EFFECTS OF CALCIUM-DEFICIENT DIETS, AGE AND THYROPARATHYROIDECTOMY UPON CALCIUM METABOLISM IN SHEEP

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CHAPTER I

INTRODUCTION

Calcium is widely available at economical prices and consequently is often fed to ruminants in excess of their recommended dietary level. As a result, most studies have been made on the effects of excess dietary calcium rather than calcium deficiency. In nutrition, calcium deficiency is a term that is usually avoided. Rather, it is referred to as insufficient intake of calcium and even though this is commonly reported, the sequelae of calcium deficiency *per se* is yet to be described.

Nutritional research is handicapped by a lack of reliable estimates for the metabolic sufficiency or insufficiency of a nutrient. This is particularly true regarding the metabolic status of calcium because its concentration in the extracellular fluid is homeostatically controlled under the influence of parathyroid hormone and calcitonin and vitamin D. Assessment of the metabolic status of calcium is more difficult in adult than young animals, because the adult can draw calcium from the skeletal reserves for extended periods of time without harm. This is not so for the young, growing animal.

The conflicting results of the effects of parathyroidectomy in ruminants as well as a lack of response when this hormone is administered to parturient paretic cows, have tended to minimize the importance of these glands to such animals.

The purpose of this investigation was to study the influence of dietary calcium, animal age and parathyroidectomy upon calcium metabolism in sheep.

CHAPTER II

LITERATURE REVIEW

The forces responsible for producing changes in the motion of calcium ions, constitute a well regulated system in the animal's body. The level of calcium in serum depends upon a balance between calcium contributed from both intestinal absorption and bone resorption on the one hand, and the calcium lost from blood by deposition in the mineral phase and excretion through the kidney and intestine on the other. Hormonal and vitamin regulation of the forces transporting calcium across biological membranes comprises the body's calcium-homeostatic mechanisms.

Calcium Kinetics

Intestinal Absorption

Phillipson and Storry (1965) found no apparent differences for the sites of calcium absorption in the ruminant and non-ruminant. The rumen epithelium appears to be impermeable to both calcium and magnesium because no significant uptake was found. In this study, using sheep, they found that losses of calcium ions occurred from the upper and middle portions of the jejunum, but losses from the duodenum and lower ileum were not found. Using everted intestine sacs, Schachter, et al. (1960) have shown that calcium is transported from the mucosal to the serosal surface, against both concentration and electrical gradients. Active transport of calcium across the intestine was confirmed by these

workers, who found that it is an oxygen-dependent, energy-requiring process, which is inhibited by metabolic poisons and is specific for calcium. The animal's age is important, because transport is greater in the younger animal. Dowdle, et al. (1960) conducted experiments which indicated that the efficiency of transport is related to the needs of the animal and that the effect of age may simply reflect the needs of a growing animal; their results show that calcium-deficient diets lead to a progressive increase in the efficiency of active transport of calcium by the intestinal wall.

A dependence of the active transport of calcium upon vitamin D has been shown (Dowdle, et al., 1960). It is now established that calcium absorption is depressed in the vitamin D-deficient animal and is restored within several hours after oral or parenteral administration of the vitamin (Nicolaysen and Eeg-Larsen, 1956; Schachter, et al., 1961; Harrison, 1961; Wasserman, 1964; Schachter, et al., 1964). Studies of the effect of actinomycin D on vitamin D-induced calcium transport (Zull, et al., 1966; Harrison and Harrison, 1966), and the demonstration of a vitamin D-stimulated incorporation of uridine into RNA of the chick gut (Norman, 1966), have led to the conclusion that this action is dependent on RNA and protein synthesis. Wasserman and Taylor (1963, 1966) have shown that the stimulation of calcium absorption by vitamin D in the rachitic chick is associated with the appearance in the duodenal mucosa of a protein fraction which forms a soluble complex with calcium. On the basis of these observations, it has been proposed that vitamin D induces the synthesis of a protein which is required for optimum activity of the calcium transport system. A similar calcium transport system for other tissues has not been found.

An active metabolite of vitamin D_3 has been isolated from porcine plasma and identified as 25-hydroxycholecalciferol (Blunt, et al., 1968). Its potency has tentatively been estimated at 1.4 times that of vitamin D_3. Stohs and DeLuca (1967) have shown that this metabolite of vitamin D_3 is the primary or only form associated with intestinal nuclei, the proposed site of vitamin D action.

Evidence for and against a function for parathyroid hormone in calcium absorption exists. The mechanism by which the intestinal tract adapts to a calcium-deficient diet is not prevented by thyroparathyroidectomy (Dowdle, et al., 1960). However, a decrease in calcium absorption following parathyroidectomy, has been demonstrated (Talmage and Elliot, 1958; Rasmussen, 1959; Schachter and Rosen, 1959).

Bone Resorption

The role of the skeleton in calcium metabolism can only be understood when the kinetics of the movement of ions between body fluids and bone mineral are fully appreciated. The basis of these relationships is a two-way movement of calcium ions in and out of bone. The forces influencing the resorption of bone mineral will be considered first.

Resorption of bone mineral is essential for maintenance of normal serum calcium and inorganic phosphorus levels. Skeletal mineral stores respond to dietary calcium and phosphorus, bone becoming saturated on high and depleted on low levels (Bauer, et al., 1929; Sherman, 1947; McLean and Urist, 1955; Malm, 1958).

Resorption is a complex process, involving the breakdown of both mineral and matrix. Several theories have been proposed for the biochemical action of parathyroid hormone in bone resorption. The

investigations of Walker, et al. (1964) and Hetlar, et al. (1961) support the theory that the hormone induces the synthesis of enzymes which destroy bone matrix. This theory has been substantiated by the facts that the administration of inhibitors of RNA or protein synthesis inhibits the bone resorption effects of parathyroid hormone in vivo (Rasmussen, et al., 1964) and in vitro (Raisz, 1965). Another theory concerns the possibility that parathyroid hormone influences the enzymic production of organic acids or chelators to dissolve bone salts (Neuman, et al., 1956; Firschein, et al., 1958; Martin, et al., 1958). The accumulation of lactate and citrate, associated with parathyroid hormone administration, has directed studies on the effect of this hormone on carbohydrate metabolism in bone. Schartum and Nichols (1962) found no evidence for a direct relationship between the nature and amount of acids produced and the degree of bone resorption. Another theory is that parathyroid hormone activates a biological ion transport system, which pumps calcium and/or phosphate ions from bone to the extracellular fluid. This theory probably has its origin from the results of Rasmussen, et al. (1967) which show that the hormone exerts its influence on mitochondria in vitro, by increasing calcium and phosphate transport. Using this in vitro system, it has also been shown that the hormone causes accelerated respiration and pyridine nucleotide oxidation of mitochondrial suspensions (Rasmussen and Ogata, 1966; Fang, et al., 1963; Aurbach, et al., 1964).

Vitamin D influences bone metabolism, but its mechanism has not been established. Vitamin D is essential for parathyroid hormone action on bone but not kidney (Rasmussen, et al., 1963; Arnaud, et al., 1966), and the vitamin in pharmacologic doses promotes bone resorption even in

the absence of parathyroid hormone (Albright, 1948). The synergistic actions of vitamin D and parathyroid hormone are suppressed by inhibitors of RNA and protein synthesis (Zull, 1966; Norman, 1965).

Bone Deposition

The calcification of bone appears to be influenced by humoral and local factors whose mechanism is unknown. Within the bone, there are factors contributing to calcification, and these include osteoblast cells and uncalcified osteoid tissue. The observation that osteoblasts are found in areas of bone formation (Pritchard, 1956), has lead to the conclusion that these cells are associated with the formation of bone, possibly with a role in the synthesis and deposition of organic matrix. The organic matrix of bone consists of 95% collagen and 5% ground substance (Bauer, et al., 1961). Under normal conditions, calcification follows closely upon formation of the bone matrix.

Howland and Kramer (1921) observed a correlation between the degree of calcification of bone matrix and the product of total calcium and total phosphorus levels of the serum. Their noting that the product of calcium and inorganic phosphorus concentration (mg%) in serum, has to exceed 30, for calcification to occur, has led to several theories regarding calcification.

The inorganic component of bone is a mass of crystals referred to as hydroxyapatite and having the formula, $Ca_{10}(PO_4)_6(OH)_2$. The concepts of Howland (1921) were extended by Neuman and Neuman (1958), who postulated that the supersaturation of blood with calcium and phosphorus causes these ions to be constantly lost from the blood by the formation of hydroxyapatite in bone. Furthermore, they postulated that the

process is crystallization rather than precipitation and that crystal growth requires less energy than does precipitation; precipitation involves the collision of a large number of ions, whereas crystallization upon preformed matrix can occur at lower concentrations. Since collagen, existing in connective tissues other than bone, does not normally undergo calcification, there must be other factors involved in the mineralization of bone.

In addition, Fleisch and Bisaz (1962) have postulated that alkaline phosphatase secreted by the osteoblasts could possibly hydrolyze pyrophosphates, which are strong inhibitors of mineralization.

Intestinal Excretion

The occurrence of calcium in the feces represents the net results of intake, absorption and excretion, originating from nonabsorbed dietary calcium and calcium which once, having been absorbed, is excreted back into the intestine. That calcium which is excreted into the intestinal tract and appears in the feces is referred to as fecal endogenous calcium and is the results of a normal physiological function which tends to decrease the calcium content of blood. Its significance to calcium homeostasis will be considered.

Fecal endogenous calcium has two possible origins: 1) the secretion of calcium from blood to lumen of the intestine and, 2) secretion into the intestine by way of the digestive juices. Nicolaysen and Eeg-Larsen (1953) found no correlation between fecal calcium and alterations in serum calcium in animals on a calcium-free diet. These results imply that this source of endogenous calcium may be constant and has no important adaptive regulatory role in calcium homeostasis.

The main source of fecal endogenous calcium probably comes from the digestive juices and its physiological significance depends upon its origin in the intestinal tract (Bronner, 1964). If the digestive juices occur within absorption sites for calcium, then the quantity of calcium appearing in the feces would be a function of the digestion and absorption processes; however, if there is no exposure of the digestive juices to calcium absorption sites, then the loss of digestive juice calcium is a direct one, unrelated to digestion and absorption. On the basis of the normal volume of digestive juices and the calcium concentration therein, it has been estimated that the calcium from digestive juices, if unabsorbed, could constitute as much as is found in the entire extracellular fluid (Thomas and Howard, 1964).

Fecal endogenous calcium in ruminants has been measured by one of two different methods utilizing calcium radioisotopes. One method, developed by Comer, et al. (1953) and modified by Hansard, et al. (1957) consists of injecting ^{45}Ca into the blood stream and measuring the appearance of isotope in the feces. The second method involves the oral administration of ^{45}Ca and measurements of the percentage of ^{45}Ca administered that appears in the feces. Studies on endogenous losses of calcium in cattle and sheep have been summarized (Agriculture Research Council, 1965). From a total of 153 determinations in cattle, the mean fecal endogenous calcium was 15.2 mg. Ca/kg. bodyweight, with 62% of the values falling between 14.1 and 16.9 mg. Ca/kg. bodyweight. No age effect is apparent from these data.

The values for sheep yielded a mean of 35 mg. Ca/kg. bodyweight which is much higher than the values found for cattle. There is no apparent explanation for this species difference.

Kidney Excretion

The importance of normal kidney function is evident from the estimates of Thomas and Howard (1964) for man in which it is shown that approximately 10 gm. Ca/day is filtered by the normal glomerular filtration rate and that less than 1% of this appears in the daily urine output. The major factors which have been reported to increase the rate of calcium excretion include sodium clearance (Walser, 1961), complexing ions such as phosphate (Jahan and Pitts, 1948), citrate (Chang and Freeman, 1950), EDTA and related compounds (Holland, et al., 1953), sulfate (Walser and Browder, 1959), and adrenocorticosteroids (Robinson, et al., 1962).

Salivary Secretion

Any nutrient appearing in the saliva and not redigested, is important to the ruminant, because of the copious flow of saliva in this animal. Although the concentration of calcium in saliva is low, Smith and Stewart (1968) found a relationship between total serum calcium concentration and salivary calcium concentration. When they made sheep hypocalcemic by the method of hemodialysis, they found a direct relationship between plasma and salivary calcium, and when the serum calcium fell below 3.8 mg./100 ml., saliva became devoid of calcium.

Disturbances in Calcium Kinetics of the Ruminant

Parturient Paresis

Parturient paresis or milk fever is considered to be a metabolic disease in which the cow cannot meet the demands of gestation and

lactation for calcium. Among the biochemical changes observed in this condition are hypocalcemia, hypophosphatemia, hyperglycemia, low concentrations of citric acid, high concentrations of pyruvic and lactic acids in blood, and occasionally ketosis and hypomagnesmia (Moodie, 1965).

Several theories have been presented for the etiology of milk fever and those listed of prime importance by Payne (1964) include: 1) a sudden extra demand for calcium and phosphorus secreted in the colostrum, 2) milk fever-prone cows may be deficient in parathyroid hormone and less able to mobilize sufficient calcium and phosphorus for lactation, 3) milk fever may be caused by another endocrine defect associated with parturition and independent of the parathyroid gland, 4) milk fever may be due to gastrointestinal stasis at the time of parturition. In addition, Moodie (1965) discusses a liver dysfunction theory as a possible cause for milk fever, and Capen and Young (1967) have evidence for the possible role of calcitonin in milk fever. These investigators found increased hypertrophic calcitonin-secreting cells were associated with the disease.

In spite of increased understanding and control of the disease, the exact nature of its cause has not been determined. Breazile and Williams (1967) examined the dependence of neuromuscular function upon the distribution of the cations Na^{+}, K^{+}, Ca^{++}, and Mg^{++} in the extracellular and intracellular fluids. These authors compared abnormalities in neuromuscular function to experimental observations in several types of paretic syndromes in cattle. The syndromes of milk fever were described as hyperexcitability resulting from an increased permeability of nerve and muscle membranes to sodium and potassium ions, brought

about by a decrease in extracellular calcium ion concentration. Hyperexcitability is followed by paralysis when the membranes are no longer capable of producing action potentials due to prolonged depolarization beyond threshold.

Grass Tetany

Grass tetany is a disease associated with decreased levels of magnesium in serum. It is often referred to as hypomagnesemic tetany. Lactating or pregnant cows are most susceptible, but other ruminants may also be afflicted. A hypocalcemic condition is often associated with grass tetany and a metabolic relationship between these two cations makes this disease relevant to calcium homeostasis.

Over 10,000 cattle were lost in recent years due to grass tetany in California and Nevada, and losses have also occurred in Georgia, Mississippi, South Carolina, Maryland, Utah, Wyoming, Idaho, Oklahoma, and Texas (Grunes, 1967). Among the factors that have been implicated in grass tetany are low content and decreased availability of magnesium in forage, the calcium and potassium content of the forage, the ratio of potassium to calcium plus magnesium in the forage, increased rate of passage of green forage compared to dry feed, high levels of milk production, age of animal, change in weather conditions, various types of stress to the animal (Lowry and Grunes, 1968), fertilization with high rates of nitrogen and potassium (Grunes, 1967), and the ability of grass tetany-prone rangeland forages to accumulate trans-aconitate (Stout, et al., 1967).

Bohman, et al. (1968) found that an oral drench of KCl, citric acid or trans-aconitate administered singly failed to produce tetany,

while the combination of KCl with either acid produced tetany 66 to 100% of the time when administered at the range of 1.57 gm./kg. body-weight.

Breazile and Williams (1967) attributed the syndromes of grass tetany to a decrease in extracellular magnesium concentration resulting in an increased permeability of nerve and muscle membranes to potassium. The accumulation of potassium ions in the extracellular fluid causes hyperexcitability of the membranes and is often manifested as mania in the animal. After prolonged hyperexcitability, the membranes become unresponsive and paralysis occurs.

Parathyroid Hormone

Chemistry

Bovine parathyroid hormone (PTH) has been isolated and purified (Hawker, et al., 1966), allowing studies to be made on its structure (Potts, et al., 1965) and the relationship of structure to function. Bovine PTH is reported to have a molecular weight near 8,500 and a tentative model of its structure has been made (Aurbach and Potts, 1967) from the determined amino acid composition (Rasmussen and Craig, 1962; Hawk, et al., 1966). Rasmussen and Craig (1962) studied structure-activity relationships and have shown a loss of biological activity with either performic or hydrogen peroxide oxidation of the hormone. The loss of activity was closely related with rate of methionine oxidation, and activity was restored by reduction with cysteine. It appears that inactivation following methionine modification is not the result of loss of the residues, but to the unfavorable conformational changes in the molecule or to the reduction in binding affinity to

receptor sites in effector tissues (Potts and Aurbach, 1965). These investigators have also shown by carboxypeptidase digestion that the first five amino-terminal residues are unessential for either biological or immunological activity. Likewise, leucine-aminopeptidase digestion indicates that the first four or five carboxy-terminal residues are dispensible for full biological activity (Potts and Aurbach, 1965).

Despite considerable modification of the PTH molecule by exopeptidase degradation, cyanogen bromide cleavage, or dilute acid hydrolysis, considerable biological activity is found in the resulting residues (Potts and Aurbach, 1965). These results imply that the biologically active fragment is within the central region of the molecule. Evidence for a folded structure or hydrogen and hydrophobic bond stabilization could not be found when assessed by optical rotation and spectrophotometric titration of tyrosine residues.

Biochemistry

The target organs for PTH are considered to be bone, kidney and intestine. Bone is generally thought to be the main target organ and most biochemical studies on PTH have involved bone. The observations that injections of PTH are followed by an increase in serum citrate (L'Heureux and Roth, 1953; Neuman, et al., 1956), and that bone tissue was the source of citrate (Martin, et al., 1958), have resulted in extensive studies into bone carbohydrate metabolism. Because the citrate ion is a strong chelator of calcium it has been postulated to play a role in demineralization of bone (Neuman and Neuman, 1958). Martin, et al. (1965) used cultures of mouse calvaria to study the alterations in bone metabolism produced by parathyroid hormone. These workers

observed decreased oxidation of citrate, increased utilization of glucose with lactate accumulation, decreased collagen synthesis and a stimulation of nucleic acid synthesis in parathyroid hormone-treated cultures. Evidence was also provided for both increased synthesis and inhibited oxidation of citrate. However, it was stated that the relationship between citrate production and bone demineralization was not necessarily a causitive one.

Hekkelman (1961) proposed that a possible block in isocitric dehydrogenase of bone might be responsible for accumulation of metabolic acids. It was suggested that the hormone decreased the amount of NADP available to the bone cells and hence to NADP-dependent enzymes. This concept was studied further by van der Straaten (1965) who observed a decrease in resorption and osteoclast proliferation in bone cultures when either NAD or NADP was added to the parathyroid hormone treated cultures. It was also observed that ATP, AMP, CMP and GMP, each inhibitory to bone NADPase activity, greatly suppressed the hormonal effect.

The influence of PTH upon the energy-linked transport of cations across the mitochondrial membrane has received considerable attention (Utzumi, et al., 1966; Sallis and DeLuca, 1960; Rasmussen and Ogata, 1966). In general, the effects of PTH upon mitochondria are enhanced release of trapped calcium, stimulated uptake of phosphate, potassium and magnesium ions and increased respiration and nucleotide oxidation. Rasmussen, et al. (1967) found evidence that these actions of PTH on mitochondria show specificity, sensitivity and physiological correlation and may well be indicative of the in vivo actions of PTH.

Physiology

The establishment of a radioimmunoassay for PTH has permitted direct measurement of plasma concentrations (Berson, et al., 1963). The concentration of parathyroid hormone was determined in 24 normal cows and values ranged from 400 to 1800μμgm./ml. with a mean of 1070 μμgm./ml. (Sherwood, et al., 1966a). A reciprocal relationship for serum calcium and PTH was reported with a negative correlation coefficient of 0.6. This relationship was further substantiated by infusions of EDTA or $CaCl_2$.

The radioimmunoassay was used to assess factors controlling the rate of PTH secretion (Care, et al., 1966). Hypercalcemic perfusions reduced PTH secretion by 20%, whereas EDTA perfusion resulted in stimulation. Hypermagnesemic perfusions also increased hormone secretion, but a specific effect for low magnesium concentration could not be determined in the experiments at hand. Sodium phosphate, given intravenously, caused an indirect increase in PTH secretion when hypocalcemia resulted (Sherwood, et al., 1966b).

Calcitonin

Chemistry

Porcine calcitonin has been isolated and purified (Putter, et al., 1967; Bell et al., 1968; Potts, et al., 1967), and its amino acid sequence determined (Potts, et al., 1968). Its molecular weight was estimated to be 4,500 on the basis of its amino acid composition. The activity of purified material is 200 to 270 MRC units per mg. of protein (Potts, et al., 1968; Bell, et al., 1968). Tashjian and Warnock

(1968) treated calcitonin with several enzymes and chemical reagents in order to study structure-function relationships. Hydrolysis by pepsin, trypsin and chymotrypsin led to complete loss of activity. Activity was not effected by treatment with carboxypeptidase, leucineaminopeptidase, neuramidase, P-OH mercuribenzoate, iodoacetate or cyanogen bromide. Loss in activity was observed when calcitonin was treated with dilute HCl or neutral Tris buffer at high temperatures, oxidation with hydrogen peroxide, and tyrosinase. Although the material used in these studies was not highly purified, the authors concluded that calcitonin either contains or is a simple peptide.

Biochemistry

Because discovery, isolation and purification of calcitonin are recent events, few studies on its biochemical actions have been made. Because the effect of calcitonin is in opposition to that of parathyroid hormone, it is of interest to note, in many cases, that the metabolic effects of PTH are reversed by calcitonin.

Calcitonin increases the number of osteoblast and amount of bone while the osteoclasts are decreased (Matrajt, et al., 1968), the effect being enhanced in intact animals as compared to parathyroidectomized animals. When calcitonin was added with parathyroid hormone to a culture of mouse calvaria, there was a depression in release of calcium and citrate compared to that of parathyroid hormone treatment (Nisbet and Norman, 1968). Whereas parathyroid hormone increases acid phosphatase activity, calcitonin causes a decrease in the level of this enzyme (Foster, V., 1968).

Physiology

Considerable experimental evidence has accumulated to show that calcitonin exerts its hypocalcemic effect by influencing bone. The intestine has been excluded by showing a hypocalcemic response with the gut removed (Aliopoulios, 1966). Hypocalcemic response to calcitonin has also been observed in nephrectomized animals, indicating that a renal mechanism is not involved (Hirsh, et al., 1964).

The primary effect of calcitonin on bone appears to be in the inhibition of bone resorption. Calcitonin inhibits the release of calcium from bone cultures (Friedman and Raiz, 1965; Aliopoulios, et al., 1966), lowers plasma radio-calcium from ^{45}Ca-labelled bone (Johnston and Deiss, 1966), reduces urinary excretion of hydroxyproline (Robinson, et al., 1967), and reduces the density of osteoclasts in bone (Matrajt, 1968).

Calcitonin produces phosphoturia in parathyroidectomized rats (Robinson, et al., 1966). In the intact animal it may be expected that calcitonin and parathyroid hormone are additive in their induction of phosphoturia.

Deftos, et al. (1968) have established a radioimmunoassay for calcitonin. Using this technique, these authors observed a direct relationship between hormone and plasma calcium concentrations. Their studies with rabbits show the presence of hormone in normal circulation, a rapid response to hypercalcemia, and a rapid (5-15 min., half-life) turnover of calcitonin in blood.

Thyroparathyroidectomy in Ruminants

Although thyroparathyroidectomy (TPX) was performed on ruminants as early as 1907 (MacCollum, et al., 1907), and has subsequently been reported on a number of occasions (Simpson, 1921; Smith, et al., 1957; Stott, et al., 1957a; 1957b; Payne and Chamings, 1964; Mayer, et al., 1966), results have been confounded by diet, age, sex, pregnancy, surgical procedure and post-surgical treatment.

Simpson performed TPX on four pregnant ewes and with the exception of one animal, no acute tetany was observed. The ewe which exhibited symptoms of tetany, did so four months after operation and abortion had occurred. A second ewe died three months after operation and post-mortem examination revealed two dead fetuses. The remaining two ewes had normal births, one having two and the other one healthy lamb.

Smith, et al. (1957) TPX six and thyroidectomized (TX) two kid goats eight to nine months of age. Of the six TPX animals, one died 57 days postoperative and the others survived 74 days, at which time they were placed on a low-calcium diet. Three of the TPX animals died 11, 71, and 143 days after being placed on the low-calcium diet.

Stott and Smith (1957a) TPX eight calves ranging in age from eight to 99 days and observed fatal tetany within nine days in seven of their animals. The results of TPX were decreased serum calcium and inorganic phosphorus, with the phosphorus increasing just before death. When the internal parathyroids and the thyroid, or external parathyroids were removed, no tetany and little effect on the serum calcium and plasma inorganic phosphorus was observed.

Thyroparathyroidectomy in nonlactating pregnant cows and non-pregnant lactating cows resulted in a marked decrease in serum calcium

and inorganic phosphorus without symptoms of milk fever or tetany (Stott and Smith, 1957b). These authors concluded that the high Ca:P ratio in natural ruminant diets permits the adult ruminant to maintain pregnancy, parturiate, and lactate in the absence of parathyroid glands. Mayer (1966) TPX adult cows and observed asymptomatic hypocalcemia, which was followed by a return to normal calcium levels without treatment. Payne and Chamings (1964) TPX 32 goats and observed no difference in response between adult males and kids; tetany was rare in both.

Summary

The mechanism by which the level of calcium is maintained in blood and extracellular fluids is one of the best regulated systems in the body. Calcium is unique among the mineral nutrients in being directly regulated by vitamin D and the two polypeptide hormones, parathyroid hormone and calcitonin. Vitamin D enhances calcium absorption and is essential for the function of parathyroid hormone. A relationship between vitamin D and calcitonin has not been established. Bone is the primary target for both hormones, PTH enhancing and calcitonin inhibiting bone resorption. The regulation of secretion is controlled by the concentration of blood calcium, low levels stimulating PTH and high levels stimulating calcitonin.

Calcium resources are readily available and relatively cheap; consequently, calcium is rarely lacking in the diet of humans or animals. In animal production practices, calcium is often fed in uneconomical abundance. As high levels of calcium have been shown to interact with other minerals, principally P, Mg, Mn and Zn, feeding of excess calcium may be biologically unsound. Conventional research techniques in

nutrition such as balance, digestion, and growth trials have failed to "draw the lines" regarding mineral requirements. When the complexity of mineral interactions is considered, it is not surprising that the conventional nutrition trials have often failed to establish exact requirements.

The measurement of serum calcium as an indicator of calcium status in the animals's body is often unreliable because of its unique regulation system. The presence of such a regulation system may, in itself, suggest a means of assessing calcium status of an animal. Therefore, an animal not meeting its calcium requirement through the diet must rely upon body resources, i.e., bone calcium. Measurement of parathyroid hormone secretion might indicate the degree at which demand for calcium is met by diet or bone. In situations where hypocalcemic states exist (parturient paresis, pseudohypoparathyroidism) measurement of thyrocalcitonin secretion may relate these disorders to overproduction of this hormone.

The purpose of the investigations reported herein is to provide more information on the factors which influence calcium homeostatis in the ruminant animal.

CHAPTER III

EXPERIMENT I

Introduction

Assessment of the metabolic status of calcium in adult animals is difficult because they are able to draw upon skeletal reserves to meet body requirements (Benzie, et al., 1955). The adult animal exhibits higher endogenous losses, lower absorption and lower utilization of dietary calcium than young animals which need dietary calcium for bone formation (Hansard, et al., 1954a; Hansard, et al., 1954b). As Ca-deficient diets are at times fed for extended periods, it would appear desirable to have response criteria which would assess the calcium status in adult animals quickly and accurately. The experiment described herein was designed to evaluate different response criteria which might be used for such purpose.

Experimental Procedure

Eight wether sheep averaging about 36 kg. and which were more than one year of age were placed in metabolism stalls and fed a basal diet containing 0.01% calcium (Table I). Feces and urine were collected quantitatively daily for 70 days and the daily aliquots were combined into 10 consecutive 7-day periods. Blood samples, obtained by jugular vein puncture, were taken initially and at the end of each 7-day balance period. When the 10-week metabolism phase was completed, these eight

TABLE I

PERCENTAGE COMPOSITION OF DIETS

Ingredients	Percent	
Ground Corn	87.00	
Urea	1.50	
Cottonseed hulls	9.29	
Calcium carbonate[a]	1.00	
Sodium chloride	0.50	
Potassium carbonate	0.71	
Trace mineral mix[b]	-----	7 gm./100 lb.
Vitamin A & D[c]	-----	10 gm./100 lb.

[a]Omitted from calcium-deficient diet and replaced with hulls.

[b]Composition of the trace mineral mixture (mg./100 gm. diet); $FeSO_4$, 42.51; $MnSO_4 \cdot H_2O$, 15.37; $Na_2B_4O_7$, 12.56; $ZnSO_4 \cdot 7H_2O$, 26.35; $CuCO_3 \cdot Cu(OH)_2$, 1.97; KI, 0.07; $CoCl_2 \cdot 6H_2O$, 0.05; CaF_2, 0.20; $Na_2MoO_4 \cdot 2H_2O$, 0.50; $Cr_2(SO_4)_3$, 0.04; Na_2SeO_4, 0.02.

[c]The mixture supplied 20,000 IU and 2,500 USP units of vitamins A and D, respectively/gm.

animals were paired with control sheep, which had been fed a commercial pelleted diet and kept in metabolism stalls. All sheep received, via the jugular vein, 20 ml. of a solution containing 100 mg. of phosphorus, supplied as KH_2PO_4. The phosphorus solution was injected into the vein on one side of the neck and samples were obtained for analysis from the opposite vein at definite intervals of time after phosphorus injection. One week after the initial phosphate injection, two sheep from each of the Ca-deficient and control groups were injected with 300 mg. of phosphorus. At the same time, two sheep from each group were injected with 459 mg. of $MgSO_4$ in 20 ml. H_2O, following above procedures. Two weeks later, four animals from each of the treated and control groups were anesthetized with halothane and the renal artery and vein were cannulated for collection of blood samples. Each animal then received 500 ml. of physiological saline via a femoral cannula and initial blood samples were collected. Immediately afterwards 300 mg. of phosphorus were injected into the femoral vein, which was followed by a collection of blood samples from the renal vein and artery at one minute intervals after the phosphorus injection.

Feed, feces, urine and plasma were analyzed for calcium, magnesium, copper, and zinc by atomic absorption spectrophotometry. All phosphorus analyses were for inorganic phosphorus using the method of Fiske and Subbarow (1925).

Results and Discussion

Table II exhibits the results of feeding a calcium-free diet for ten consecutive weeks on the balance of calcium and phosphorus during this period. Feed consumption was good throughout the experiment, thus

TABLE II

THE EFFECT OF FEEDING A CALCIUM-DEFICIENT DIET ON EXCRETION AND RETENTION OF CALCIUM AND PHOSPHORUS

Periods	1	2	3	4	5	6	7	8	9	10	
Days	0-7	7-14	14-21	21-28	28-35	35-42	42-49	49-56	56-63	63-70	
Calcium[1]											S.E.[2]
Intake, gm.	0.39	0.38	0.39	0.40	0.42	0.42	0.43	0.40	0.36	0.35	
Feces, gm.	8.8	3.3	3.0	2.8	3.4	5.2	3.6	4.0	3.6	2.5	0.24
Urine, mg.	15.4	19.0	10.8	18.1	18.9	12.2	11.2	10.6	8.6	7.4	1.33
Retention[1]	-8.43	-2.93	-2.62	-2.42	-3.00	-4.99	-3.18	-3.61	-3.33	-2.16	
Phosphorus[1]											
Intake, gm.	9.0	8.7	8.9	9.2	9.7	9.6	9.7	9.6	7.2		
Feces, gm.	14.0	7.6	8.9	14.5	8.3	7.8	7.8	6.3	6.3		0.46
Urine, gm.	3.7	2.2	2.8	2.6	2.9	2.3	3.3	2.6	1.9		0.17
Retention[1]	-8.7	-1.1	-2.8	-7.9	-1.5	-0.5	-1.4	+0.7	-1.0		

[1] Average per week (all values represent the average of 8 animals).

[2] Standard error.

intakes of calcium and phosphorus were fairly constant during all periods. Fecal calcium excretion was highest during the first week of the test. Although there was some variation in the fecal calcium values during the subsequent time intervals, all values were significantly lower than that of the first period. As sheep do not excrete much calcium in the urine, calcium balance (Table II) closely followed the trends of fecal calcium.

Urinary phosphorus values did not differ ($P < .05$) during the entire trial. Fecal phosphorus values are characterized by two definite peaks during the first and fourth weeks; these peaks were higher ($P < .01$) than at all other periods. Partial explanation for the first peak has been offered above, but no explanation is offered for the high excretion found during the fourth week. Results which relate to the question are presented in Figure 1, in which are presented the effects of feeding a Ca-deficient diet on plasma calcium and phosphorus levels. Plasma calcium decreased in a manner which did not differ from linearity during the first three weeks, but by the end of the fourth week the level had returned to that obtained initially and so remained for the duration of the experiment.

These results are in accord with those obtained in an earlier experiment (Nelson, et al., 1966) in which rats were used. In another experiment, Nelson and Tillman (unpublished data) found that plasma calcium, after the initial decrease, returned to normal and remained high in a sheep fed diet 1 for 23 weeks. The increase in plasma calcium level is in general agreement with the concept (Sherwood, et al., 1966) that the lowest level of plasma calcium, found at the end of the third week, stimulated parathyroid hormone secretion, which caused the

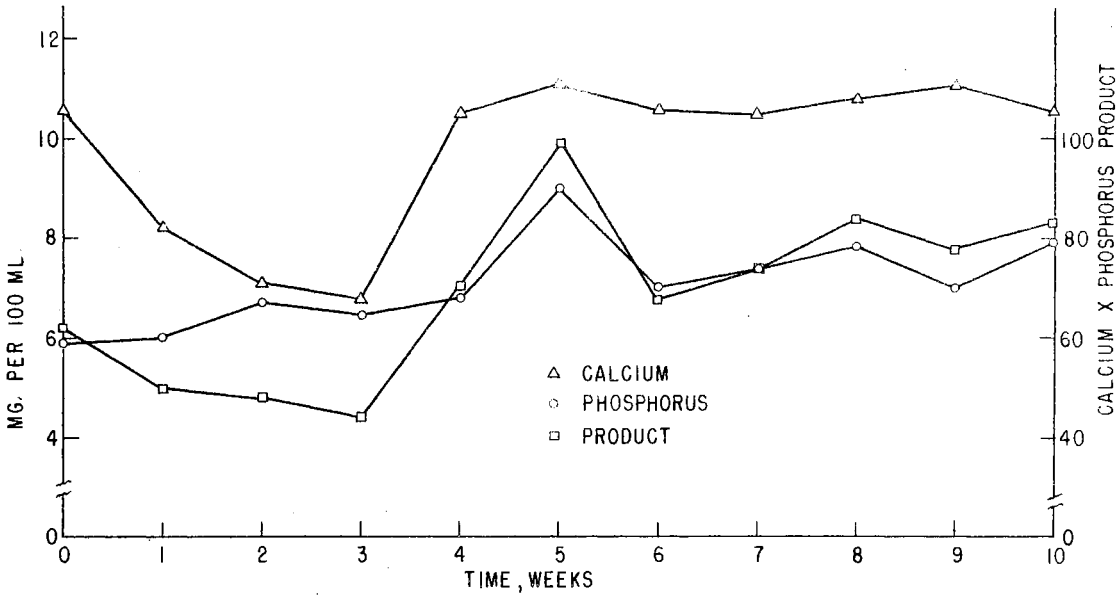

Figure 1. Effect of Ca-Deficient Diet on Plasma Calcium and Phosphorus Levels and Ca X P Product in Plasma

increase in plasma calcium level found at the end of the fourth week (Figure 1). If the classical mode of action of parathyroid hormone is to cause increased excretion of urinary phosphorus (Albright and Reifenstein, 1948), it is notable that urinary phosphorus excretion did not increase during the fourth week (Table II) or during the remainder of the experiment. It is of interest to note that instead, there was a great increase in fecal phosphorus excretion during the fourth week. Whether the increase in plasma calcium level is related to the increased fecal phosphorus excretion noted during the same period is an open question. Plasma phosphorus level increased with time on diet, a phenomenon noted and explained by other workers (Krook and Lowe, 1964). The product of total plasma calcium and plasma inorganic phosphorus described a triphasic curve over the experiment and agree with the results that Krook and Lowe (1964) obtained on horses. The sequence of events in this curve was as follows:

1. During the first decreasing phase, hypocalcemia exerted a greater effect on the product value than did hyperphosphatemia.
2. During the first increasing phase, hyperphosphatemia exerted a greater effect on the product value than did hypocalcemia.
3. In the second decrease, the body seemed to be making a greater compensation for hyperphosphatemia.

It is generally accepted that a normal level for plasma or serum calcium is in the range of 9 to 12 mg./100 ml. (Long, et al., 1965), thus a measurement of plasma calcium between 7 and 21 days would indicate a dietary deficiency, but this would not be true at any other time.

After 28 days, the product of plasma calcium and plasma inorganic phosphorus increased beyond the initial level and the data indicate that a product higher than 70 could indicate an inadequate level of dietary calcium on the basis of these observations. In this connection, Krook and Lowe (1964) obtained similar results when horses were fed an adequate level of calcium, with abnormal calcium to phosphorus ratios of 1:3 and 1:6.

Plasma mineral data are shown in Table III. Plasma magnesium and copper levels changed ($P < .01$) during the ten weeks of feeding a calcium-free diet. Both minerals appeared to vary inversely with the plasma calcium values, increasing during hypocalcemia and returning to previous levels when hypocalcemia was corrected. Changes in plasma zinc values were similar to those of copper and magnesium, but not significant. Calcium and magnesium are biologically antagonistic (Morris and Odell, 1963), which might explain the inverse relationship observed between these minerals in the plasma of calcium-deficient sheep. A relationship between calcium and copper has not been established. Another possible explanation for these observations between lowered calcium and increased magnesium, copper and zinc is that these cations may have a common protein carrier in blood. When plasma calcium is decreased, more carrier sites may be available for binding other cations.

The effects of injecting intravenously 100 mg. of phosphorus or an equal volume of saline upon plasma minerals in calcium-deficient and control sheep are shown in Table IV. Except for the 60 minute time period, the rate of disappearance of phosphorus between the CP and DP groups followed a similar course; moreover the Ca-deficient animals maintained lower plasma inorganic phosphorus levels than did controls.

TABLE III

EFFECT OF FEEDING A CALCIUM-DEFICIENT DIET UPON PLASMA MINERAL LEVELS

	Time on diet, weeks										
	1	2	3	4	5	6	7	8	9	10	S.E.[1]
Ca,[2] mg./100 ml.	8.25	7.13	6.74	10.48	11.05	10.56	10.42	10.78	11.02	10.58	0.17
P,[2] mg./100 ml.	5.95	6.70	6.41	6.78	9.01	6.85	7.34	7.83	6.99	7.93	0.92
Mg,[2] mg./100 ml.	2.70	2.46	2.89	2.77	3.07	2.79	3.14	2.78	2.84	2.58	0.004
Cu,[2] ug./100 ml.	127	154	139	143	124	136	113	109	111	109	2.68
Zn,[2] ug./100 ml.	105	110	154	103	112	109	87	94	105	97	5.70

[1] Standard error.

[2] All values represent the average of 8 animals.

TABLE IV

THE EFFECTS OF INTRAVENOUS INJECTION OF 100 MG PHOSPHORUS UPON SERUM CA, P, AND MG VALUES IN CALCIUM-DEFICIENT AND CONTROL SHEEP

Post-injection time, min.	Phosphorus (mg./100 ml.)				Calcium (mg./100 ml.				Magnesium (mg./100 ml.)			
	CP	DP	CS	DS	CP	DP	CS	DS	CP	DP	CS	DS
1	9.7	7.7	8.9	6.5	10.9	10.0	11.1	10.6	2.7	2.6	2.7	2.7
20	8.9	7.2	8.7	7.0	10.7	9.3	10.8	10.0	2.6	2.3	2.5	2.6
40	7.9	6.4	8.5	5.2	10.5	9.0	10.4	9.8	2.5	2.3	2.5	2.5
60	6.9	7.0	8.9	7.8	10.3	9.4	10.4	9.5	2.4	2.5	2.5	2.4
120	6.0	5.2	7.3	6.5								

CP - Control sheep, phosphate injected.

DP - Ca-deficient sheep, phosphate injected.

CS - Control sheep, saline injected.

DS - Ca-deficient sheep, saline injected.

Calcium-deficient sheep injected with saline had lower plasma phosphorus values than saline-injected controls ($P < .001$) and all phosphate-injected animals ($P < .001$) except those tested at the 60 and 120 minute periods.

Plasma calcium values for the calcium-deficient animals, which were injected with 100 mg. phosphate, were lower than those of phosphate-injected controls, saline-injected controls, and saline-injected, calcium-deficient sheep. Plasma calcium values for the saline-injected calcium-deficient controls and the phosphate-injected controls did not differ significantly. Plasma magnesium values tended to be lower for the calcium-deficient, phosphate-injected sheep. These data indicate that the elimination of injected phosphorus results in a concomitant loss of calcium and possibly magnesium ions from the calcium-deficient sheep. These results indicated that a larger quantity of phosphorus so injected might show a greater difference between the Ca-deficient and control sheep; however, when 300 mg. of phosphorus was injected, the nature of the response (Table V) was similar to that found with the lower level. The Ca-deficient sheep were able to maintain the plasma inorganic phosphorus level lower than control sheep following an intravenous injection of either 100 or 300 mg. of phosphorus, except for the 60 minute test period. The action of PTH on kidney leads to an increased concentration of phosphorus in the urine (Arnaud, et al., 1967). The lower levels of serum phosphorus observed in the phosphate injected, Ca-deficient sheep could be attributed to a higher excretion of phosphate in the urine of these sheep.

When renal A-V differences were measured (Figure 2), it was found that the Ca-deficient sheep had a significantly wider ($P < .05$) A-V

TABLE V

THE EFFECTS OF INTRAVENOUS INJECTION OF 300 MG. PHOSPHORUS UPON SERUM CA AND P IN CALCIUM-DEFICIENT AND CONTROL SHEEP

Injection Time, Min.	Phosphorus, mg./100 ml.		Calcium, mg./100 ml.	
	Control	Ca-deficient	Control	Ca-deficient
0	5.9	5.6	11.3	14.0
1	10.0	8.0	11.2	10.1
20	8.3	7.4	9.9	12.7
40	7.5	7.3	11.6	10.7
60	7.0	7.3	11.6	10.2

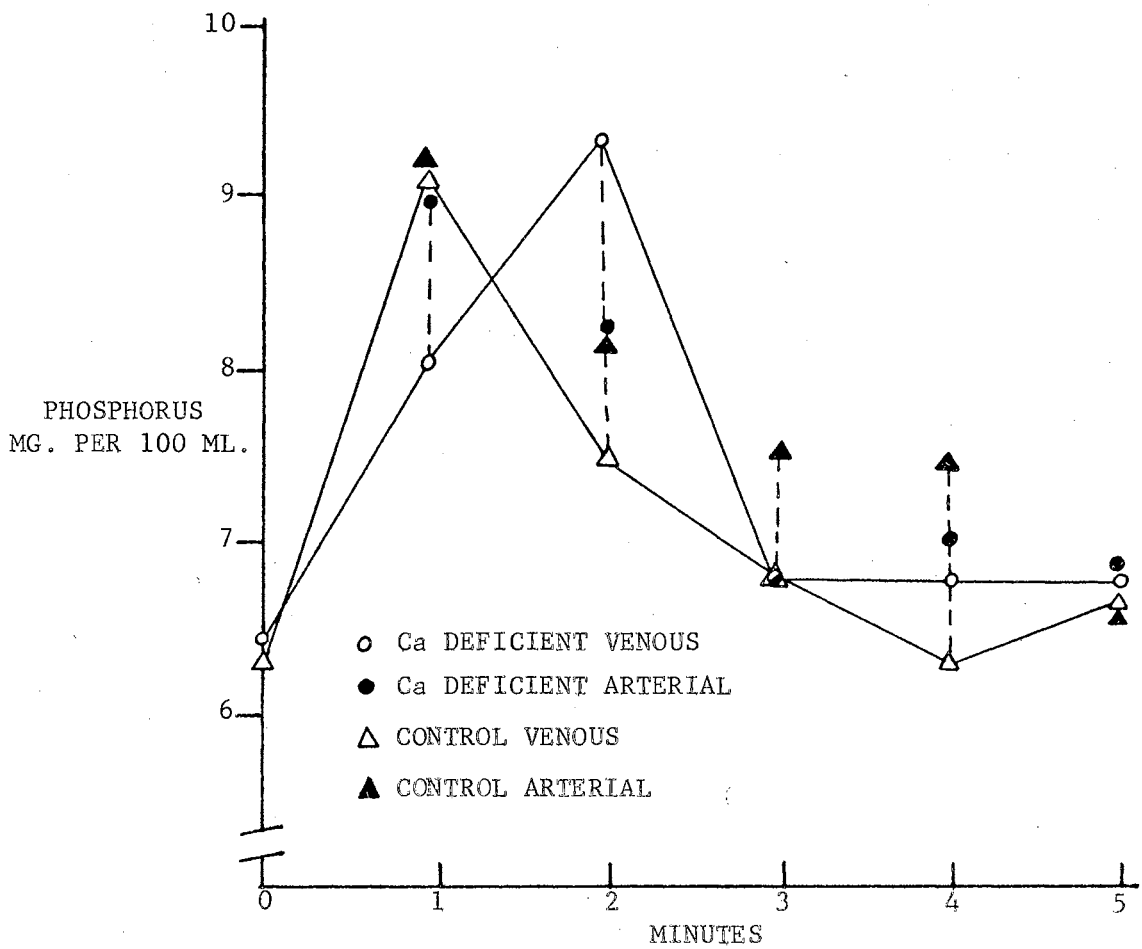

Figure 2. Effect of an Intravenous Injection of 300 Mg. Phosphorus Upon A-V Differences in Plasma Phosphorus

spread in serum inorganic phosphorus at one minute after injection, but afterwards there were no significant differences between treatments, indicating that the mechanism for elimination of excess phosphorus from the blood stream in Ca-deficient sheep acts quicker than that of the control sheep. The response appears to be an immediate one to the injection of phosphate and did not follow the response of sheep to the calcium-deficient diet, where in the balance trial phosphorus was excreted mainly in the feces. The mechanism might be of such magnitude to provide a method of determining the calcium status in sheep and suggests that a measure of PTH activity might provide a more sensitive test.

The effects of injecting 100 mg. of magnesium into the control and Ca-deficient sheep are shown in Table VI. Ca-deficient sheep had higher serum magnesium levels than controls before and after injection of magnesium, except for the 20 minute period. When the changes in serum magnesium levels were calculated as a percent of the initial value, the values in Ca-deficient sheep showed a higher percentage increase during the 5 and 10 minute post-injection periods. Since it was observed that serum magnesium concentrations increased during a 10-week period when the animals were fed the calcium-free diet (Table III), it could be postulated that the magnesium pools of these animals were saturated. If this should be true, it is possible that calcium-deficient sheep had higher post-injection levels of magnesium due to an inability of the injected magnesium to equilibrate with the magnesium pools of the body.

Serum calcium decreased in both the control and calcium-deficient animals after the injection of magnesium; the controls had larger absolute and percentage decreases in serum calcium. It appears that some

TABLE VI

EFFECT OF INTRAVENOUS INJECTION OF 100 MG. MAGNESIUM IN CONTROL AND CA-DEFICIENT SHEEP

	Time after injection							
	Initial	1 min.	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
Controls								
Mg, mg./100 ml.	2.48	3.68	3.17	2.98	3.05	3.20	2.83	2.82
Change, mg./100 ml.		+1.20	+ .69	+ .50	+ .57	+ .72	+ .35	+ .34
% Change		48%	28%	20%	23%	29%	14%	14%
Ca, mg./100 ml.	10.40	9.05	8.97	9.26	9.91	9.61	9.26	9.22
Change, mg./100 ml.		-1.35	-1.43	-1.14	- .49	- .79	-1.14	-1.18
% Change		13%	14%	11%	5%	8%	11%	11%
Ca-Deficient								
Mg, mg./100 ml.	2.83	4.48	3.80	3.62	3.33	3.06	3.26	3.15
Change, mg./100 ml.		+1.65	+ .97	+ .79	+ .50	+ .23	+ .43	+ .32
% Change		48%	34%	28%	18%	8%	15%	11%
Ca, mg./100 ml.	9.07	8.60	8.50	8.79	8.95	7.65	8.50	8.09
Change, mg./100 ml.		- .47	- .57	- .28	- .12	- .42	- .57	- .98
% Change		5%	6%	3%	1%	5%	6%	11%

mechanism is active in the control group for eliminating both calcium and magnesium when the serum cation concentration is increased by magnesium injection. This mechanism appears to be inhibited in calcium-deficient animals. Thus, further studies on the response to magnesium injection may reveal a method for estimating calcium status in animals and deserves further investigation.

Summary

When adult sheep were fed a calcium-deficient diet, serum calcium decreased linearly for three weeks. Between weeks 3 and 4, the rate of decline in serum calcium was overcome by the mobilization of storage calcium, resulting in a return of serum calcium levels to pre-existing levels. Mobilization of calcium, once initiated, was rapid and the hypocalcemic condition corrected to normal calcium concentration within a one-week period and was so maintained throughout the duration of this trial. Renal clearance of injected phosphorus was more pronounced in calcium-deficient than in control sheep indicating an involvement of parathyroid hormone.

CHAPTER IV

EXPERIMENT II

Introduction

The parathyroid gland has an important role in calcium homeostasis of most mammals. As normal plasma calcium levels existed in the thyroparathyroidectomized (TPX) adult cows (Stott and Smith, 1957), and non-lactating TPX cows (Mayer, et al., 1966), and relatively low increases of plasma calcium followed parathyroid hormone (PTH) administration to non-parturient cows (Hibbs, et al., 1947; Jackson, et al., 1962), it is generally believed that these glands are not important to calcium metabolism in ruminant animals. However, the results of Experiment I indicated an influence of PTH upon the maintenance of plasma minerals. The trials described herein, were conducted in an attempt to relate the role of the parathyroid glands to the responses obtained in Experiment I.

Methods

Two trials were conducted for the purpose of studying the effects of age and a calcium-free diet on TPX sheep.

Trial 1. Three adult sheep, about 18 months of age, were thyroparathyroidectomized (TPX), while three sham-operated sheep of the same age served as controls. All animals were placed in metabolism stalls

and each fed 908 gm./day of a "purified" diet (Table VII) for two weeks prior and subsequent to surgery, after which they were fed 908 gm./day of the purified calcium-free diet shown in Table VII. Daily fecal and urinary samples were collected and pooled during 7-day periods. Aliquots of the pooled samples were dried, weighed and ashed. The ash was dissolved in 4 NHCl and analyzed for calcium and magnesium by atomic absorption spectrophotometry.

The following surgical procedure was performed on all animals: Food was withheld for 24 hours and water for 12 hours prior to surgery. All animals received intravenously one mg./kg. bodyweight of promazine hydrochloride as a preanesthetic sedative and anesthesia was induced ten minutes later by the intravenous administration of a 2.5% solution of thiamylal sodium at a rate of approximately 13 mg./kg. Following endoctracheal intubation, anesthesia was maintained with a halothane/oxygen mixture in a semi-closed circuit.

The approach to the external parathyroid gland was made with the animal in lateral recumbency. A skin incision was made from a point two cm. below the base of the ear and one cm. behind the vertical ramus of mandible extending downwards and backward for approximately five cm. and terminating just above the jugular furrow. Subcutaneous tissues were separated by blunt dissection, great care being taken to prevent hemorrhage. The external jugular vein was located and the occipital vein identified, ligated and sectioned. Further blunt dissection between the external jugular vein and the brachiocephalicus revealed the carotid sheath; the external parathyroid gland usually being located in the adipose tissue just dorsal to the carotid sheath. The gland, which is spherical in outline, dark pink in color, and approximately three mm.

TABLE VII

COMPOSITION OF PURIFIED DIETS

Ingredient	Basal Diet %	Ca-Free Diet %
Corn starch	34.37	34.37
Glucose[a]	24.37	24.37
Cellulose[b]	31.05	31.57
Urea[c]	4.20	4.20
Corn oil[d]	1.00	1.00
Choline chloride	0.10	0.10
Vitamins A & D[e]	0.02	0.02
K_2CO_3	2.21	1.32
K_2HPO_4	-----	1.69
$CaHPO_4$	1.32	-----
$MgSO_4$	0.12	0.12
$MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$	0.27	0.27
Na_2SO_4	0.25	0.25
NaCl	0.62	0.62
Trace minerals[f]	0.10	0.10

[a]Cerelose. Corn Products Company, Argo, Illinois.

[b]Solka-Floc. B-W20. Brown Co., Berlin, New Hampshire.

[c]Crystalline urea. Courtesy John Deere Chemical Co., Pryor, Oklahoma.

[d]Mazola. Santoquin added to give 0.0125% in total diet.

[e]Containing 20,000 IU and 2,500 USP units/gm. of vitamins A and D, respectively. Courtesy NOPCO Chemical Co., Harrison, New Jersey.

[f]Composition of the trace mineral mixture (mg./100 gm. diet); $FeSO_4$, 42.51; $MnSO_4 \cdot H_2O$, 15.37; $Na_2B_4O_7$, 12.56; $ZnSO_4 \cdot 7H_2O$, 26.35; $CuCO_3 \cdot Cu(OH)_2$, 1.97; KI, 0.07; $CoCl_2 \cdot 6H_2O$, 0.05; CaF_2, 0.20; $Na_2MoO_4 \cdot 2H_2O$, 0.50; $Cr_2(SO_4)_3$, 0.04; Na_2SeO_4, 0.02.

in diameter, was isolated by blunt dissection and the blood supply occluded by application of a haemostat. The operation was relatively bloodless. Subcutaneous tissues were closed with chromic gut and the skin with braided polyester sutures. The opposite external parathyroid gland was removed in a similar manner. Frozen sections of the isolated parathyroid glands were cut and stained with hematoxylin and eosin thereby permitting histological identification during surgery.

After removal of the external parathyroids, the animal was then placed in dorsal recumbency and a midline incision made on the ventral aspect of the neck, extending posteriorly for five cm. from a point just behind the cricoid cartilage. The subcutaneous tissues were separated by blunt dissection to expose the thyroid glands, which lie on either side of the trachea, extending from the 2nd to the 6th tracheal rings and having a connecting isthmus at the level of the 5th ring. Thyroid arteries were ligated as they entered the anterior poles of the glands and the entire thyroid glands, including the associated internal parathyroid glands were excised. No attempt was made to identify the internal parathyroid glands.

Thyroid and parathyroid glands of control animals were similarly exposed (sham-operated) but were left intact. In all cases post-anesthetic recovery was rapid and healing was by primary union without complication.

Trial 2. Five sheep, about six months of age, were TPX, while five sham-operated animals of the same age served as controls. Methods and procedures were identical to those described in Trial 1.

Results

Trial 1. Table VIII shows the values for serum calcium and magnesium in the adult sheep (Trial 1) during the post-operative period. Sheep A1 was completely TPX while one superior parathyroid was left in A3 and complete removal of one thyroid lobe in A2 was not confirmed. Sheep A1 had a precipitous decrease in serum calcium during the two weeks following surgery. The period was complicated by inappetance, which possibly contributed to the decrease in serum calcium. Halse (1958) has observed hynomagnesemia in intact, starving cows. Upon return of appetite, serum calcium values increased from 4.9 mg./100 ml. at 14 days to 7.8 at 24 days, but subsequently decreased until the animal died 43 days post-operation. Tetany was not observed. Sheep A3 responded with a decrease in serum calcium only after being placed on the calcium-free diet. Serum calcium in A2 and A3 remained lower than the control sheep after feeding the calcium-free diet.

In the control adult sheep, serum calcium values were affected only when they were placed on the calcium-free diet (Table VIII). Differences in serum calcium levels between control and TPX adult sheep existed during the first week post-operation and after the feeding of the calcium-free diet.

Calcium balances on the adult sheep are shown in Figure 3. Sheep A1 was in positive calcium balance during the first two weeks' post-operation when the basal diet was being fed. During this same period, A2 was in positive balance the first week but the balance became negative the second week. Sheep A3, which retained one external parathyroid gland, was in negative calcium balance for both these weeks. The mean

TABLE VIII

EFFECT OF THYROPARATHYROIDECTOMY ON SERUM CA, AND MG

Post Surg. Days	TPX						Control					
	A1		A2		A3		B1		B2		B3	
	Ca[1]	Mg[1]	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
0	11.1	3.0	12.0	3.0	11.0	3.2	10.5	3.0	11.1	3.8	10.8	2.8
1	9.9	2.8	9.7	2.7	11.1	3.2	10.1	3.1	10.2	3.4	10.0	3.1
2	9.3	2.9	9.3	2.6	10.2	3.2	11.2	3.2	10.7	3.4	10.4	3.2
3	6.9	2.6	9.5	2.6	10.6	3.5	11.1	3.5	12.2	3.7	11.2	3.0
4	8.4	2.8	9.8	2.5	11.0	3.7	11.9	3.2	11.6	3.6	10.9	3.3
5	8.4	2.8	9.5	2.5	11.5	3.6	11.4	3.0	11.4	3.4	11.9	2.9
6	7.5	2.3	9.4	2.3	10.6	3.3	11.8	3.3	11.0	3.2	11.5	3.4
7	7.2	2.3	10.0	2.7	10.1	3.9	11.5	2.9	11.4	3.8	10.6	2.8
8	6.3	1.6	10.2	2.3	11.1	3.5	11.3	3.0	11.9	3.2	11.4	2.8
9	5.8	1.5	10.0	2.3	11.7	3.5	11.4	3.4	11.7	3.4	11.3	2.5
10	6.0	1.3	10.4	2.[illegible]	11.8	3.4	11.1	3.1	11.1	3.2	8.8	2.9
11	4.9	1.4	9.7	2.[illegible]	9.8	3.7	11.1	2.9	11.9	3.5	11.2	2.8
12	5.7	1.5	9.7	2.4	11.6	3.7	9.8	2.9	11.3	3.3	11.2	2.7
13	4.2	1.7	8.8	2.6	10.6	3.5	10.1	3.0	11.5	3.4	11.0	2.8
14	4.9	2.1	8.6	2.6	10.2	3.4	11.4	2.9	10.3	3.4	10.6	2.9
[2]												
15	5.0	2.1	9.7	2.5	9.9	3.2	9.9	3.1	10.1	3.3	9.3	2.9
16	4.7	2.1	9.3	2.6	9.4	3.4	9.8	3.3	10.7	3.5	10.6	2.9
17	5.0	2.2	9.8	2.9	9.2	3.3	9.9	3.0	10.3	3.5	10.4	3.1
18	5.2	2.2	9.5	2.7	9.0	3.3	10.2	3.2	10.4	3.4	10.7	3.1
19	5.9	2.6	9.9	2.7	8.8	3.3	9.8	3.4	9.9	3.5	10.6	3.2
20	6.7	2.7	9.3	2.8	8.6	3.5	9.9	3.2	9.8	3.7	10.5	3.1
21	7.3	2.9	8.9	3.1	8.5	3.8	11.0	3.5	9.8	3.7	8.5	3.1
22	6.9	2.6	10.2	3.0	9.6	3.7	10.0	3.3	11.0	3.6	10.0	3.0
23	6.7	2.6	9.7	3.0	8.9	3.5	10.1	3.1	8.8	3.8	10.4	3.3
24	7.8	2.8	10.2	3.0	9.4	3.4	10.1	2.8	10.2	3.5	10.4	3.1
25	7.5	2.9	9.9	2.7	9.3	3.3	9.9	2.9	10.2	3.5	10.6	3.0
26	7.4	2.8	10.2	2.7	9.1	3.3	9.7	2.6	10.4	3.5	10.5	2.7
27	6.9	2.5	9.9	2.3	9.5	3.0	10.1	3.0	10.5	3.2	10.3	2.9
28	6.5	2.4	9.8	2.4	8.9	3.2	9.8	3.0	10.2	3.5	10.7	2.9
29	7.0	2.6	9.9	2.8	9.1	3.2	9.6	2.1	9.9	3.4	10.0	3.1
30	6.7	2.4	9.6	3.1	8.7	3.5	9.0	3.0	9.9	3.3	9.6	3.1
31	5.6	2.3	9.3	2.7	8.3	3.4	8.7	3.1	9.2	3.4	9.2	3.2
32	5.5	2.1	9.2	2.8	8.2	3.6	9.1	2.8	8.2	3.7	10.0	2.7
33	5.3	2.2	9.0	2.4	8.2	3.2	8.7	2.7	10.9	3.4	9.2	2.7
34	4.9	2.2	8.7	2.5	8.1	3.4	8.9	2.8	9.8	3.4	10.0	3.0
35	3.7	2.2	9.2	2.3	8.2	3.4	9.1	2.8	9.8	3.7	10.2	3.2
36	3.3	2.1	9.7	2.3	8.9	3.3	8.9	2.8	9.9	3.4	10.4	3.1
37	3.0	2.1	9.4	2.5	8.3	3.2	9.2	3.0	10.5	3.5	10.2	3.3
38			9.4	2.5	9.6	3.5						
39					9.4	3.3						

[1] mg./100 ml.

[2] Animals placed on calcium-free diet.

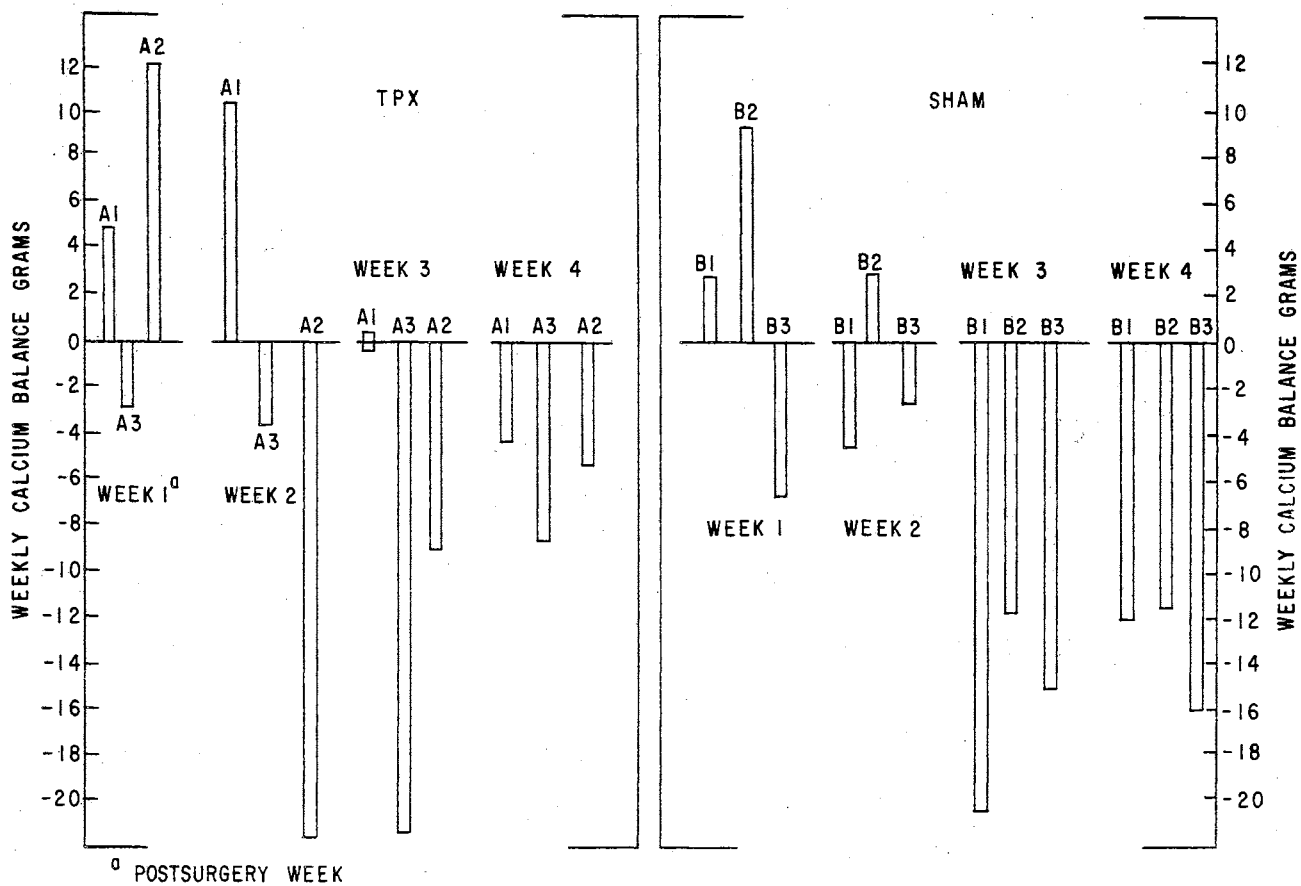

Figure 3. Calcium Balance for TPX and Control Adult Sheep

calcium balance for the control animals was negative for the same period. After feeding the calcium-free diet, the calcium balance for all animals became negative (sheep A1 < A2 < A3 < B1, < B2, < B3). These data indicated that TPX decreased calcium mobilization from body stores and thereby decreased fecal endogenous calcium.

Trial 2. Complete thyroparathyroidectomy was performed in all five of the six-months old sheep. The parathyroid glands were confirmed histologically. The post-operative serum calcium and magnesium values for TPX sheep are shown in Table IX, while values for the controls are shown in Table X. Serum calcium declined more rapidly in the young TPX sheep than in TPX adults. Feed consumption was excellent in the young sheep during the first three weeks' post-operation. Sheep Y3 and Y4 died before being placed on the calcium-free diet. The latter died during the night of the 13th day after surgery, while the former was observed going into tetany 15 days' post-operation and died within 30 minutes of the onset of tetany. Sheep Y2 died on day 23 and Y1 on day 25; 9 and 11 days, respectively, after being placed on the calcium-free diet. Sheep Y5 died on day 34, 20 days after being placed on the calcium-free diet. This animal exhibited muscular weakness at day 21 and was unable to stand for several days. It recovered the ability to stand, but became recumbent again the day before death.

Figure 4 exhibits the calcium balance of the control and TPX sheep during the first two weeks. Due to early mortality of the young TPX sheep, balance studies during the calcium-free diet phase could not be conducted. The TPX animals were in positive calcium balance following surgical removal of the glands, as was the case with adult sheep A1 (Trial 1).

TABLE IX

EFFECT OF THYROPARATHYROIDECTOMY ON SERUM CA AND MG

Post Surg. Days	Y1		Y2		Y3		Y4		Y5	
	Ca[1]	Mg[1]	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
0	11.0	2.5	10.6	3.3	10.0	2.4	10.5	2.4	10.2	2.4
1	9.1	2.6	9.5	2.6	8.7	2.5	8.5	2.3	9.2	2.3
2	8.3	2.6	7.7	2.7	7.1	2.3	6.2	2.3	8.8	2.3
3	8.3	2.6	8.8	3.0	7.0	2.3	6.6	2.3	7.1	2.3
4	8.4	2.6	8.9	2.9	7.0	2.4	7.3	2.5	7.7	2.1
5	7.4	2.5	7.2	2.9	6.9	2.2	6.7	2.4	7.1	2.2
6	6.8	2.6	9.0	2.9	6.0	2.1	10.2	2.8	6.3	2.2
7	7.0	2.6	10.4	3.4	7.2	2.3	7.3	2.3	6.9	2.1
8	5.1	2.4	6.9	2.4	7.4	2.3	6.8	2.4	7.3	2.2
9	6.4	2.4	8.9	3.2	7.9	2.8	6.3	2.1	7.4	2.2
10	7.1	2.6	8.3	2.9	7.7	2.7	5.5	2.0	7.9	2.5
11	7.0	2.7	6.8	2.8	7.8	2.9	5.1	2.1	7.7	2.6
12	7.0	2.4	6.4	2.7	7.3	2.6	4.7	2.1	7.4	2.5
13	6.4	2.3	6.2	2.6	6.8	2.5	3.4	1.6	7.4	2.4
14	6.8	2.6	5.8	2.6	5.8	3.0			6.7	2.5
[2]										
15	5.9	2.3	6.2	2.7	6.2	2.3			6.7	2.0
16	5.6	2.5	6.7	2.6					7.4	2.4
17	5.3	2.2	6.6	2.5					7.0	2.5
18	5.2	2.4	6.6	2.2					7.0	2.3
19	5.1	2.4	5.7	2.0					6.6	1.7
20	5.8	2.4	5.8	2.0					6.6	2.4
21	5.0	2.3	5.2	1.9					6.1	2.0
22	5.1	1.8	4.1	1.7					5.7	1.7
23	4.6	1.7							5.6	2.2
24	4.3	1.7							5.7	2.3
25	3.2	1.4							5.6	1.6
26									5.4	1.8
27									5.1	1.5
28									5.0	1.7
29									4.2	1.7
30									4.5	1.7

[1] mg./100 ml.

[2] Animals placed on calcium-free diet.

TABLE X

SERUM CA AND MG VALUES IN CONTROLS

Post Surg. Days	X1		X2		X3		X4		X5	
	Ca[1]	Mg[1]	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
0	10.0	2.4	10.5	2.2	10.5	2.6	10.5	2.7	10.0	2.8
1	8.7	2.2	9.6	2.6	10.4	2.9	10.0	2.9	9.9	2.8
2	8.7	2.6	10.6	2.7	9.9	2.8	9.6	2.9	9.6	2.7
3	9.9	2.6	10.0	2.7	10.3	2.8	10.1	3.1	9.4	2.8
4	10.0	2.5	10.0	2.6	10.3	2.9	10.3	2.9	9.4	2.9
5	10.9	2.3	10.2	2.7	10.1	2.8	10.1	3.1	9.6	2.7
6	11.2	3.5	10.5	2.9	10.3	3.1	10.0	3.1	9.7	2.6
7	11.2	2.8	10.6	3.1	9.7	3.0	10.0	3.0	10.8	3.1
8	11.4	2.9	12.5	3.3	10.4	2.9	10.9	3.0	10.3	3.0
9	11.0	3.0	11.2	2.9	7.2	2.9	10.7	3.0	11.5	3.3
10	11.5	3.2	9.9	2.9	8.6	2.8	11.8	3.4	10.7	3.2
11	11.0	3.3	9.8	3.0	9.6	2.6	11.1	3.4	10.8	3.0
12	8.9	2.8	11.1	3.0	10.3	2.8	11.3	3.2	10.9	3.1
13	8.7	2.9	10.9	3.1	10.4	2.8	10.5	3.0	11.2	3.2
14	10.9	3.3	10.3	3.1	10.0	2.7	11.1	2.9	10.9	2.9
[2]										
15	10.9	3.3	10.1	3.1	9.2	2.7	10.8	3.0	11.8	2.5
16	10.4	3.4	9.8	3.1	9.4	2.9	12.1	3.0	11.9	3.3
17	10.2	3.4	9.6	3.1	9.0	2.8	12.0	3.8	11.2	3.2
18	10.2	3.2	8.8	3.1	9.6	3.0	11.6	3.2	12.0	3.3
19	9.8	3.2	9.9	3.3	10.4	3.0	11.2	3.2	11.6	3.6
20	10.6	3.1	9.8	3.4	10.2	2.8	11.7	3.2	11.7	2.9
21	11.0	3.1	9.3	3.0	10.2	2.9	10.9	3.0	11.2	2.8
22	10.9	3.2	10.6	3.5	10.2	2.8	10.8	3.1	12.4	2.7
23	11.5	3.1	10.7	3.3	10.8	3.1	11.7	3.2	11.5	3.1
24	11.0	3.1	10.1	2.7	10.2	3.0	10.7	3.3	11.4	3.1
25	11.7	3.3	10.9	3.0	10.4	3.1	11.0	3.1	11.7	2.9
26	10.7	3.1	9.6	2.9	9.8	3.1	10.7	3.1	11.6	3.0
27	11.6	2.8	9.9	3.4	10.4	2.8	10.8	3.0	11.6	3.2
28	10.8	3.0	9.6	2.9	11.0	3.0	11.9	2.6	11.2	3.2
29	11.9	2.6	10.6	3.0	10.6	2.9	11.6	2.8	11.7	2.9
30	11.8	2.8	11.4	3.1	10.6	3.1	11.7	3.3	11.2	2.8

[1] mg./100 ml.

[2] Animals placed on calcium-free diet.

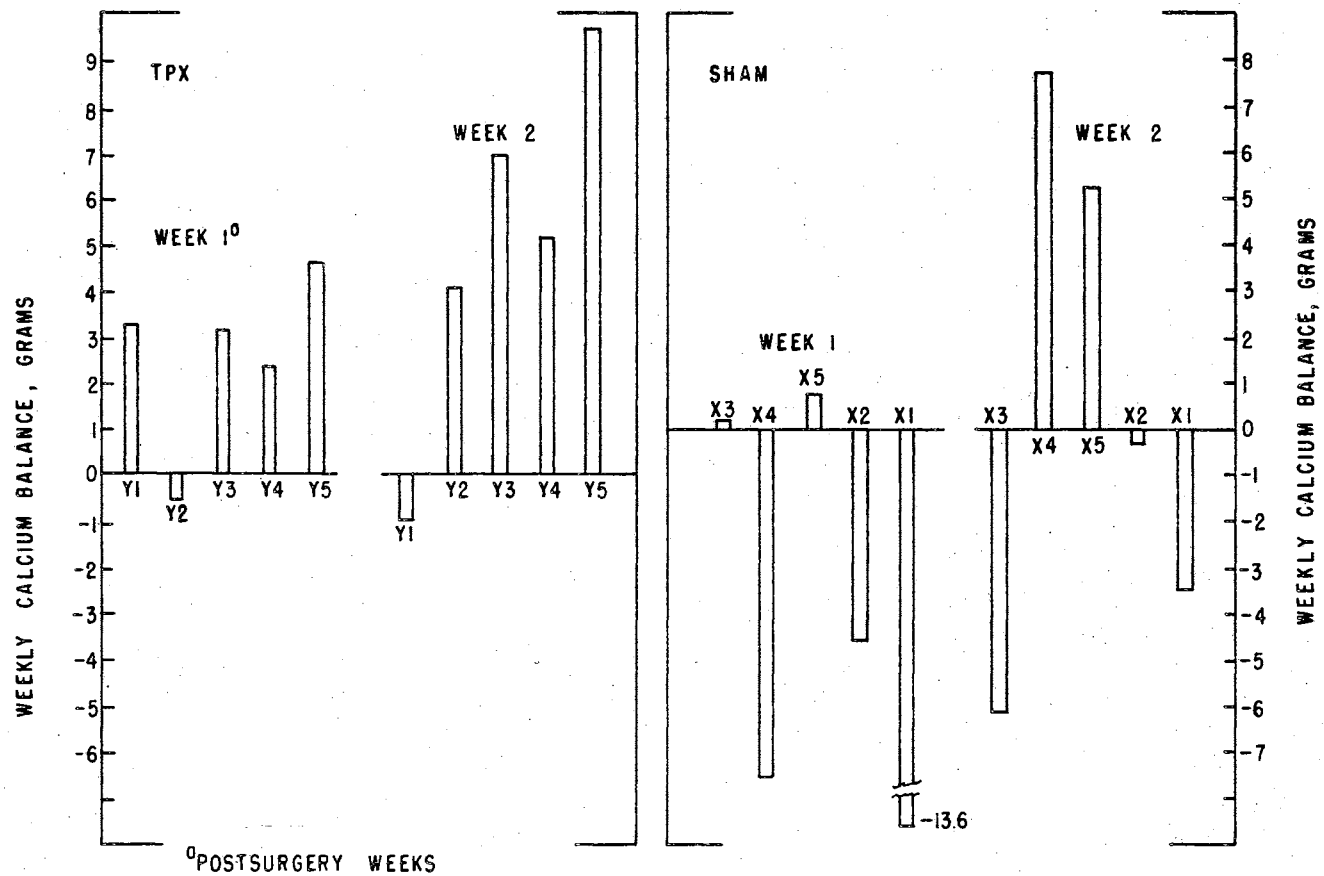

Figure 4. Calcium Balance for TPX and Control Young Sheep

Simple linear regression analysis on serum calcium values in young TPX sheep indicated that the rate of decline in serum calcium was greater when animals were fed the calcium-free diet. The prediction equation for estimating serum calcium values in TPX sheep fed the basal diet was $\hat{Y} = 8.519 - 0.147\ X$ with $S_{y \cdot x} = 0.63$; where $\hat{Y}$ is the estimate for serum calcium, X is the number of days post-operation, and $S_{y \cdot x}$ is the standard error of the estimate. The corresponding prediction equation for TPX sheep fed a Ca-deficient diet was $\hat{Y} = 10.941 - 0.250\ X$ with $S_{y \cdot x} = 0.30$. The slopes of the regression lines represent the rate of change in serum calcium in mg./100 ml./day. The slope was greater when the TPX sheep fed the Ca-free diet (b = -0.250 mg./100 ml./day) than when the basal diet was fed (b = -0.147). It cannot be determined from these data whether this is a diet effect or simply an increased rate that occurs independent of the dietary level of calcium.

Payne and Chamings (1964) observed an initial fall in plasma magnesium of thyroparathyroidectomized goats, followed by a return to normal. In these trials, neither thyroparathyroidectomy nor feeding a calcium-free diet affected serum magnesium. Values decreased when feed intake decreased, the latter often ceased prior to death of the thyroparathyroidectomized sheep. Post-mortem examination revealed no significant lesions in any of the animals.

Discussion

Recent results (Mayer, et al., 1967; Sherwood, et al., 1966) indicate the importance of the parathyroid gland for calcium homeostasis in the ruminant animal. Thyroparathyroidectomy has been shown to be fatal in the young (Stott and Smith, 1957a) but not the adult ruminant (Stott

and Smith, 1957b). The data presented in the present study indicate that age is an important criterion in assessing responses to thyroparathyroidectomy and that the level of dietary calcium may be critical to the response mechanisms. The rate of decline in serum calcium is rapid in young TPX sheep and is often followed by fatal tetany, even when the animal is maintained on a normal diet. In contrast, the TPX adult sheep is more tolerant to thyroparathyroidectomy, being capable of correcting hypocalcemia and maintaining serum calcium above symptomatic levels, even after an extended time on a calcium-free diet. The higher calcium requirements for growing animals (Hansard, et al., 1954) may be a factor in this difference.

The positive calcium balance observed in young TPX sheep indicates that thyroparathyroidectomy did not appreciably affect the absorption of dietary calcium. Mayer (1967) has shown that administration of parathyroid extract in adult parathyroidectomized cows resulted in increased bone resorption and fecal endogenous calcium. Decline in serum calcium during positive calcium balance in young TPX sheep implies a reliance upon hormonal mobilization of storage calcium for maintenance of serum levels. This in turn indicates that the 0.4% calcium diet used in this experiment may not be adequate for maximum bone growth in sheep of this age.

Summary

The rate of decline in serum calcium was rapid in young thyroparathyroidectomized sheep and often followed by fatal tetany, even when the animal was maintained on a normal diet. Positive calcium balance observed in the young thyroparathyroidectomized sheep indicated no

appreciable effect on calcium absorption. In contrast, adult sheep were more tolerant to thyroparathyroidectomy, being capable of correcting hypocalcemia and maintaining serum calcium levels above symptomatic levels even after extended time on a calcium-free diet. In these trials neither thyroparathyroidectomy nor feeding a calcium-free diet affected serum magnesium. Serum magnesium decreased only when feed intake decreased.

CHAPTER V

EXPERIMENT III

Introduction

The results of Experiment II indicate that the secretion of PTH is essential for maintaining normal serum calcium levels in sheep fed a calcium-deficient diet. It was found that the young TPX sheep exhibited a continuous decrease in serum calcium for two weeks' post-operation, even when maintained on a normal diet.

Results of the two preceeding experiments indicate that PTH is essential for maintaining normal sheep blood calcium levels, especially in lambs. Calcitonin is also involved in maintaining blood calcium levels (Deftos, et al., 1968) and is secreted by the thyroids (Chausmer, et al., 1966) and parathyroids (Copp and Henze, 1964) of sheep. In parturient paretic cows, recumbent for a prolonged time, elevated serum glutamic-oxaloacetate transaminase (SGOT) was associated with liver or muscle lesions (Gould and Grimes, 1960). Similar observations have been reported by Boyd, et al. (1964). The following experiment was conducted in order to study the effects of TX and TPX operations upon serum levels of Ca, P, Mg and the activities of cellular enzymes in serum.

Experimental Procedure

Five sheep, about eight months of age, were assigned to each of the

following surgical treatments: TPX, TX, and control. Controls were sham-operated. Experimental procedures were identical to those described in Experiment II, except that all animals were maintained on the complete basal purified diet (Table VII) throughout the experiment.

The following serum enzymes were measured under zero order kinetics: glutamic-oxalacetate transaminase (SGOT) according to the methods of Bergmeyer (1965); creatine phosphokinase (CPK) according to the methods of Weismann, et al. (1966); isocitric dehydrogenase (ICDH) according to the methods of Freedland, et al. (1965); and alkaline phosphatase (AP) according to the methods of Bowers and McComb (1966).

Results and Discussion

The TPX sheep fed a complete basal diet were not capable of maintaining normal serum calcium levels (Table XI). With the exception of sheep 398, serum calcium decreased in the TPX sheep until death, which occurred from 9 to 23 days after operation. It is not known why the one TPX sheep (398) was capable of maintaining normal serum calcium levels, but it is possible that accessary parathyroid glands, other than those removed, were present in this animal. The animal died seven months after surgery, but serum calcium values were not observed to this time.

Removal of the inferior parathyroid glands by thyroidectomy had no effect on calcium homeostasis in sheep as measured by blood calcium and phosphorus levels. As 88% of the total parathyroid weight resides in the external parathyroid glands of the bovine (Mayer, 1966), these results were expected. Thyroidectomy had no effect upon serum calcium levels of sheep. If calcitonin had its origin in the thyroid gland in

TABLE XI

SERUM CALCIUM (MG./100 ML.) IN TPX, TX AND CONTROL SHEEP

Treatment	TPX					TX					Control				
Animal No.	684	398	471	382	304	410	236	372	91	84	282	712	66	86	204
Days post-operation															
0	10.9	11.9	11.4	11.9	10.0	12.4	11.44	13.88	12.40	11.91	10.27	11.00	10.37	13.34	12.61
1	8.9	10.0	9.3	12.0	9.1	12.1	12.01	11.88	11.02	12.87	10.89	12.95	10.35	13.52	11.31
2	10.3	9.5	8.2	10.0	5.9	9.8	11.70	11.62	14.09	10.87	9.41	11.60	10.71	11.47	10.66
3	7.7	11.5	8.5	9.1	6.0	9.9	12.84	13.18	11.70	11.73	10.71	12.61	11.86	11.52	11.41
4	7.7	10.9	7.9	7.1	5.9	11.6	11.80	12.14	10.95	11.75	10.37	13.10	11.23	12.35	13.16
5	7.6	10.6	6.9	7.0	4.9	11.5	13.47	13.36	11.34	11.67	11.44	12.90	10.53	12.27	11.57
6	7.2	10.8	6.7	7.4	4.8	11.3	13.39	12.06	11.73	12.69	10.22	12.82	11.34	14.33	13.34
7	7.5	12.1	5.8	8.4	4.2	11.0	13.42	10.66	12.32	12.90	11.28	13.18	11.49	12.64	12.82
8	7.1	11.1	6.5	7.9	4.7	10.0	12.90	12.19	11.70	10.48	12.01	10.09	12.27	13.21	12.74
9	7.5	12.9	6.0	5.9	4.8	12.2	12.19	11.44	10.89	11.00	10.92	11.13	10.79	12.74	11.31
10	10.0	11.8	6.2	5.5		11.0	11.23	11.62	11.96	10.09	11.88	12.19	11.54	10.74	11.44
11	7.3	11.0	6.5	5.4		11.1	11.67	10.97	11.96	10.10	10.84	11.91	11.18	11.96	12.32
12	6.1	10.9	5.9	4.9		10.0	11.10	10.97	11.62	10.24	11.62	11.75	11.80	10.53	11.08
13	5.7	10.5	6.3	5.5		9.5	10.50	9.49	12.09	10.43	11.02	10.50	11.62	11.44	12.04
14	3.6	10.4	5.9	5.6		9.8	11.78	11.28	11.18	11.70	11.49	10.61	11.41	10.17	9.98
15	5.0	10.1	5.8	4.7		9.5	10.61	11.10	11.57	11.02	11.18	12.09	11.80	11.86	11.62
16	4.8	10.7	6.1	4.6		13.5	10.56	11.60	10.50	10.06	13.31	12.77	13.88	10.82	11.62
17	4.7	10.3	5.9			9.8	12.53	13.49	12.64	10.53	12.74	13.86	12.01	12.56	11.99
18	4.3	11.6	5.7			9.9	13.57	12.66	12.04	12.04	13.94	13.55	13.42	12.19	13.88
19	6.5	11.6	7.6			10.2	10.95	12.19	10.87	9.96	12.64	11.65	11.49	13.57	12.95
20	5.5	12.0	6.4			12.4	10.00	13.29	10.06	9.62	11.54	11.86	11.52	11.15	11.05
21	5.8	11.8	7.1			13.9	9.83	10.92	9.70	9.91	11.26	11.28	10.58	11.28	11.00
22	5.0	9.8	5.2			10.5	10.01	10.01	10.00	9.67	11.31	11.75	11.05	10.27	11.93

sheep, its removal had no effect upon serum calcium levels.

Serum calcium values for 5 TPX sheep from Experiment II and 3 from the present experiment were pooled over the first 15 post-operation days and used to determine the effect of time on serum calcium levels. The regression equation was $\hat{Y} = 9.682 - 0.277\ X$ with $S_{y \cdot x} = 0.577$. From these values it was estimated that the average sheep would die within 20.6 days after thyroparathyroidectomy. The average value in these sheep was 18.3 days. Two animals were omitted from the present experiment because one died within 9 days post-operation and the other one continued to show normal serum calcium values after surgery.

Thyroidectomized sheep had higher serum inorganic phosphorus values (Table XII) than TPX or control sheep ($P < .01$). There was no difference between the serum inorganic phosphorus values of TPX and control animals. However, it should be noted that in this experiment, as in Experiment II, most of the TPX animals had elevated serum inorganic phosphorus levels three to five days prior to death, values often reaching 12-15 mg./100 ml.

Values for SGOT, CPK, ICDH and alkaline phosphatase are shown in Tables XIII, XIV, XV and XVI. With the exception of alkaline phosphatase, all enzymes measured were elevated after prolonged hypocalcemia in the TPX sheep. Elevated SGOT levels have been attributed to liver and/or muscle necrosis, and elevated CPK levels to muscle necrosis (Schmidt, et al., 1963), and ICDH to liver necrosis (Freedland, et al., 1965). From these data it is evident that a general breakdown of several tissues was occurring in prolonged hypocalcemia. Correlations between serum levels of calcium and individual enzymes were not significant; however, the onset of hyperphosphatemia appeared to be

TABLE XII

SERUM INORGANIC PHOSPHORUS (MG./100 ML.) IN TPX, TX AND CONTROL SHEEP

Treatment	TPX					TX					Control				
Animal No.	684	398	471	382	304	410	236	372	91	84	282	712	66	86	204
Days post-operation															
0	8.2	5.9	6.3	7.3	8.5	8.8	8.1	6.8	7.5	7.7	11.6	10.7	14.5	7.5	6.9
1	8.0	5.6	5.5	5.9	6.6	9.8	7.0	8.3	8.1	8.1	10.9	10.2	6.8	7.4	8.0
2	7.9	6.4	5.6	7.2	7.8	10.1	6.1	8.5	5.9	7.0	10.8	7.8	6.7	7.0	9.5
3	7.7	8.0	4.9	8.3	7.7	10.1	6.3	5.3	9.7	7.8	9.3	7.0	8.2	6.1	7.1
4	7.5	5.8	5.3	7.3	6.2	9.2	7.0	8.2	7.9	7.2	10.1	6.6	8.8	5.1	6.9
5	8.8	6.3	5.3	7.7	9.6	10.9	7.3	8.8	8.8	8.0	9.1	9.4	8.9	5.8	7.6
6	8.2	7.8	6.8	11.3	8.5	9.3	8.4	9.8	7.2	7.8	8.7	5.6	8.7	6.8	8.4
7	7.6	5.3	5.3	9.1	10.9	10.5	6.5	8.6	6.7	7.4	8.4	6.8	7.6	5.5	6.3
8	7.6	6.0	6.4	8.5	14.1	10.4	5.8	8.4	6.6	7.9	7.6	8.4	6.1	6.9	4.6
9	7.6	4.6	7.0	5.9	15.1	8.1	7.5	9.6	9.7	7.2	5.8	7.0	5.2	6.6	6.9
10	6.7	4.8	6.5	8.2		11.1	6.8	8.2	7.1	8.8	8.3	7.1	5.3	7.9	5.7
11	7.0	5.9	6.9	9.5		10.9	6.5	7.3	6.2	8.5	6.8	7.2	5.8	7.0	5.8
12	7.4	6.3	5.8	8.0		11.6	6.7	7.3	6.6	10.4	8.7	8.3	7.9	6.0	5.1
13	7.6	6.9	6.1	7.9		12.0	7.4	7.0	6.9	7.6	8.1	7.5	7.0	8.0	7.9
14	7.4	4.9	6.5	8.7		9.8	6.5	7.0	6.6	7.7	9.4	6.7	7.3	6.4	6.7
15	7.4	7.9	5.7	8.8		9.9	8.4	6.4	5.6	8.2	7.5	5.8	7.4	8.6	7.7
16	10.3	4.5	5.4	12.0		13.4	8.0	7.2	6.9	8.7	9.5	9.8	8.4	7.1	6.0
17	9.1	6.0	4.5			11.3	7.1	5.2	6.7	8.3	5.4	6.6	8.9	7.9	8.7
18	9.2	5.0	4.3			7.9	6.3	7.7	7.3	8.4	5.3	8.0	7.1	5.1	5.6
19	9.8	7.6	4.5			9.9	7.3	8.1	7.6	7.9	5.2	6.4	6.7	3.9	6.1
20	9.9	5.1	4.4			8.9	7.2	8.7	7.1	7.1	4.5	6.4	6.6	3.5	5.9
21	10.4	4.9	4.1			8.5	7.6	7.3	10.1	8.8	4.7	5.8	6.4	4.2	5.1
22	13.4	5.5	4.9			11.3	7.8	8.8	9.6	8.6	6.8	7.6	5.7	3.6	5.6

TABLE XIII

SGOT LEVELS, I.U./LITER, IN TPX, TX AND CONTROL SHEEP

Treatment	TPX					TX					Control				
Animal No.	684	398	471	382	304	410	236	372	91	84	712	66	282	86	204
Days post-operation															
0	48	36	80	56	45	42	62	44	40	36	66	68	42	99	65
1	52	36	78	55	63	47	67	45	67	37	60	65	41	83	55
2	48	36	57	48	58	40	57	41	42	32	53	62	38	67	46
3	43	36	47	42	48	35	54	40	42	28	44	60	41	52	48
4	37	36	40	46	52	32	45	35	45	30	40	56	57	47	44
5	29	36	37	49	49	29	40	36	37	28	48	51	44	47	67
6	41	36	35	56	48	33	46	32	37	27	42	56	39	39	51
7	37	36	40	48	48	34	43	33	39	20	39	37	29	42	45
8	38	29	20	51	126	30	39	32	37	55	39	37	42	30	37
9	37	36	32	53		34	56	42	33	27	34	40	42	42	57
10	39	40	52	56		29	56	37	30	24	36	33	30	35	47
11	27	42	47	68		35	64	37	37	27	44	35	28	32	52
12	44	43	44	49		40	150	48	45	31	47	60	27	30	72
13	33	45	56	56		45	212	37	32	27	39	40	22	59	73
14	67	33	31	62		34	274	47	38	33	44	28	35	26	77
15	105	37	76	62		36	274	49	41	26	39	36	25	32	110
16	120	30	91	76		38	264	34	23	21	53	33	27	32	116
17	137	29	104	96		34	249	47	39	26	61	29	25	30	186
18	170	36	139	248		48	282	54	41	28	55	35	25	31	299
19	179	32	219			43	359	55	40	29	61	39	28	24	300

TABLE XIV

CPK LEVELS, I.U./LITER, IN TPX, TX AND CONTROL SHEEP

Treatment	TPX					TX					Control				
Animal No.	684	398	471	382	304	410	236	372	91	84	712	66	282	86	204
Days post-operation															
5	2.2	2.0	2.0	1.7	4.8	2.0	3.0	1.0	0.5	0	2.6	3.9	1.5	2.0	6.7
9	1.0	1.5	2.0	0	18.4	1.5	2.0	5.4	6.2	2.2	1.7	0	0	0	7.2
12	1.0	2.0	1.7	2.0		2.2	2.5	8.9	3.9	2.0	7.6	1.7	1.0	2.0	1.2
15	1.2	1.7	6.9	2.0		2.5	6.9	2.0	2.5	0.7	3.5	0	2.0	1.2	5.2
16	1.2	1.0	14.6	2.5		2.1	2.5	4.2	1.0	0	0.2	1.2	1.0	2.2	4.4
17	0	0	22.2	8.9		3.0	8.9	2.7	3.7	2.7	7.9	2.0	0	0.7	2.5
18	4.2	3.0	26.6	19.7		2.5	5.4	1.0	3.0	0	2.0	2.2	1.2	2.5	13.6
19	1.0	0	35.3	81.4		3.9	2.5	4.4	4.9	1.7	8.6	2.0	0.5	2.0	58.0
22	29.6	1.5	72.0			4.9	109	5.0	3.5	2.0	17.3	3.9	1.5	2.7	208

TABLE XV

ICDH LEVELS, I.U./LITER, IN TPX, TX AND CONTROL SHEEP

Treatment	TPX					TX					Control				
Animal No.	684	398	471	382	304	410	236	372	91	84	712	66	282	86	204
Days post-operation															
0	9.3	3.7	3.1	9.3	4.5	3.3	3.5	3.2	9.7	2.7	2.1	4.5	2.1	2.9	1.4
1	17.0	6.0	2.1	1.9	3.7	2.3	1.9	2.1	9.1	3.9	22.5	2.1	9.7	7.9	4.8
2	28.5	2.5	2.9	5.4	5.4	4.7	3.5	4.0	11.2	3.1	22.8	1.7	5.4	3.3	4.5
3	39.1	2.9	5.2	6.2	1.5	6.0	2.0	1.0	11.8	3.5	16.5	3.7	7.4	1.7	10.8
4	25.0	2.5	3.9	7.5	4.8	3.8	4.0	3.0	6.4	4.6	2.9	11.6	7.2	7.9	3.8
5	18.0	1.9	5.0	9.1	3.9	7.6	4.8	3.5	12.4	5.2	3.0	3.3	6.2	7.0	2.9
6	21.0	3.3	4.5	10.3	4.8	6.4	6.0	5.2	3.5	4.8	3.0	3.3	5.0	10.3	9.9
7	17.0	7.4	4.1	12.0	7.5	6.1	4.0	4.7	7.2	2.7	8.7	1.2	1.5	12.4	3.9
8	10.0	7.9	7.9	19.2	18.4	7.0	7.7	3.5	6.4	3.1	4.3	2.7	3.7	0.8	2.1
9	13.0	1.5	1.2	8.1		6.3	5.4	5.4	9.5	4.1	2.9	1.9	4.1	8.1	6.2
10	3.5	5.0	6.2	11.2		6.3	4.6	4.3	5.2	8.5	2.1	4.1	4.5	7.2	4.1
11	1.4	5.2	7.2	11.6		6.4	5.0	3.8	8.9	5.6	5.2	2.5	2.9	7.2	5.6
12	10.3	5.0	14.3	8.5		6.4	4.0	8.9	10.5	6.0	4.0	3.3	3.7	5.2	3.1
13	17.2	6.8	18.4	10.1		6.2	2.9	3.7	6.2	4.1	3.5	4.0	4.1	4.8	4.0
14	22.1	6.4	20.1	9.7		6.2	3.3	2.0	4.5	4.8	3.1	3.7	3.7	4.8	6.4
15	27.3	13.2	18.0	11.2		8.1	4.0	4.2	5.6	6.0	3.3	3.3	5.0	3.5	6.4
16	23.6	5.0	19.5	12.2		6.2	5.4	2.7	6.2	4.8	3.7	3.7	4.1	4.3	5.8
17	28.5	4.5	19.5	22.3		5.6	4.1	1.0	3.9	3.5	1.5	4.1	2.9	5.8	5.2
18	32.7	5.8	21.7			6.4	4.1	4.4	4.5	2.1	3.3	4.5	3.9	5.0	6.8
19	38.5	5.8	19.0			6.2	5.0	5.9	4.2	4.6	2.5	4.1	3.5	3.7	12.6
20	46.5	3.3	21.1			4.8	9.1	4.2	4.3	5.0	2.3	3.9	3.3	3.3	22.3

TABLE XVI

SERUM ALKALINE PHOSPHATASE LEVELS, I.U./LITER, IN TPX, TX AND CONTROL SHEEP

Treatment	TPX					TX					Control				
Animal No.	684	398	471	382	304	410	236	372	91	84	712	66	282	86	204
Days post-operation															
0	209	107	146	143	110	109	136	167	206	218	125	171	190	176	164
1	200	104	153	134	106	116	116	130	181	188	120	157	183	157	151
2	195	109	169	153	99	118	129	169	180	202	114	199	234	167	173
3	199	118	181	167	127	121	129	146	151	187	121	181	209	143	137
4	187	102	155	173	121	125	127	139	123	188	148	171	201	158	173
5	168	107	140	159	151	106	132	141	120	140	170	190	210	160	130
6	153	120	134	139		113	137	146	118	120	194	217	224	164	125
7	218	104	139	141		99	139	153	129	143	111	224	268	164	144
8	120	104	116	107		127	157	136	118	185	120	215	211	130	107
9	150	129	160	127		125	160	174	174	238	130	297	259	185	151
10	144	129	146	107		118	137	151	174	206	143	269	253	188	143
11	79	113	164	97		113	155	141	171	185	130	245	224	167	143
12	113	107	157	125		92	139	114	176	192	139	252	245	148	136
13	95	116	167	104		92	116	106	155	171	132	217	222	137	120
14	100	104	111	88		102	90	85	153	130	118	206	201	118	100
15	117	109	114			93	102	81	160	146	129	218	250	294	118
16	150	111	106			93	107	106	141	157	130	200	231	120	109
17		106	95			85	100	97	129	153	113	202	225	125	99
18		123	95			76	93	120	153	153	109	211	275	132	93
19		109	74			70	90	102	130	129	100	199	225	130	90
20		93				75	69	70	111	106	100	70	220	113	69

associated with elevated serum enzymes in the TPX sheep. In TPX sheep 398, which did not produce hypocalcemia, there was no elevation of phosphorus or enzymes in serum. The possible association of hyperphosphatemia and elevated serum enzyme concentrations in hypocalcemic, TPX sheep might have an explanation in the membrane theory of Rasmussen and Tenenhouse (1968), who postulated that calcium ions are involved as an integrator of metabolic events and cyclic 3', 5'-adenosine monophosphate (3', 5'-AMP) serves as an important regulator for the permeability of cellular membranes to Ca^{++} or of the binding of this ion to membranes. Accordingly, Ca^{++} ions play a function in the status of cellular membranes, calcium-associated representing a closed state and calcium-disassociated representing an open state. In application of this theory, it is assumed that the ATP-Ca complex represents an associated state by the strong chelating capacity of ATP, and the conversion of ATP to 3', 5' AMP represents a conversion from a strong to a weak chelator of calcium, or from an associated to a disassociated state. Thus, as the extracellular Ca^{++} concentration is decreased due to TPX, less is available for maintaining the ATP-Ca associated membrane state. The elevation of serum inorganic phosphorus could represent a hydrolysis product of the normally Ca-bound nucleotides, phospholipids and other phosphorus-containing molecules of the membrane which bind Ca^{++}. Associated with an altered membrane structure, a leakage of enzymes from the cell could occur.

It was also observed that one animal from each of the TX (No. 236) and the control group (No. 204) had elevated serum enzyme levels toward the end of the trial. During the time these experiments were conducted, it was discovered that the purified diet used was probably deficient in

vitamin E (Buchanan-Smith, personal communication). As vitamin E-deficiency is known to result in muscle (Horwitt, 1965) and liver necrosis (Schwarz, 1965), it was considered that the effects of TPX on serum enzymes observed in this experiment might be due to or augmented by vitamin E-deficiency. This idea was tested by injecting animals 204 and 236 with 700 IU of dl alpha-tocopheryl acetate and measuring SGOT levels over an eight-day period. The results, which are in Table XVII, show that the elevated SGOT concentrations were readily reversible by the administration of vitamin E. Thus, it is not conclusive that TPX and the resultant hypocalcemia were solely responsible for the elevated serum enzyme concentrations observed. The evidence for a membrane-structure relationship between both Ca^{++} (Rasmussen and Tenenhouse, 1968) and vitamin E (Tappel, 1964) could make either or both of the factors important to the interpretation of these data.

Summary

Young thyroparathyroidectomized sheep, fed a diet containing the recommended level of calcium, developed hypocalcemia which eventually resulted in death. Serum inorganic phosphorus and certain serum enzyme concentrations were elevated prior to death of the TPX sheep. Serum calcium levels were not effected in thyroidectomized sheep, but inorganic phosphorus levels were increased.

TABLE XVII

EFFECT OF VITAMIN E INJECTION UPON SGOT LEVELS

Time After Injection (Hrs.)	Animal 204 SGOT I.U./liter	Animal 236 SGOT I.U./liter
0	1317.3	849.0
6	1112.4	
12	1122.2	605.0
24	858.7	585.5
48	536.7	458.6
78	380.6	458.6
96	236.1	264.4
144	170.8	190.3
192	95.6	148.3
240	49.8	117.1

CHAPTER VI

GENERAL SUMMARY AND CONCLUSIONS

When ruminant animals are fed a calcium-deficient diet, serum calcium decreases until the rate of decrease is overcome by the mobilization of storage calcium. As shown in Experiment I, the mobilization of storage calcium, once initiated is rapid, the hypocalcemic condition corrected to normal calcemia within a period of one week. Plasma phosphorus increased during the hypocalcemic phase and the early mobilization phase, only to decrease during the middle and late mobilization phase.

These data are consistent with the classical modes of action of PTH: parathyroid hormone is stimulated by low calcium levels, causes the demineralization of bone salt, and voids the excess phosphorus by inhibiting the renal reabsorption of phosphorus. Many clinicians rely upon serum calcium values or the serum calcium x phosphorus product as an indicator of calcium status in the body. If the forces controlling calcium levels in serum are so dominant, as indicated in this experiment, as to maintain normal calcium and phosphorus levels, then it appears that only when calcium or phosphorus are abnormal, can any definite diagnosis be made. Thus, the animals in this experiment did not show gross abnormalities in their serum calcium or phosphorus levels after being fed a calcium-deficient diet for 10 weeks, during which time the homeokinetic forces were being exerted.

The importance of the parathyroid glands in the ruminant animal is evident from the data obtained in Experiments II and III. The need for PTH secretion appears to be a function of the amount of calcium in the diet and the physiological age of the animal. Thus, adult sheep are tolerant to TPX provided sufficient calcium is available in the diet. The young ruminant, on the other hand, was not tolerant to TPX when calcium was supplied as 0.4% of the diet. Two possible interpretations of these data are evident. Growing ruminants may have calcium demands which are greater than the rate of absorption. Under such a condition, the animal is temporarily dependent upon bone calcium, which eventually may be replaced. If, in the case, the use of bone calcium is dependent upon PTH, PTX would result in the observed hypocalcemia. Another possibility concerns the fact that the 0.4% calcium used in this diet was not optimal for growth of the young ruminant. Thus, there would exist competitive forces between extracellular fluid and bone tissue for calcium. If, initially, the factors affecting bone deposition of calcium were greater than those for retaining calcium in the extracellular fluid compartment, hypocalcemia would result. The hypocalcemia would, in turn, stimulate PTH secretion and bone demineralization. In this case, TPX would remove the force mobilizing calcium from bone to blood and calcium would remain within the bone. The fact that TPX animals in positive calcium balance had decreasing serum calcium levels, tends to make the latter interpretation more acceptable.

The homeokinetic mechanisms involved in calcium metabolism are critical to the animal under nutritional and physiological stresses. Measurement of PTH and calcitonin as the forces influencing calcium

metabolism could reveal the metabolic status of this mineral in the animal's body.

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