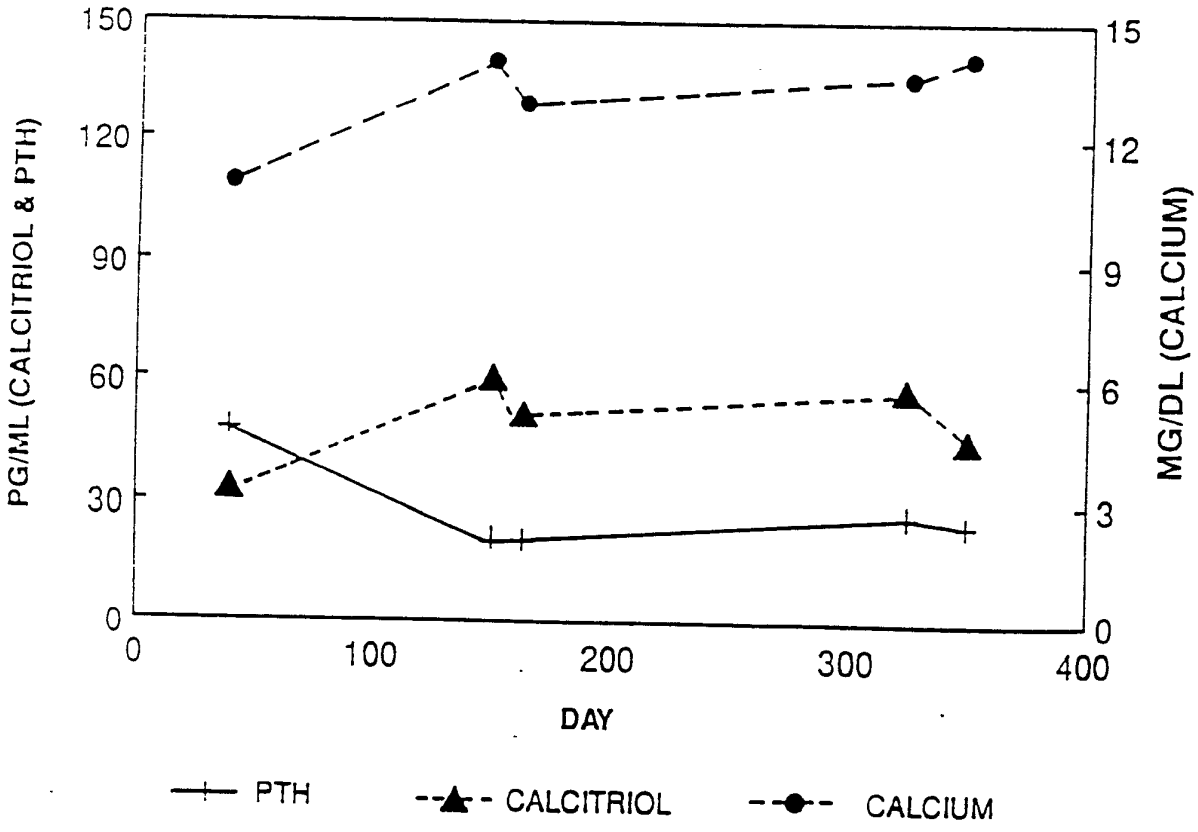


FIG. 15B

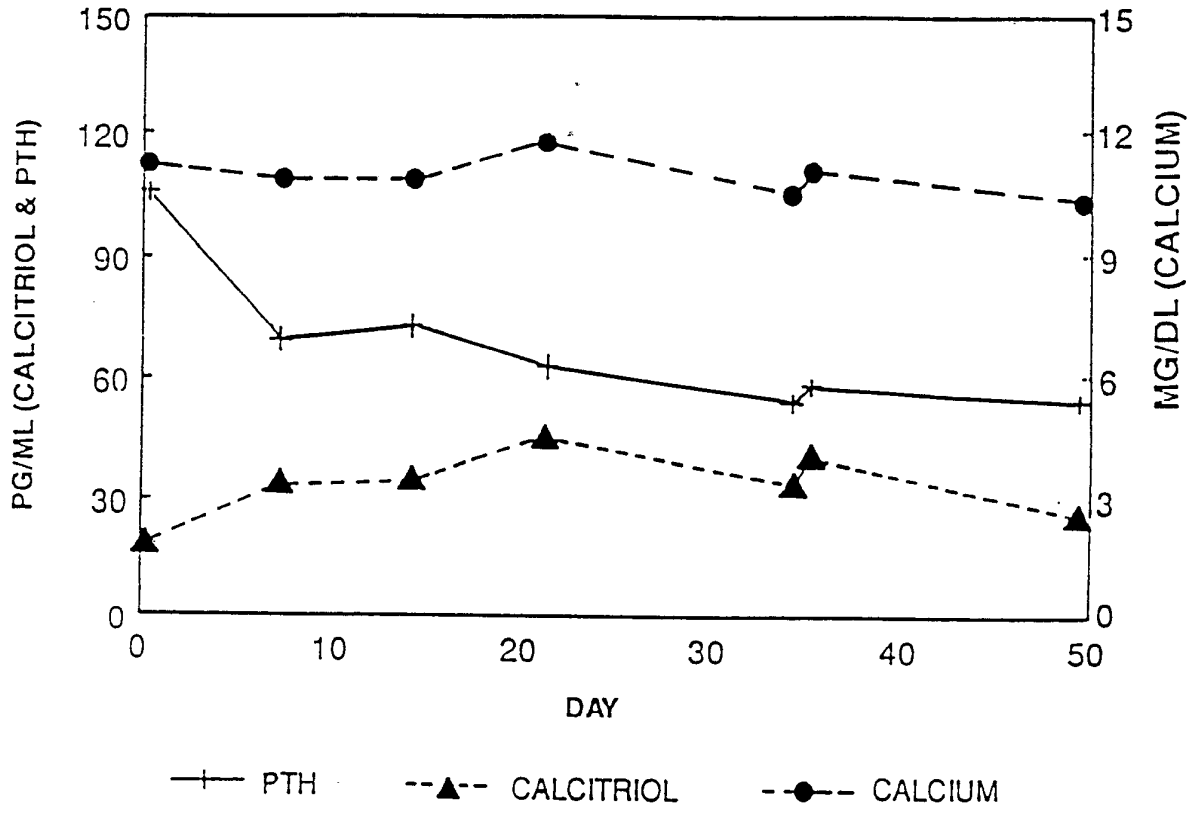


FIG. 16A

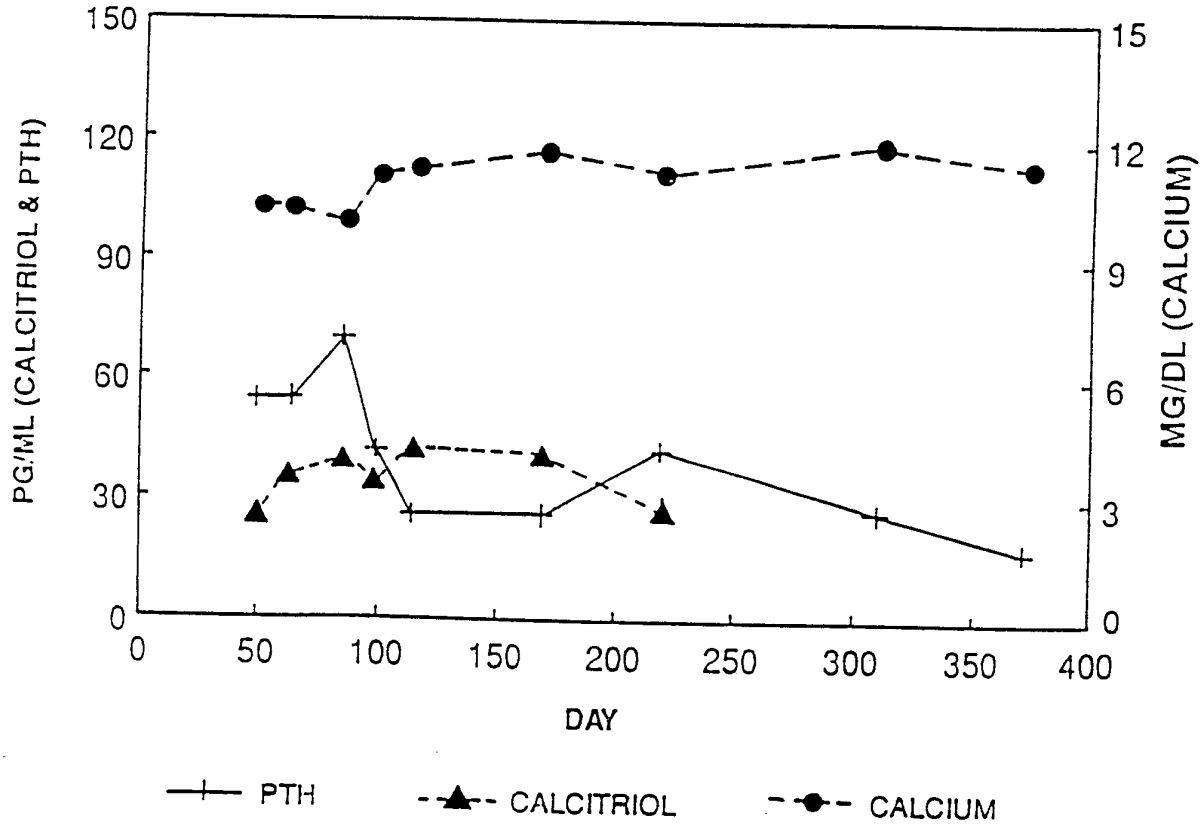


FIG. 16B

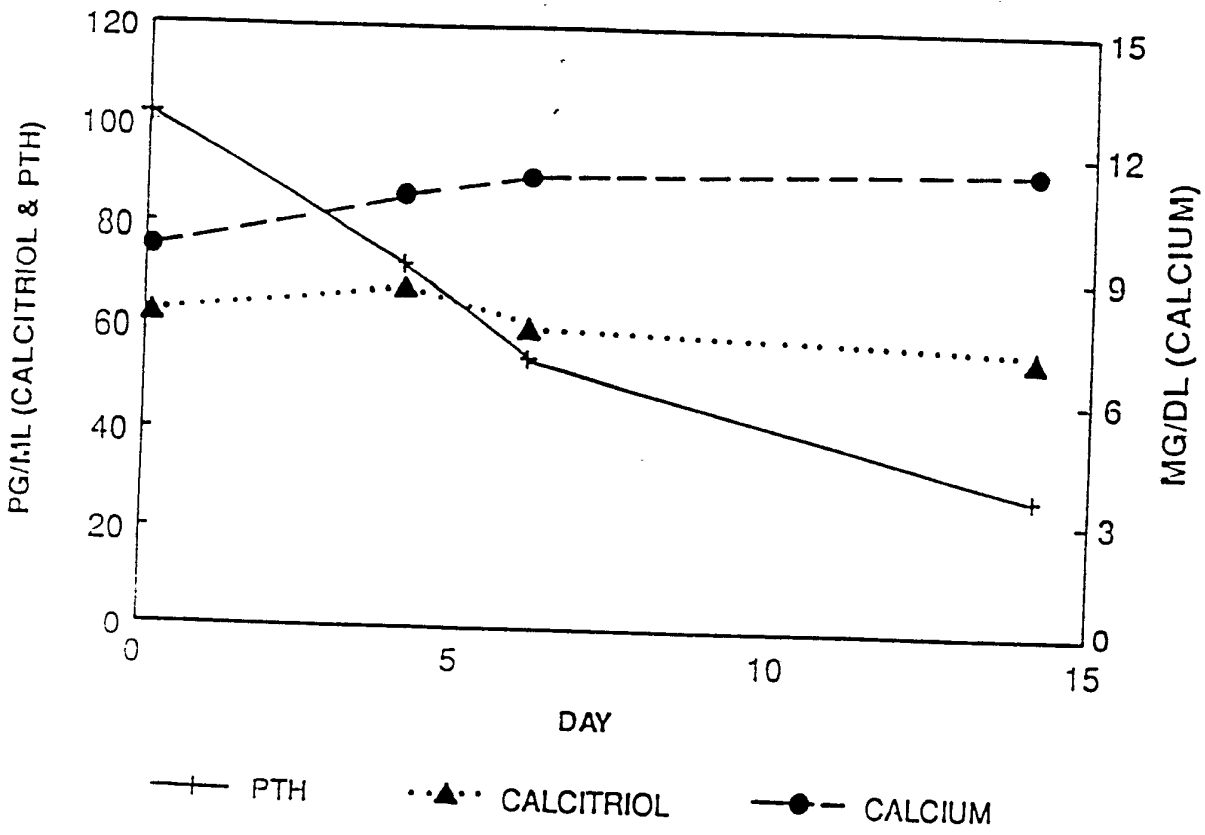


FIG. 17A

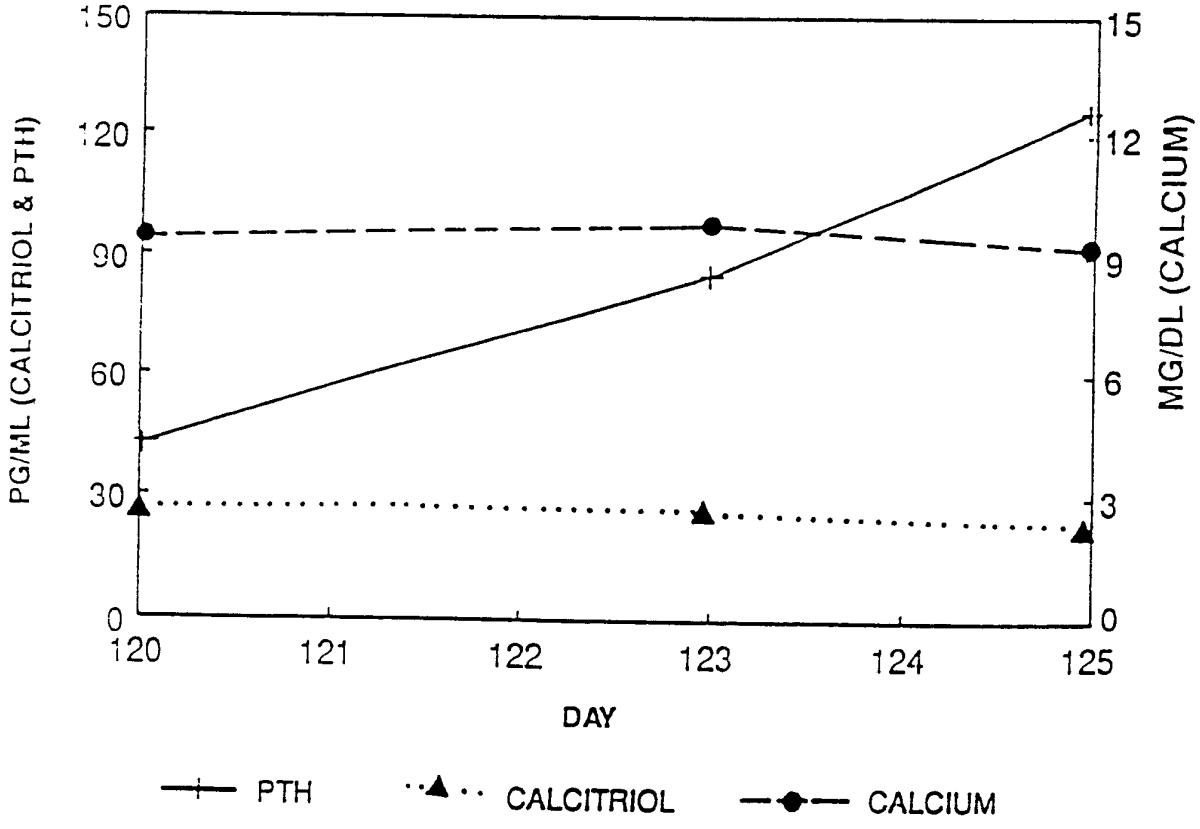


FIG. 17B

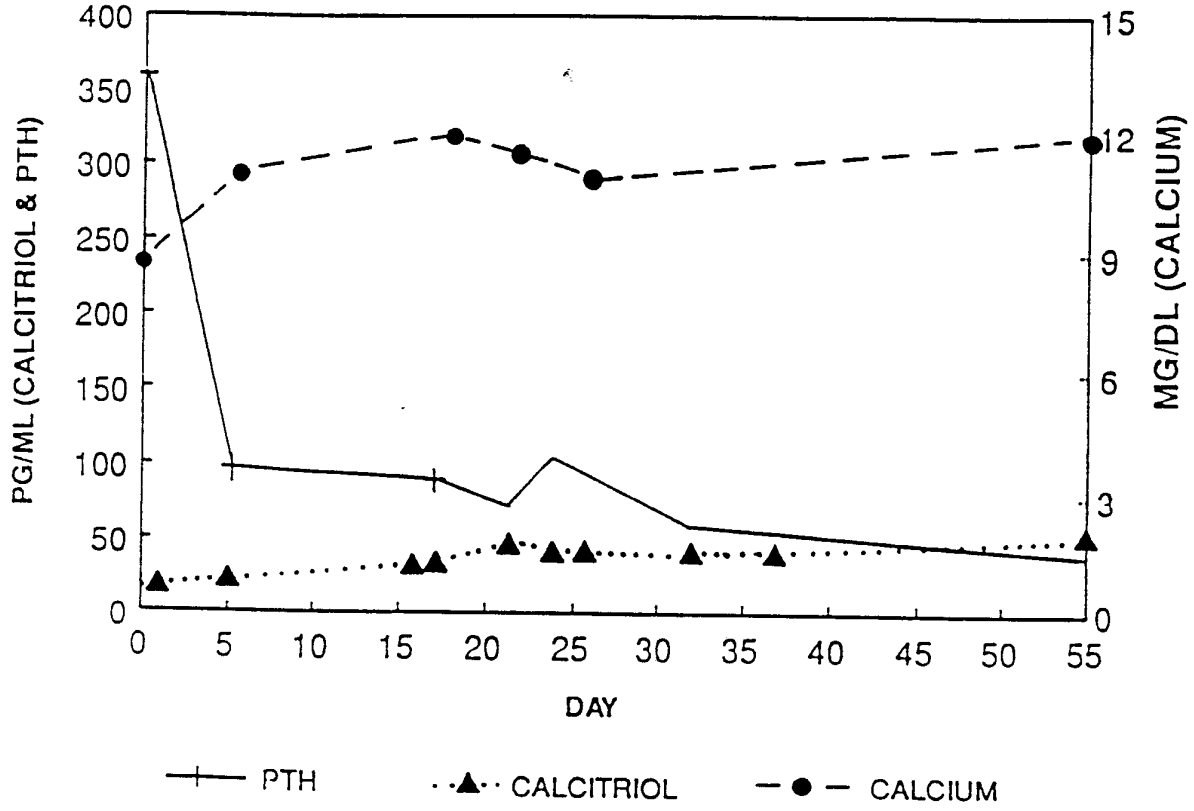


FIG. 18A

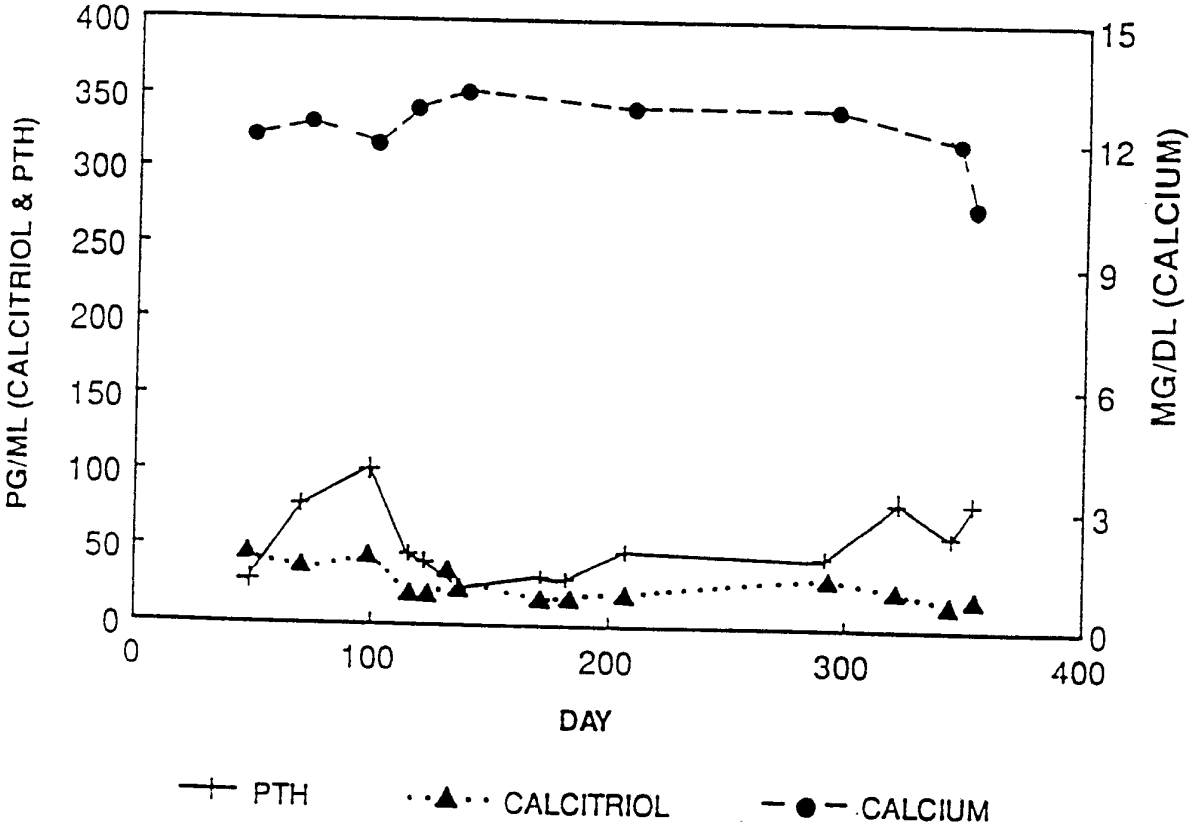


FIG. 18B

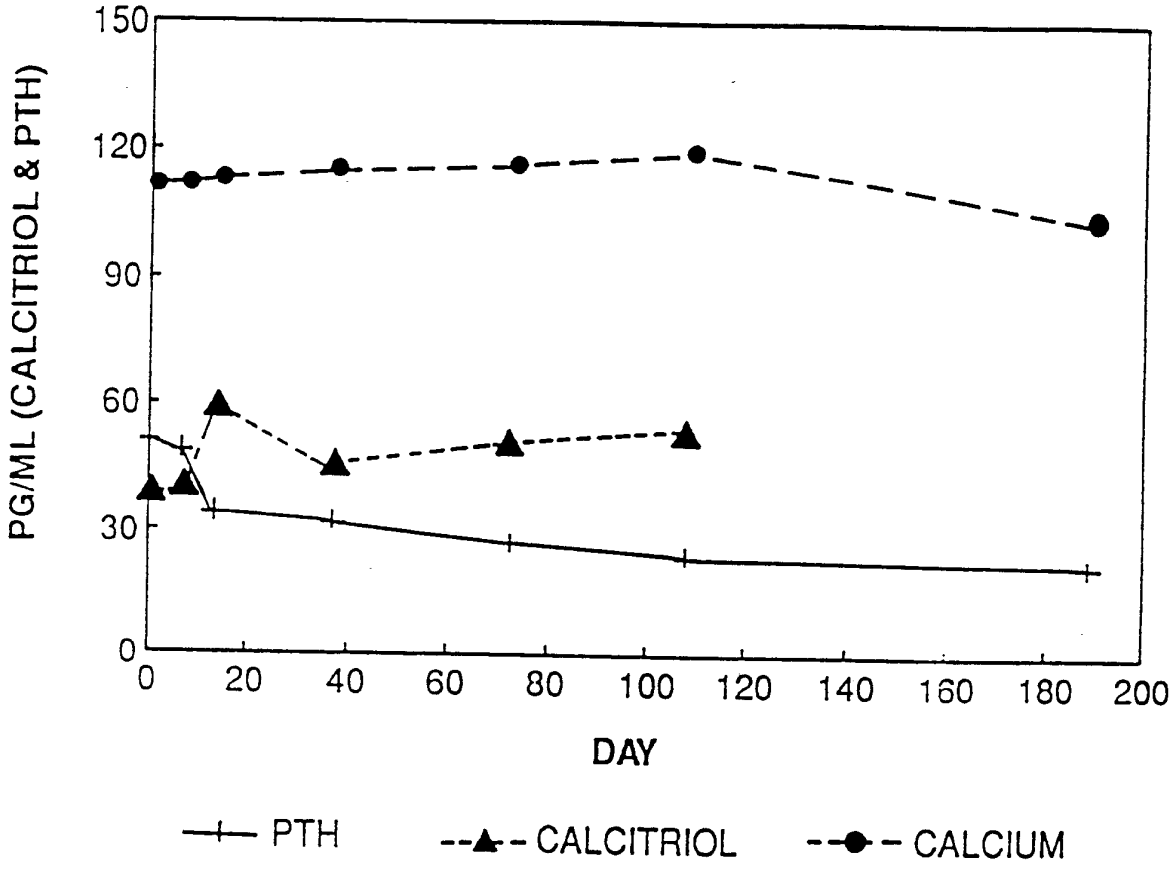

150
120
90
60
30
0
PG/ML (CALCITRIOL & PTH)
0
20
40
60
80
100
120
140
160
180
200
DAY
PTH
CALCITRIOL
CALCIUM

FIG. 19

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/02017

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A01N 45/00 C07J .75/00
US CL: 514/167 552/653

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US	514/167 552/653

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

APS/CAS on line

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US, A 4,230,701 (HOLICK ET AL) 28 OCTOBER 1980 See entire document	1-15
X	Rocaltrol Brochure, p.p. 1-12, HOFFMAN LA ROCHE (1979) See entire document	1-15
X	SLATATOPOLSKY ET AL, Parathyroid Humone Secretion: Perturlations in Chornic Renal Failure, Conr. Nephrol., 64: 16-24(1988)	1-15
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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
31 JULY 1991	16 AUG 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	THEODORE J. CRIARES

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