EFFECTS OF ACTIVATED CHARCOAL ON SERUM VITAMIN A, PARATHYROID HORMONE, ALKALINE PHOSPHATASE, CALCIUM, AND PHOSPHOROUS LEVELS IN PATIENTS ON HEMODIALYSIS

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INTRODUCTION

The prevalence of hypervitaminosis A appears to have increased in the last two decades in those persons receiving 20-30 times the recommended RDA for dermatological conditions and in some food faddists who ingest large doses of vitamins (1). One group of persons who are frequently seen to have excessive blood levels of vitamin A are patients undergoing chronic hemodialysis for end stage renal disease (ESRD)(2,3,4). Blood levels of vitamin A are often elevated to at least two times the normal level in unsupplemented uremic patients (5).

The use of chronic hemodialysis for the ESRD patient has prolonged life, alleviated the symptoms of uremia and provided a means by which many people can maintain a good and productive quality of life. This prolongation of life, however, has presented a number of new problems (5,6,7). Many of these problems involve the hypothesized accumulation of toxins and the depletion of essential nutrients.

One area that has received a great deal of attention is the control of bone disease in ESRD. Dr. Belding Scribner, who established the first chronic hemodialysis unit in the United States in 1960 in Seattle, Washington, noted that

after two years almost all chronic hemodialysis patients developed severe, debilitating bone disease (6). Around 1967, research indicated that the chronically elevated phosphorous level seen in these patients "turned on" the parathyroid glands in order to bring the serum calcium back to its 2:1 (calcium:phosphorous) ratio. By giving aluminum hydroxide binders, the serum phosphate could be maintained within a normal range.

It was soon noted that even if the serum phosphorous levels were brought into the normal range, the parathyroid glands did not necessarily, indeed often did not, return to a normal level of secretion (7). Thus, the bone disease often persisted. Because of overactivity of the parathyroid glands, hyperplasia of the glands generally developed. Parathyroidectomies were performed with reimplantation of a small amount of parathyroid tissue in the forearm. Still the bone disease persisted.

In the 1970s, DeLuca established the pathway by which active vitamin D is synthesized and functions in the body. From his research, it was established that cholecalciferol is hydroxylated by the liver as well as the kidney (1). It became apparent that in ESRD, the kidney is incapable of hydroxylating cholecalciferol to 1,25-dihydroxycholecalciferol, the active form. Within the last five years,

1,25-dihydroxycholecalciferol has become commercially available, and many ESRD patients are currently taking this medication. In spite of these great strides in identification of the pathways and the increased availability of medications to treat this widespread metabolic disorder, bone disease in patients with ESRD continues to persist. This persistence tends to indicate that not all of the factors involved have as yet been identified (8). It seems appropriate to further investigate the possible role that hypervitaminosis A may play in the bone disease seen in ESRD. Therefore, the following study was conducted.

PROBLEM STATEMENT

Does activated charcoal affect the blood levels of vitamin A, parathyroid hormone, alkaline phosphatase, calcium, and/or phosphorous in patients with end stage renal disease maintained on chronic hemodialysis?

HISTORICAL PERSPECTIVE

There are two major categories of renal failure, acute tubular necrosis (ATN) and chronic renal failure often called end stage renal disease (ESRD). ATN is generally a result of forces other than intrinsic renal disease which cause the kidney to cease functioning to the point where life cannot be sustained. Examples of the causes of ATN are prolonged use of nephrotoxic drugs, allergic reactions, hypotension during surgery, prolonged shock, or any other cause of decreased blood flow to the kidneys. ATN may last from a few days to as long as four months. If the patient can be maintained by dialysis, and the cause of the renal failure treated, then complete recovery of renal function is to be expected.

Chronic renal failure, on the other hand, is a result of a disease process inherently affecting the kidney. There is little that can be done to halt its inevitable course, a course which, without intervention by dialysis or transplantation, will lead to death from accumulated metabolic toxins.

Because of the persistent incidence of renal osteodystrophy in the chronic ESRD patient, it appears there very well may be other factors involved in this

often severe bone disease. It is one of these potential factors that the following study was designed to investigate, the effect of hypervitaminosis A on renal osteodystrophy.

The possibility that hypervitaminosis A was contributing to renal osteodystrophy was illustrated by the following case. A patient on hemodialysis transferred to the Community Dialysis Unit, The Methodist Hospital's chronic outpatient dialysis facility, to undergo training for Chronic Ambulatory Peritoneal Dialysis (CAPD). He was noted at the time of his transfer to be hypercalcemic and had already undergone partial parathyroidectomy. Prior to his transfer to the Methodist unit, the patient had had an extensive workup looking for a primary tumor. The workup was negative. Several months after completing CAPD training, the patient began complaining of increasing pruritus and, for the first time, heel pain. The patient was again admitted and worked up for a tumor. Again the results were negative. As an outpatient, serum parathyroid hormone, calcitonin, and 1,25-dihydroxycholecalciferol ($1,25\ (OH)_2D_3$) levels were drawn and were within normal limits. The chief of the renal service noted that vitamin A levels were high in the majority of the dialysis population, and that skeletal lesions had been reported in hypervitaminosis A

(4,9). The patient's serum vitamin A level proved to be greater than 1300 IU/100 ml, approximately six times normal. A dietary history showed no abnormally high vitamin A intake. Since activated charcoal had been used in the treatment of pruritus, the patient began a regimen of oral activated charcoal with meals. Within one month, the heel pain had decreased, although without major resolution of the pruritus, and the serum calcium returned to within normal limits. After taking the charcoal for one and a half months, the patient stopped taking the charcoal on his own volition. The bone pain recurred. The charcoal was restarted and the symptomatic bone pain again subsided. In view of this case, it seemed worthwhile to see if similar results could be achieved in other patients.

In order to study hypervitaminosis A, four separate entities must be considered: dialysis, renal osteodystrophy, the effect of hypervitaminosis A on bone, and the use of activated charcoal. Each will be reviewed separately in the context of the study. A statement of their possible interaction will be made at the conclusion of this review of the literature.

Dialysis

It was not until the early 1940s that an effective method was developed for treatment of end stage renal

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time. This was a costly procedure, however, and funding became a limiting factor in gaining access to the procedure. For the most part, the funding problem was solved in the United States by the passage and promulgation in 1973 of Public Law 92-603 (11). This law provided payment for chronic dialysis for patients with end stage renal disease who qualified for Social Security. Now, not only the technical means, but the economic means for conducting long term maintenance dialysis had been achieved. Approximately 98% of the American population would be eligible for Social Security coverage for ESRD maintenance dialysis and/or transplantation.

At the present time there are two types of dialysis, hemodialysis and peritoneal dialysis (PD). The basic principle for both types of dialysis is osmosis or diffusion of solutes across a semi-permeable membrane. In hemodialysis, the semi-permeable membrane is a man-made cellulose acetate membrane (10). The cellulose acetate membrane of cellophane was first developed and used for sausage casing. The membrane has micropores which allow small molecules such as sodium, potassium, calcium, phosphorous, urea, and creatinine to diffuse through the membrane but retain larger molecular weight solutes such as albumin, red blood cells, and hormones. The diffusion occurs between two

fluid compartments: blood and the dialysate. Blood contains the higher solute concentration. The dialysate contains the following concentration of solutes: sodium 135 mEq/l; potassium 2.0 mEq/l; calcium 3.5 mEq/l; phosphorous 0; magnesium 0; urea 0; and creatinine 0.

Peritoneal dialysis (PD) uses the body's own peritoneum as the semi-permeable membrane. Blood from the vessels in the peritoneal membrane is again one of the fluid compartments. Dialysis solutions containing sodium 132 mEq/l, potassium 0, calcium 3.5 mEq/l, phosphorous 0, magnesium 1.5 mEq/l, urea 0, and creatinine 0 are infused into the peritoneal cavity through a Teneckhoff catheter (a permanent peritoneal catheter), allowed to equilibrate for 20 minutes to 4 hours, and then drained. The process is repeated until serum levels of blood area nitrogen (BUN), creatinine, and potassium are lowered to the desired levels. Because larger molecular weight solutes are dialyzable in PD and the efficiency of this dialysis may vary from one patient to another, for the purpose of this study only patients on hemodialysis were selected instead of PD patients.

Renal Osteodystrophy

As previously stated, the interest in renal osteodystrophy has grown rapidly in recent years due to the high incidence of bone disease in patients with chronic

renal failure and in those treated with maintenance dialysis. The term renal osteodystrophy includes all skeletal disorders that occur in patients with renal failure.

The major chemical and metabolic features of renal osteodystrophy include hypocalcemia, hyperphosphatemia, hyperplasia of the parathyroid glands,and high circulating levels of parathyroid hormone (PTH), impaired intestinal absorption of calcium, abnormal metabolism and action of vitamin D, bone disease, and soft tissue calcifications (7). Radiographic features of hyperparathyroidism, osteosclerosis, osteoporosis, and osteomalacia have all been observed in patients with chronic renal failure (7).

Subperiosteal reabsorption, most often seen in the phalanges of the hand, is probably the most easily recognized feature of secondary hyperparathyroidism seen on X-rays. Osteosclerosis, or increased areas of bone density which appear in bands, is the result of remineralization of bones that have shown previous reabsorption. In children with longstanding renal failure, the defective mineralization leads to abnormalities which are typical of rickets. The appearance of "renal rickets" is quite common in children with end stage renal disease. The infrequent pseudofracture or Looser's zone is the only sign of osteomalacia,

and generally occurs in areas of mechanical stress or at the site of entry of nutrient vessels. Osteopenia, decreased density of bone, a nondiagnostic feature of osteomalacia, is frequently seen. However, osteomalacia, secondary hyperthyroidism and osteoporosis can all lead to decreased bone density, making it impossible to determine the cause of osteopenia by X-ray alone. Fractures, most commonly in the ribs, are not infrequent in patients with renal osteodystrophy (7).

In general, the radiologically-confirmed incidence of renal osteodystrophy is as high as 66% of the dialysis population (7). Severity of bone disease increases with the length of dialysis (7). Improvement may occur when the ionized calcium in the dialysate is present in a concentration of 6.0 to 7.4 mg/100 ml (7). Since approximately 50-60% of the serum calcium is ionized, a dialysate concentration of 6.0 to 7.4 mg/100 ml will cause a net increase to the patient of ionized calcium during dialysis (7).

It has been suggested that an important factor causing hypocalcemia is phosphate retention that occurs transiently and perhaps undetectably very early in renal failure. Such transient hyperphosphatemia may temporarily decrease blood calcium, causing the parathyroid glands to secrete more parathyroid hormone (PTH). The increased PTH would

decrease renal tubular reabsorption of phosphorous and calcium towards normal until a new steady state is reached. See Figure 1 (7,12).

There is little doubt that an elevated serum phosphorous in patients with advanced renal failure will decrease serum calcium levels and produce parathyroid hyperplasia. In an attempt to decrease the incidence of soft tissue calcifications, it is recommended that the serum calcium and phosphorous product be kept below 70 (13). The incidence of soft tissue calcification is very high when the product of the serum calcium concentration times the serum phosphorous concentration is greater than 75 and is infrequent when it is below 70 (7). To lower the serum phosphate levels, aluminum hydroxides are given orally with meals. The aluminum binds with phosphorous in the gut, forming a non-absorbable compound preventing GI absorption of phosphate.

The homeostasis of calcium in the body is dependent not only on the regulation of serum phosphate, but on two different hormones, vitamin D and parathyroid hormone (PTH). The precise control of calcium by these factors, and perhaps others, contributes to the structural integrity of bone.

Over the last decade, research has shown that the liver and the kidney are jointly responsible for producing

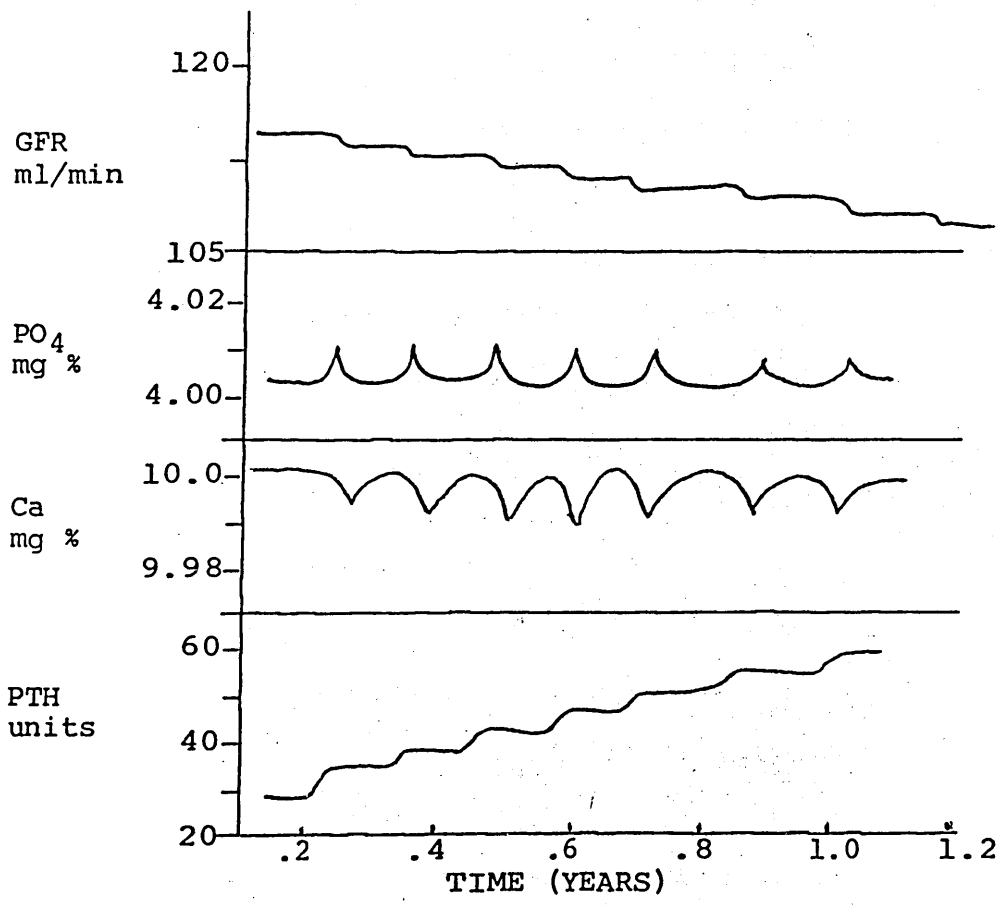

Figure 1. Possible sequence of events occurring early in the course of chronic renal disease which lead to parathyroid hyperplasia (7).

Key: GFR = Glomerular Filtration Rate

PO_4 = Phosphorous

Ca = Calcium

PTH = Parathyroid Hormone

1,25 $(OH)_2D_3$, vitamin D_3, the active form of vitamin D. The normal sources of vitamin D for humans are either from the diet or through ultraviolet irradiation of the pro-vitamin, 7-dehydrocholesterol, a compound found abundantly in the skin (14). Studies indicate that 7-dehydrocholesterol is first converted to pre-vitamin D_3 which remains in the skin and is slowly converted to vitamin D_3. This conversion to vitamin D_3 is temperature-dependent, but may average between 2.5 to 10.0 ug/day (100-400 IU), values near the minimal daily requirement (14,15). Some renal failure patients have had moderately successful resolution of the pruritus associated with hyperparathyroidism by daily exposure to ultraviolet light (16). Dietary vitamin D is absorbed in the proximal small bowel, carried in the plasma bound to a specific vitamin D-binding protein, and transported primarily to the liver.

The renal conversion of 25-hydroxycholecalciferol (25-$(OH)D_3$) to 1,25 $(OH)_2D_3$ appears to be the most closely regulated step in the bioactivation of vitamin D. The enzyme responsible for this conversion is 25-hydroxycholecalciferol-1α-hydroxylase and is located in the mitochondria of the renal cortex. Physiological factors regulating this conversion are not known at this time, but may be due to changes in the intracellular or intramitochondrial

concentrations of calcium, phosphorous, and hydrogen ions. Effectors may include PTH, calcitonin, $1,25(OH)_2D_3$, or other forms of vitamin D (14). All have received considerable interest, but their effect remains unclear. Fairly well documented facts include: (1) high levels of $1,25(OH)_2D_3$ inhibit 25-hydroxycholecalciferol-1α-hydroxylase, (2) as calcium is lowered this inhibitory effect is lowered, (3) increased dietary calcium intake decreases the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, (4) increased PTH increases the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, (5) increased intestinal calcium absorption increases the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, (6) dietary phosphate restriction augments GI absorption of calcium, (7) estrogen increases $1,25(OH)_2D_3$ generation, progesterone exhibits the same response but to a lesser degree, (8) prolactin and growth hormone stimulate the renal production of $1,25(OH)_2D_3$, (9) absence of insulin decreases $1,25(OH)_2D_3$ generation, and (10) cortisol and other glucocorticords suppress intestinal calcium absorption and decreases $1,25(OH)_2D_3$ production.

One of the target tissues for vitamin D is the bone, but the precise mechanism of action at this site is controversial. It is unclear if vitamin D has a direct effect or whether the observed effects are a result of

increased levels of calcium and phosphorous in the blood and extracellular fluid. Clinical observations made in uremic and dialysis patients suggest that vitamin D and its metabolites are effective in inducing the mineralization of osteoid, the unmineralized bone matrix (14). This mineralization often occurs before there are measurable changes in serum calcium and phosphorous. Dialysis using a mildly elevated ionized calcium does not in and of itself prevent osteodystrophy. However, treatment with vitamin D can reverse the bone disease in some cases. Therefore, evidence from in vivo observations support theories that vitamin D enhances and normalizes bone synthesis and mineralization in spite of animal studies indicating that $1,25(OH)_2D_3$ significantly increases bone reabsorption (14).

The second hormone affecting calcium homeostasis is parathyroid hormone (PTH). PTH is secreted by the parathyroid glands from two precursors, pre-pro-PTH and pro-PTH. The amino acid content is reduced by proteolytic cleavage from 115 amino acids to 90, then 84 respectively. Serum calcium and serum magnesium are the most important factors in the secretion of PTH. A calcium decrease as small as 0.1-0.2 mg/dl is an important stimulus in PTH secretion (14). The mechanism is as yet unclear. Hypermagnesemia suppresses, and hypomagnesemia enhances, PTH secretion.

It is known that severe magnesium depletion increases PTH secretion, and there is evidence suggesting that severe magnesium depletion in uremic patients decreases PTH secretion in spite of hyperplastic glands and marked hypocalcemia (14).

To a lesser extent beta-adrenergic agonists, such as isoproterenol, epinephrine, and norepinephrine, stimulate PTH secretion in vitro (17). Preliminary results indicate that treatment with propranolol, a beta-adrenergic blocker, lowers serum PTH levels in uremic patients maintained on hemodialysis (17). Hypertension is a common consequence of chronic renal insufficiency and is frequently seen in dialysis patients. Beta-blocking antihypertensive agents, e.g., propranolol, are frequently used.

From studies by Berson and Yalow, there has been shown the existence of fragments of PTH in plasma that are immunologically different from the hormone extracted from human parathyroid glands (18). These investigators showed that: (1) PTH fragments had varying half-lives, (2) the carboxyl PTH fragment was the most common fragment found in uremic patients, (3) the half-life of these fragments was generally prolonged in uremic patients. The molecular weight of these fragments was around 7,000 versus 9,500 daltons found in native PTH from the parathyroid gland (14). There is

evidence that these PTH fragments are made in the peripheral circulation, particularly by the liver and kidney (17). There are two recognized PTH fragments. The amino terminal PTH consists of amino acids 1-34 and is considered more biologically active. The second fragment identified is the carboxyl terminal, and to date it does not appear to be biologically active. However, its predominant occurrence in uremic patients, and the frequent incidence of bone disease, lead one to believe that it may well prove to be of physiological importance in uremia.

As stated, it is felt that the biologically active portion of PTH is in the first 34 amino acids from the N-terminal. Animal studies indicate that there is a threefold increase in activity when the PTH N-terminal is used than when the intact PTH was used (19). In addition, studies by Canterbury et al. suggest that the physiological significance of the peripheral metabolism of PTH may lie in providing close control of the effects of PTH on bone and does not simply represent catabolism of the intact hormone (20). Therefore, serum calcium levels may control the secretion of PTH from the parathyroid glands as well as modify the rate of formation of biologically active fragments by the liver and kidney. The fragments may then be responsible for calcium mobilization from the skeleton (14).

Bone cell function is regulated by the concentrations of calcium and phosphorous ions in bone cells. PTH acts by controlling the intracellular concentration of these two ions. PTH regulation of these ions has three general effects: (1) PTH increases the ratio of osteocytic to osteoclastic bone reabsorption, (2) it increases the rate of conversion of mononuclear phagocytes to osteoclasts and probably prolongs the life of individual osteoclasts, and (3) it affects the rate of synthesis of bone collegan by depressing osteoblastic activity (14).

Hypervitaminosis A and Bone Metabolism

The first report of pathological fractures caused by hypervitaminosis A was reported by Collazo in 1929 (11). There have been a number of animal studies on chicks and rats that show the toxic effects of vitamin A. These toxic effects in animals and humans are characterized by periosteal calcifications which are not consistent with the radiological findings attributed to those typical of secondary hyperparathyroidism (21). In addition, there are a number of case reports of skeletal pathology attributed to hypervitaminosis A in otherwise normal patients (21,22). Microradiographic studies on the human skeleton from two patients with hypervitaminosis A showed increased bone reabsorption (21,22).

In one of these patients, seven weeks after cessation of vitamin A intake, a bone biopsy showed that reabsorption surfaces had decreased (22). The serum levels of elevated vitamin A in the four patients reported above were 525, 625, 412, and 2459 IU/100 ml, with normal being less than 275 IU/100 ml. Three of the four patients had hypercalcemia, and serum alkaline phosphatase was elevated in three patients. A PTH level was drawn in only one of the four patients, and it was within normal limits. In this same patient, hydrocortisone was given with a concomitant decrease in the calcium levels.

This patient is of considerable interest. The duration of his illness was six years, and over these six years he had a number of renal complications, namely nephrocalcinosis, renal insufficiency, and nephrolithiasis. His renal function was greatly reduced, creatinine clearance (Cr Cl) was 17 cc/min with normal limits between 100-120 cc/min (21). It should be noted that dialysis is generally necessary when the Cr Cl is less than 10 cc/min. When the hydrocortisone was given his Cr Cl rose to 48 cc/min. All four patients complained of headaches, fatigue, bone pain, and arthralgias. Such complaints are frequently seen in patients on dialysis (7).

In 1977, Chertow et al. reported results from in vivo and in vitro studies on vitamin A stimulation of parathyroid hormone (8). From their studies in vitro, Chertow et al. suggest that vitamin A stimulates PTH secretion through a specific effect on basic cell function. They base this conclusion on: (1) The observed response of vitamin A on PTH secretion is both dose and time related. The longer the time and the higher the dose, the greater the PTH secretion. (2) The absence of stimulation by retinoic acid on PTH secretion. (3) The suppressive effect of calcium in the presences of vitamin A--secretion rates of PTH in the presence of vitamin A and a high calcium media were markedly less than seen with vitamin A and normal calcium media. However, vitamin A and a high calcium media did significantly stimulate secretion when compared to the suppressive effect on PTH secretion of calcium alone, and furthermore, vitamin A and a low calcium media also stimulated PTH secretion. (4) Antagonism of the vitamin A effect by vitamin E and hydrocortisone--vitamin A penetrates lipid membranes and alters membrane surface area and tension independently, and it is believed PTH is stored in the Golgi membranes as granules (14). It is possible that vitamin A may, through its presence in or interaction with membranes, modify the fusion properties of the granule or cell

membranes, while vitamin E and hydrocortisone, on the other hand, are membrane "stabilizers" and are known to antagonize the membrane effects of vitamin A. (5) The specific ultrastructure effects associated with the stimulation of PTH by vitamin A--in their ultrastructure studies vitamin A increased membranous intracellular microvillous interdigitations. This effect may in turn facilitate fusion between the secretion granule and cell membrane, and stimulate emicytosis and release of PTH. (6) That vitamin A increased lysosomal cathepsin D activity within the parathyroid gland, but did not increase the cathepsin D into the media--vitamin A is known to stimulate cathepsin D release from liver lyosomes through its effect on the lysosomal membrane; but because it does not increase cathepsin D into the media, this suggests that this is an intracellular effect. This effect supports the suggestion that PTH release is a result of proteolytic activity.

Chertow et al. also conducted in vivo studies of 11 normal males. Two intramuscular injections of vitamin A palmitate were given between 16 and 20 hours apart. The results indicated the PTH secretion was significantly increased in the subjects receiving injected vitamin A when compared with the saline injections given to the control group (8).

Chertow et al. concludes that these observations warrant further study, particularly in those groups where hypervitaminosis A and elevated PTH levels occur over a prolonged period of time, specifically those in renal failure and during pregnancy (8).

It has been reported by a number of investigators that hypervitaminosis A is extremely common in severe renal insufficiency and in the dialysis population (4,23,24). Even though the reasons for this two- to threefold increase in the dialysis population are not totally clear there are a number of reasonable documented possibilities. The dietary intake of some vitamins is less than optimum because certain foods high in these particular vitamins are substantially restricted because they are also high in potassium. This, however, is not the case in vitamin A. The typical renal diet can easily provide the RDA for vitamin A (23). Vitamin A has a molecular weight of 286, but circulates in plasma attached to a specific carrier protein, the molecular weight of which has been estimated to be approximately 22,000 daltons in man (24). This retinol-binding protein is normally filtered through the glomeruli of the kidney and reabsorbed in the tubules. Because of its high molecular weight, it is not readily dialyzed out. One study does report that 10% of the vitamin A is dialyzed out, but even

this does not represent an appreciable loss (24). Since the kidney is the normal excretory pathway for vitamin A, patients on dialysis have no means of excreting the vitamin. It is also generally felt that high vitamin A levels are a result of high circulating retinol binding protein (4,25). Why the retinol binding protein is high is not known. Whether these are all of the reasons for hypervitaminosis A in dialysis patients or not, the fact remains that the vast majority of these patients have chronic hypervitaminosis A (2,4,8).

Farrington et al. have recently published results of a study examining some of the biochemical, radiological, and histological indicators of bone disease in hemodialysis patients (26). Their results showed there was a statistically significant correlation between serum vitamin A levels and plasma calcium concentrations, but serum vitamin A did not correlate with age, duration of dialysis, or plasma calcium, phosphate, alkaline phosphatase, hydroxyproline or PTH concentrations. In those patients with hypercalcemia, their vitamin A levels were higher than in those with normal calcium levels. Subperiosteal calcifications were not detected in any patient. When vitamin A supplementation was withdrawn, the serum vitamin concentration remained higher but serum calcium levels fell. Four out of the six

hypercalcemic patients in this group became normocalcemic and also had a drop in serum alkaline phosphatase.

O'Fearghail et al., in response to Farrington et al.'s study, reported that there was seen a positive correlation between vitamin A levels and plasma alkaline phosphatase and PTH levels, but no correlation between vitamin A levels and serum calcium (27). Also in response to Farrington et al.'s study, Stewart et al. noted that his group found a positive correlation between hypercalcemia and the higher levels of vitamin A. They also saw no correlation between vitamin A levels and alkaline phosphatase, but did see a correlation between vitamin A levels and PTH. Those patients with the highest vitamin A levels had a significantly higher incidence in low bone density, increased PTH levels, and hypercalcemia after dialysis (28).

It is obvious from the above reports that there have been a number of differing biochemical and radiological findings in the reports of the effects of hypervitaminosis A and its impact on bone disease. Perhaps it follows that one explanation might be that vitamin A not only affects the secretion of PTH by the parathyroid glands, but also has a direct effect on the bone. Stewart said it very nicely, "perhaps not too much should be made of correlations, or the lack of them, between various parameters until causal

links have been established by further controlled investigations" (28).

Activated Charcoal

The use of sorbents in renal failure have been used off and on for the past decade in an effort to bind some of the uremic toxins. Those efforts have been made in an attempt to decrease, perhaps eliminate, the need for dialysis. Nephrologists have been especially interested in orally ingested sorbents which may extract uremic toxins from gastrointestinal fluids.

One of the major hinderances in the use of oral sorbents is that the "uremic" toxin or toxins are as yet unidentified. In 1964, Yatzitis described his lowering of blood urea nitrogen levels in uremic patients with 20-50 g of oral activated charcoal daily for 4 to 20 months (7). Other works have not had as satisfactory results; 50 g of activated charcoal in one study caused nausea to the point where the study had to be discontinued. Yatzidis' work was poorly controlled. His subjects were on 20 g protein diets and had creatinine clearances between 10 to 15 cc/min which is at or above the recommended levels for the inception of dialysis (7). Thus, the decrease in blood urea nitrogen may have not been a specific benefit from the oral activated charcoal. Studies since have confirmed that activated

charcoal does little to bind BUN or creatinine, but will bind ammonia. Sparks reasoned that the inefficiency of activated charcoal ingestion in lowering BUN and creatinine levels in uremic dogs might be due to lipids in the intestinal tract blocking surface sites otherwise available to nitrogen wastes (7). If this should indeed be the case, there is reason to believe that the activated charcoal would preferentially bind fat soluble compounds. Hence, vitamin A might be more readily absorbed than other compounds.

Summary

Renal osteodystrophy is a frequent, sometimes painful and debilitating disease. Over the last decade great strides have been made in studying the metabolic pathways of what appear to be the major abnormalities in calcium, phosphorous, 1,25 $(OH)_2D_3$, and PTH. These discoveries have been achieved in a very short period of time, yet much remains to be clarified. There appears to be sound documented evidence that hypervitaminosis A plays a role in bone reabsorption. Whether this excess in vitamin A plays a substantial role or any role at all in renal osteodystrophy is as yet unclear. The present study was conducted in an attempt to address this issue.

HYPOTHESES

The hypotheses which were examined were:

1. There is no significant difference in the mean blood level of vitamin A before and after oral administration of activated charcoal in a group of chronic hemodialysis patients.
2. There is no significant difference in the mean blood level of parathyroid hormone before and after oral administration of activated charcoal in a group of chronic hemodialysis patients.
3. There is no significant difference in the mean blood level of the alkaline phosphatase before and after oral administration of activated charcoal in a group of chronic hemodialysis patients.
4. There is no significant difference in the mean blood level of calcium before and after oral administration of activated charcoal in a group of chronic hemodialysis patients.
5. There is no significant difference in the mean blood level of phosphorous before and after oral administration of activated charcoal in a group of chronic hemodialysis patients.

METHODS

The sample consisted of ESRD patients undergoing chronic hemodialysis treatment at the Community Dialysis Unit at The Methodist Hospital. Selection criteria were: (1) the subject was over 18 years of age, (2) each had three or more chemical or clinical signs of renal osteodystrophy, and (3) each had been on dialysis for at least two years.

The subjects were evenly divided at the onset of the study into two groups of 15 patients each, 18 years of age or older (see Appendix). Each patient had a parathyroid hormone level of 3,000 nleq/ml (normal levels, 50-500 nleq/ml) or higher, with an alkaline phosphatase in excess of 100 mlU/ml, and bone pain. Patients were randomly selected to be either in the control group or in the treatment group. One patient became nauseated the first day and was transferred from the treatment group to the control group. A second patient became discouraged after two days that the oral activated charcoal had not "cured" his "arthritis." He was also transferred from the treatment group to the control group.

Each patient in the treatment group served as his/her own control since the same parameters were measured pre-

and post treatment. The function of the control group was to detect any seasonal variation in serum vitamin A levels. The treatment group received a total of nine tablets (one tablet--350 mg activated charcoal) per day divided into three tablets per meal for a total of 3,150 mg of activated charcoal per day. The study period lasted for four weeks. All subjects were on vitamin supplementation. Only one out of the 30 subjects was on vitamin supplementation that included 5,000 IU units of vitamin A. No changes in the vitamin supplements was made during the study period. Care was taken so that medications were not changed unless medically necessary.

The control group received no charcoal. Baseline blood samples were drawn on both groups before treatment was begun. The blood was analyzed for vitamin A, carotene, parathyroid hormone, alkaline phosphatase, calcium, and phosphorous (29,30,31,32,33,34). At two weeks, the treatment group had serum calcium, phosphorous and alkaline phosphatase determinations to detect any rapid decreases and/or change in these laboratory guidelines. After four weeks, blood samples were drawn and again analyzed for vitamin A, carotene, PTH, calcium, phosphorous, and alkaline phosphatase. The intake of activated charcoal was calculated and prescribed exclusively by a physician using guidelines recommended for the treatment of pruritus.

The blood was drawn from the blood lines used in the normal hemodialysis treatment procedure by technicians, LVNs, and RNs trained and approved by the Renal Section of The Methodist Hospital to conduct the dialysis procedure. No extra venipuncture for the purpose of drawing blood for the study was done at any time. Analyses were done according to standard procedures by qualified persons in service at The Community Dialysis Unit, The Methodist Hospital.

The data were statistically analyzed using analysis of variance based on the design of the experiment (one factor mixed design on four factors). Multiple linear regression was also used to examine the correlation between several of the serum levels. Comparisions of the distributions of several of the values were done by chi-square analysis. The minimum level of probability that was accepted was $p<0.05$.

RESULTS AND DISCUSSION

The study was designed to investigate the lowering of vitamin A levels in patients by using oral activated charcoal, and to study several physiological parameters of calcium metabolism in hemodialysis patients. This study investigated the effects of oral activated charcoal on these same physiological parameters. The physiological parameters selected for study were PTH, alkaline phosphatase, calcium, and phosphorous. These were selected because the major chemical and metabolic findings in renal osteodystrophy include hypocalcemia, hyperphosphatemia, hyperplasia of the parathyroid glands, high circulating levels of PTH, impaired intestinal absorption of calcium, abnormal metabolims and action of vitamin D, bone disease, and soft tissue calcification (7).

Demographic Distribution

All of the subjects participating in the study had three or more of the major chemical and metabolic features of renal osteodystrophy. Of the 30 patients, 7 were female and 23 were male (see Table 1). The seven females were somewhat evenly distributed between the three age-range

classifications. The male population, however, was predominantly younger than 25 years of age.

Table 1

Age Distribution of the Study Participants

Age (Years)	Study Group (n=13)		Control Group (n=17)	
	Male	Female	Male	Female
18-35	1	1	8	1
36-50	4	2	4	0
51 and over	2	3	3	1

Vitamin A

Serum vitamin A levels were consistently and overwhelmingly elevated in all patients except one. Of the 60 determinations of vitamin A, only five--one in the study group and four in the control group--fell within the normal range of 65-275 IU/dl (see Table 2). Of the five determinations that fell within normal limits, four out of the five were at the upper limits of normal in excess of 250 IU/dl. Five determinations were greater than 800 IU/dl, which is more than three times the normal level.

The mean serum vitamin A level in the control group was significantly lower than those in the study group by chi-square analysis. The activated charcoal did not appear to

have any effect on the vitamin A or carotene levels in the study group. Carotene levels of all subjects were within normal limits. Normal carotene levels are 50-300 mEq/DL.

Table 2

Serum Vitamin A Levels in IU/dl for Study and Control Group Subjects[a]

	Study Group (n=13)				Control Group (n=17)			
	<275	275-500	500-800	>800	<275	275-500	500-800	>800
Baseline	0	4	7	2	1	10	6	0
Four Weeks	1	3	7	2	3	9	4	1

Note: Chi-square = 4.35, $p<0.05$

[a]Normal values for vitamin A are 65-275 IU/dl

Parathyroid Hormone

A high circulating level of PTH is by definition a major chemical feature of renal osteodystrophy. Thus, it was selected as one of the physiological parameters to be studied. One would expect to find a lowering of PTH, as the serum vitamin A levels were lowered due to decreased bone reabsorption.

None of the study group had PTH levels within normal limits. One subject in the control group who had had a partial parathyroidectomy had the only two PTH

determinations that were within normal limits (see Table 3). All others were elevated.

Table 3

Serum Parathyroid Hormone (PTH) Levels in nleq/ml for Study and Control Group Subjects[a]

	Study Group (n=13)					Control Group (n=17)				
	>500	500-2000	2000-9000	9000-50000	<50000	>500	500-2000	2000-9000	9000-50000	<50000
Baseline	0	1	7	3	2	1	0	3	12	1
Four Weeks	0	1	8	2	2	1	0	6	8	2

[a]Normal PTH values are less than 500 nleq/ml.

The assay used to determine hPTH 44-68 (human parathyroid hormone 44-68) is a homologous anti-human PTH serum and is monospecific for an antigenic site, that is capable of producing an antibody, to the middle region of PTH. Use of this antiserum permits the detection of fragments of PTH as well as intact molecules. This antigenic site is contained in several circulating fragments of PTH as well as the intact hormone (31). Thus, this assay gives an index of PTH secretion. In renal failure, three specific categories or ranges have been well studied (31). Patients just beginning hemodialysis generally have PTH levels between 500-6000 nleq/ml. Chronic hemodialysis patients

with severe secondary hyperparathyroidism (severe osteitis fibrosis) range from 9000-100,000 nleq/ml. The hyperparathyroidism seen in chronic renal failure is considered to be secondary since the abnormality does not originate as a primary lesion of the parathyroid gland. Levels between 500-2000 nleq/ml in chronic hemodialysis with nonparathyroid hypercalcemia (sarcoidosis, or vitamin D toxicity, or high calcium dialysis bath) imply relatively suppressed parathyroid function. There was no correlation by regression analysis between vitamin A levels and PTH in this study at baseline determinations, nor was there any correlation between vitamin A levels and PTH in this study at final serum determinations. The oral activated charcoal had no effect on PTH.

Calcium

Even though serum calcium levels are usually maintained within normal limits in renal osteodystrophy, the total body calcium is decreased in patients on long term hemodialysis. Maintenance of normal serum calcium levels is due to bone reabsorption. Calcium levels were determined to identify major shifts in the serum levels. If PTH levels had been lowered by lowering serum vitamin A levels, serum calcium levels would drop as remineralization of bone occurred, and the alkaline phosphatase, as an indicator of bone activity,

would be simultaneously increased. If, on the other hand, PTH levels had been elevated, serum calcium would have been increased.

At most determinations, serum calcium levels in both groups were within normal limits; 34 determinations out of a total of 39 in the study group and 19 determinations out of a total of 34 in the control group were within normal range (see Table 4). It should be noted that a sizeable number of control subject levels were below normal. The fact that the majority of the subjects had normal levels is consistent with the chemical features of renal osteodystrophy. ANOVA showed no significant correlation between calcium and vitamin A or PTH. As seen in vitamin A and PTH, oral activated charcoal had no effect on calcium.

Table 4

Serum Calcium Levels in mg/dl for Study and Control Group Subjects[a]

	Study Group (n=13)			Control Group (n=17)		
	>8.5	8.5-10.3	<10.3	>8.5	8.5-10.3	<10.3
Baseline	1	12	0	5	12	0
Two Weeks	2	11	0	-	-	-
Four Weeks	0	11	2	9	7	1

[a]Normal calcium values are 8.5-10.3 mg/dl.

Phosphorous

Since hyperphosphatemia is a hallmark of renal osteodystrophy, it was also measured as a physiological indicator for this study. In hemodialysis patients, serum phosphate levels are primarily regulated by patient compliance in taking aluminum hydroxide medications. Therefore, only major shifts in serum phosphorous levels could have been considered to be related to the study itself rather than patient compliance. A significant drop in phosphorous in the study group would have been expected if PTH levels had fallen since phosphorous would be incorporated in bone remineralization. Conversely, increases in serum phosphorous levels would occur if PTH levels had increased.

The hyperphosphatemia found in the control group was consistent with the clinical findings seen in renal osteodystrophy (see Table 5). Phosphorous levels in the study group was predominantly within normal limits and there were no significant differences between the two groups by analysis of variance. Oral activated charcoal had no effect on phosphorous in this study.

Alkaline Phosphatase

Alkaline phosphatase is an enzyme which hydrolyzes phosphate esters (35). This enzyme is produced by a number of tissues, especially bone, intestine, liver, and placenta.

Table 5

Serum Phosphorous Levels in mg/dl for Study and Control Group Subjects[a]

	Study Group (n=13)			Control Group (n=17)		
	>3.5	3.5-6.0	<6.0	>3.5	3.5-6.0	<6.0
Baseline	3	7	3	1	6	10
Two Weeks	1	7	5	-	-	-
Four Weeks	2	5	6	2	5	10

[a]Normal phosphorous values are 3.5-6.0 mg/dl.

In the absence of specific disease states, most of the serum alkaline phosphatase is derived from bone (35). None of the female patients selected for the study were pregnant nor did any of the patients have significant liver disease. Alkaline phosphatase is present in osteoblasts and probably plays a role in the regulation of mineralization by controlling the concentration of pyrophosphate. Osteoblasts and osteoclasts are generally felt to be the sites of bone mineralization and reabsorption. When either bone mineralization or reabsorption exceeds the normal remodeling, then the serum alkaline phosphatase is increased. An increase in the serum alkaline phosphatase would indicate increased bone mineralization or reabsorption.

The alkaline phosphatase levels confirmed the presence of abnormally elevated bone activity (see Table 6). A significant percentage of both the study group's and the control group's alkaline phosphatase levels exceeded 90 mlU/ml, the upper limits of normal. As seen in the other measured parameters, oral activated charcoal had no effect on serum alkaline phosphatase, as shown by ANOVA.

Table 6

Serum Alkaline Phosphatase Levels in mlU/ml for Study and Control Group Subjects[a]

	Study Group (n=13)			Control Group (n=17)		
	>90	90-400	<400	>90	90-400	<400
Baseline	5	8	0	1	11	5
Two Weeks	5	7	1	-	-	-
Four Weeks	5	6	2	1	10	6

[a]Normal alkaline phosphatase values are 25-90 mlU/ml.

There was a slight negative correlation in the control group between the vitamin A and the alkaline phosphatase levels (R=-.4, p=0.05). The alkaline phosphatase levels were higher when the vitamin A levels were lower. This finding was unexpected, and inconsistent with conclusions previously reported by Chertow et al. (8).

There does not appear to be a logical physiological explanation to account for the negative correlation in the control group's alkaline phosphatase and vitamin A. In reviewing the composition of the two groups, there does not appear to be a random distribution of subjects, particularly in the age category. Slightly over half of the male members in the control group were in the 18-35 year old range. Four of these eight controls were between the ages of 18-25 years old. It is possible that this data confirms the conclusion of Frame et al. that hypervitaminosis A in the young growing child has a greater, more profound effect on the bones than on older adults (21). This finding bears further study. It would be of interest to have a random study of subjects who are still within the period of normal bone growth. A second study of randomly selected subjects 25 years of age or older would probably constitute a more homeogenous patient population.

CONCLUSIONS

The data presented in this study confirm previous findings that hypervitaminosis A is extremely prevalent in the chronic hemodialysis population. Vitamin A levels do not appear to be stable as was previously reported (24). Because of the delay in acquiring the activated charcoal, there were two baselines drawn for a number of the subjects. These two baselines were highly variable; 6 out of 25 rose more than 50%. Presumably this alteration of serum vitamin A levels was due to dietary changes which were not monitored in this study. If the study is repeated, dietary histories, including ethnic food habits, would be important to obtain as well as evaluation of the influence of diet on changes in serum vitamin A levels. Two subjects out of five did have normal serum vitamin A determinations. One of these subjects had both vitamin A determinations within normal limits. The other subject had one vitamin A determination within normal limits, and the second vitamin A determination was only mildly elevated.

Literature review historically confirms and underlines the observation that the elevated serum vitamin A levels

cause extensive joint and bone abnormalities in otherwise normal humans and animals. To date, the overwhelming evidence indicates that the primary cause of renal osteodystrophy is the aberration of normal pathways of calcium, phosphorous, and vitamin D metabolism (2,3,4,7). In spite of advances in the understanding of these anomalies, renal osteodystrophy is still a major problem in chronic renal failure. It seems likely that other factors influence the progression of this debilitating entity.

Another metabolite involved could be vitamin A. As stated in the review of literature, the symptoms and clinical findings of hypervitaminosis A in otherwise healthy persons are similar to those seen in renal osteodystrophy.

Failure of the activated charcoal to lower the serum vitamin A may have been due to an inadequate dose. Another study using larger amounts of activated charcoal might be justified. On the other hand, the study time period of one month may have been too short to see the effects of the activated charcoal on vitamin A. The highly absorbent tablets were difficult to swallow and easily crumbled. Thus, patient compliance in taking the activated charcoal must be considered as a factor.

Serum vitamin A levels could perhaps be decreased by dietary restriction. The practice of vitamin fortification

and the availability of foods naturally rich in vitamin A would make the diet limited in food selection and probably imcompatible with patient acceptance for long periods of time. However, for experimental purposes, this avenue for testing the effects of vitamin A on renal osteodystrophy would have merit.

The ideal course would be to find an oral preparation that would bind vitamin A in the GI tract preventing absorption, and one with only minimal side effects. These data also strongly discourage the practice of prescribing vitamin preparations that contain vitamin A to patients on hemodialysis.

Since all carotene values were within normal levels, it appears that vitamin A absorption is normal in hemodialysis patients. The resultant hypervitaminosis A is apparently a result of inadequate excretion. The pursuit of the question of whether vitamin A is a significant contributing factor affecting the incidence and/or progression of renal osteodystrophy appears to have clinical merit.

APPENDIX

CONSENT FORM

Concent Form
Texas Woman's University

1 I hereby authorize Sherry B. Snead to preform the following procedure:

One tube (15cc) of blood will be drawn and analyzed for vitamin A and carotene levels. There will be two randomly selected groups based on the results of the vitamin A level, elevated parathyroid hormone, alkaline phosphatase, calcium, and phosphorous levels. The presence of bone pain will also be considered. Group I will be prescribed activated charcole to bring down the vitamin A levels. Group II will be the control group and will make no change in what they already do. After one month, blood will again be drawn and analyzed for vitamin A, carotene, and parathyroid hormone. Calcium and phosphorous levels will be drawn during the study to make sure your calcium and phosphorous levels do not drop too low.

2. The procedure listed in paragraph 1 has been explained to me by

_____
Name

3. (a.) I understand that the procedure described in paragraph 1 involve the following possible risk or discomforts:

No extra needles will be involved. Blood will be obtained as it always is, from the blood tubing. The extra blood will not drop your hematocrit. Therefore, there is no added risk or discomfort to you other than what you may experience during your regular dialysis treatment.

(b.) I understand that the procedures and investigation described in paragraph 1 have the following potential benefits to myself and/or others:

As you may already know, bone disease in dialysis patients is a big problem. That is why you take phosphate binders, to control the phosphorous in your blood. This helps control some of the bone disease. but not all. It has been shown that vitamin A can cause the parathyroid

hormone levels in normal people to be elevated. Since most dialysis patients have elevated vitamin A levels because it is not easily dialized out, it may be that high parathyroid hormone levels and high alkaline phosphatase levels in dialysis patients may in part be due to high vitamin A levels. If this is true, by keeping vitamin A levels down, we may be able to help correct a small part of the bone disease.

(c.) I understand that - No medical service or compensation is provided to subjects by the university as a result of injury from participation in research.

4. An offer to answer all of my questions regarding the study has been made. If alternative procedures are advantageous to me, they have been explained. I understand that I may terminate my participation in the study at any time.

_____ _____

Subjects Signature Date

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