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I am submitting herewith a thesis written by George E. Beckmann III entitled "Magnesium Depletion and Repletion Effects on Magnesium, Calcium and Potassium in Bone, Muscle, Cerebrospinal Fluid and Plasma of Sheep." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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MAGNESIUM DEPLETION AND REPLETION EFFECTS ON MAGNESIUM, CALCIUM AND POTASSIUM IN BONE, MUSCLE, CEREBROSPINAL FLUID AND PLASMA OF SHEEP

A Thesis

Presented for the

Master of Science

Degree

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George E. Beckmann III

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ABSTRACT

The major objectives of this experiment were to evaluate the effect of magnesium depletion and subsequent repletion upon the mineral levels (Mg, Ca, and K) in bone, skeletal muscle, cerebrospinal fluid and plasma in yearling and mature wethers.

Two separate identical trials were conducted using 12 yearling wethers (trial I) and 12 mature wethers (trial II). The animals were divided into a control group of four wethers fed a magnesium supplemented diet, and a treatment group of eight wethers fed first a magnesium deficient diet, and then, at the point of magnesium depletion, switched to a magnesium supplemented diet (repletion). Four times during each trial, biopsy samples of bone, skeletal muscle, cerebrospinal fluid and plasma were taken from each wether. Records of feed consumption, fecal excretion, and urine excretion were recorded and samples of each were collected from each animal.

The yearling treatment wethers were depleted of bone magnesium more rapidly and to a lower level than the mature wethers, which showed a similar decreasing trend. Repletion had no immediate effect on the magnesium level in the bone in either trial.

The plasma magnesium level of treatment wethers in both trials dropped rapidly during depletion, and increased rapidly during repletion.

There was no significant effect found in either trial on the mineral status of skeletal muscle or cerebrospinal fluid due to magnesium depletion or repletion. However, the skeletal muscle of the treatment wethers in trial I showed a slight decreasing trend in magnesium level.

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CHAPTER I

INTRODUCTION

A metabolic disorder of ruminants called "grass tetany" or hypomagnesemic tetany has been recognized for a long time. It principally affects cows grazing lush, rapidly growing spring pasture (Grunes et al., 1970). This metabolic disturbance probably affects less than 1 percent of the ruminant female population, but the incidence may be considerably higher in individual herds or flocks (Fontenot, 1972). Symptoms of hypomagnesemia include hyperexcitability, anorexia, muscular twitching, tetanic convulsions, and, in many cases, acute death (Aikawa, 1971).

Intense research concerning hypomagnesemia has been conducted, but there are still many unanswered questions related to the origin, etiology, nature and occurrence of the disease. The relationship between grass tetany and low plasma magnesium values indicates a possible magnesium depletion of the body stores or metabolic pools within the animal. Knowledge of the metabolism of magnesium and related minerals, and assessment of the mineral levels in different metabolic pools during magnesium deprivation is imperative in order to answer the questions concerning this disorder.

The objectives of this experiment were to study the metabolism and evaluate the levels of magnesium, calcium and potassium in bone, skeletal muscle, cerebrospinal fluid, and plasma of sheep during a magnesium depletion-repletion trial.

CHAPTER II

REVIEW OF LITERATURE

I. HISTORY OF HYPOMAGNESEMIA

For over a century, a metabolic disturbance which may result in tetany has been known to occur among ruminants consuming rapidly growing lush green pastures. This disease, "grass tetany," was first related to low serum magnesium by Sjollema in the Netherlands in 1928 (Grunes et al., 1970). Grass tetany is frequently referred to as hypomagnesemia, grass staggers, lactation tetany, and wheat pasture poisoning. Pfander (1973) reported that during the 1930's, wheatfield poisoning killed a number of cows in the United States.

Fontenot et al. (1973) and Grunes et al. (1970) reported that virtually all the cases of grass tetany occur in sexually mature females in the late stages of pregnancy or during early lactation. However, the disease has been diagnosed in calves and steers (Crookshank and Sims, 1955). In the United States, hypomagnesemia primarily affects beef cows grazing pasture, but occurs in ewes and occasionally in dairy cows. The incidence is variable from herd to herd and sporadic within each herd.

Physical symptoms of grass tetany include hyperexcitability, incoordination, loss of appetite, muscular twitching, profuse salivation, grinding of teeth, viciousness, general tetanic contractions, pounding

heart beat, comatose condition, convulsions and death (Aikawa, 1971; Hughes and Cornelius, 1960; Sjollema, 1932; Stewart, 1954).

Because cattle with grass tetany exhibit physical symptoms similar to milk fever, another metabolic disorder, a more accurate diagnosis for hypomagnesemia can be made if the magnesium level of the blood is known (Grunes et al., 1970).

II. ABSORPTION AND EXCRETION OF MAGNESIUM

Magnesium homeostasis and balance are dependent upon an animal's ability to absorb the element from two sources: (1) the dietary magnesium (exogenous), and (2) the magnesium present in secretions and desquamated epithelial cells of the digestive tract (endogenous) (Care, 1967; Rook and Storry, 1962; Wilson, 1964). Research is inconclusive concerning the site of magnesium absorption in the ruminant. Under normal conditions, the small intestine appears to be the major site of net magnesium absorption in ruminants (Care and van't Klooster, 1965; Field, 1961). Smith (1962) reported that in calves up to one month of age, net magnesium absorption can also occur in the large intestine as well as the small intestine. Recent studies by Axford et al (1975), Ben-Ghedalia et al. (1975) and Tomas and Potter (1976) indicate that the reticulo-rumen and colon are the principal sites of net magnesium absorption.

The mechanism(s) for the regulation of magnesium absorption within the ruminant gut have been reviewed by Wilson (1964). He concluded that absorption can occur in several different ways; simple

diffusion, facilitated diffusion or active transport. Other authors (Care and van't Klooster, 1965; Scott and Dobson, 1965) suggest a process of facilitated magnesium re-entering the gut in digestive secretions. However, McAleese et al. (1961) found only two to five percent of an intravenous dose of ^{28}Mg in sheep feces indicating little endogenous loss. According to Sanwal (1974), Smith reported that a large loss of magnesium in the feces (usually 70-95 percent of intake) appeared to be characteristic of adult cattle and sheep, and seemingly most of this fecal loss was unabsorbed food magnesium. Wilson (1964) states that the loss of magnesium secreted in digestive secretions is about 200 mg. daily, and is equal to the total extracellular magnesium content. This level of endogenous magnesium indicates that re-absorption could be very important in maintaining magnesium homeostasis.

The regulation of extracellular magnesium is a result of a balance between absorption and excretion, and renal excretion is probably the most important single factor in the excretion process (Wilson, 1964). Urinary excretion appears to be controlled by a filtration-reabsorption mechanism in which magnesium behaves as a threshold substance, appearing in the urine only when the magnesium load filtered in the kidney by the glomeruli exceeds that reabsorbed by the tubules (Rook and Storry, 1962). Chicco et al. (1972) calculated a threshold value for sheep fed a purified diet to be 1.63 mg/100 ml plasma.

III. FACTORS AFFECTING MAGNESIUM UTILIZATION

There are many factors that have been studied which may influence magnesium utilization. Some of these factors include: interrelationship of magnesium with various other minerals, distribution of magnesium in the body, environment and numerous other factors.

Potassium

Magnesium is similar to potassium in distribution within the body, both being primarily intracellular ions (Care, 1967). According to Fontenot (1972), early reports from Dryerre and Nicholson and Shearer indicate the incidence of grass tetany appeared to be higher in animals grazing pastures which had been fertilized with potassium. Several authors have shown that in experiments with animals fed varying levels of potassium and magnesium, the higher potassium levels resulted in lower serum magnesium levels (House and Van Campen, 1971; Newton et al., 1972; Sanwal and Hansard, 1972; Suttle and Field, 1967). In addition, House and Van Campen (1971) and Newton et al. (1972) report that feeding a high potassium level increases fecal magnesium by interfering with magnesium absorption rather than increasing endogenous excretion into the intestine. Other authors report no effect on blood serum magnesium from feeding a high potassium ration to sheep (Daniel et al., 1952; Pearson et al., 1949).

Calcium and Phosphorus

Research by Nugara and Edwards (1961), O'Dell et al. (1960) and Toothill (1963) has indicated that in non-ruminants high levels of calcium and/or phosphorus have generally increased the magnesium requirement or impaired magnesium utilization. Research with ruminants concerning the influence of calcium and phosphorus on magnesium utilization is quite limited (Fontenot, 1972). Wise et al. (1963) reported a significant reduction in serum magnesium in calves when the dietary phosphorus level was increased in the presence of low dietary calcium. However, in steers no effect on magnesium utilization was shown by feeding high levels of calcium (Garces and Evans, 1971).

Because a substantial amount of the phosphorus in plants is in the phytate form, Dutton and Fontenot (1967) fed phytic acid, as compared to a mixture of monosodium and disodium phosphate, to sheep, and found no significant effect on magnesium absorption, retention or serum magnesium. The lack of effect from phytate or phytic acid is probably due to the action of rumen bacterial phytases which hydrolyze the phytate phosphorous (Reid et al., 1947).

Magnesium in Bone

Wilson (1964) reported that more than 50 percent of the body magnesium was found in bone, between .4 and .7 percent of the bone ash. Reports by Blaxter et al. (1954) in calves, Brant et al. (1958) in dogs and Forbes (1966) in rats indicated a drop of 30 to 60 percent in the level of bone magnesium of magnesium-depleted animals. These findings

suggested that the bone of young animals acted as a reserve of magnesium, and could be mobilized and translocated to other areas of the body for other processes (Blaxter and Rook, 1954; Duckworth and Godden, 1941; MacIntyre and Davidsson, 1958). In contrast to the young animal, the bone magnesium of the mature adult is apparently not readily mobilized in a magnesium-deficient state. Field (1960) calculated from the uptake of ^{28}Mg by the skeleton of a 5-year-old sheep that only about 2 percent of the total skeletal magnesium is available for release to satisfy physiological needs. Research by Rook (1961) supported this theory that skeletal reserves in the mature cow are small because there was an immediate and precipitous fall of serum magnesium in lactating cows given diets supplying only 1.5 grams of magnesium daily.

Rook and Storry (1962) concluded in their review that the release of skeletal magnesium is apparently not under direct hormonal regulation, but is probably controlled by the concentration of magnesium ions in the extracellular fluids bathing the bone mineral surface. MacIntyre *et al*. (1963) suggested the parathyroids have a controlling effect on plasma magnesium in rats. They proposed that a fall in plasma magnesium leads to stimulation of the parathyroids which in turn results in increased renal retention of magnesium and calcium, and hence restoration of the plasma magnesium level. Wilson (1964) states that he is not sure whether this effect on magnesium was primary or secondary to changes in calcium or phosphorus metabolism. Todd *et al*. (1962) found that in lactating cows, parathyroid extract had no effect on plasma magnesium, but increased the output of magnesium in the milk with a

concomitant decrease in urinary magnesium. Therefore, the parathyroid hormones probably only affect calcium, and magnesium release is a secondary effect.

In research with ^{28}Mg, McAleese *et al*. (1961) found that the uptake of the isotope in the long bone was much greater in the control lambs than in the magnesium deficient lambs. Since bone can act as a reserve for magnesium (Duckworth and Godden, 1941; Smith, 1959), the bone of an animal deprived of magnesium must compete with the soft tissues for that mineral when supplied in restricted quantities. Because of the priority for magnesium in the depleted soft tissues, and also because the magnesium released from the bone is replaced by calcium ions (McAleese and Forbes, 1961), the process of magnesium repletion in the bone is a relatively difficult and slow process due to both mechanical blocking and competition from other tissues.

Magnesium in Skeletal Muscle

According to Rook and Storry (1962), the magnesium content of the soft tissues varies widely, from .06 to .13 percent of the dry matter. This range of concentration reflects the variation in the soft tissues due to the level of water, fat and fibrous material in the tissue. If allowance is made for the extracellular magnesium in tissue, the concentration in intracellular water is about 30 meq./liter in all tissues (Wilson, 1964). As the concentration in extracellular water is about 2 meq./liter, there is a large concentration gradient across the cell wall.

McAleese et al. (1961) showed that the relative rate of exchange of ^{28}Mg varies for different tissues. The greatest exchange occurred in organs and tissues, such as the heart, liver and kidney, which are associated with high metabolic activity. Wilson (1964) concludes that in brain, skeletal muscle, testis and erythrocytes, on the other hand, exchange takes place in two stages; one stage which is rapidly exchangeable, and another stage which has a much slower turnover time. This is in agreement with Care (1967) who suggested that there are two components of magnesium in the soft tissues. One component is rapidly exchanged while the other is rather stable. The concentration of labile magnesium in the intracellular water was about the same as that in extracellular fluid. This indicated possibly soft tissue, including skeletal muscle, acts as a reserve for extracellular magnesium.

Under conditions of magnesium deprivation and from cattle with grass tetany, no measurable decline of magnesium concentration in the skeletal muscle has been demonstrated (Blaxter and Rook, 1954; Blaxter et al., 1954). However, in magnesium-deficient rats, declines of magnesium concentration in soft tissues have been reported (MacIntyre and Davidsson, 1958; McAleese and Forbes, 1961).

Magnesium in Cerebrospinal Fluid

Rook and Storry (1962) conclude that the concentration of magnesium in the cerebrospinal fluid is slightly higher than in plasma. Care (1967) states that magnesium can pass to and from the cerebrospinal fluid, but a mechanism exists whereby the concentration of magnesium in

the cerebrospinal fluid is maintained in the presence of a low plasma magnesium concentration. This is in agreement with Aikawa (1971) who concluded in his review that the central nervous system appears to be protected either by a barrier or by a regulating mechanism which keeps the physiologically active concentration of magnesium within certain limits, even during severe magnesium deprivation and possibly during tetany.

Magnesium in Plasma

Wilson (1964) reported that magnesium in the plasma exists in ultrafiltrable and nonfiltrable forms. Evidence suggests that the ultrafiltrable magnesium exists mainly as free ions, while the remaining nonfiltrable magnesium is bound to protein, mainly albumin and globulins. Of the total magnesium concentration in plasma, about 67 percent is ultrafiltrable. The majority of magnesium in this form (ultrafiltrable) is ionic, the physiologically active form of magnesium (Rook and Storry, 1962). The concentration of magnesium in plasma or serum showed considerable variation among species, but for all species, the majority of healthy animals maintained a plasma concentration in the range of 1.7 to 3.0 mg/100 ml. In cases of hypomagnesemic tetany, the magnesium level generally fell below 1.0 mg/100 ml (Blaxter et al., 1954). Since the plasma magnesium represents only part of the extracellular magnesium which comprises about 1 percent of the total magnesium in the animal body (Rook and Storry, 1962), plasma is an insignificant reservoir for magnesium. The magnesium content of the cerebrospinal

fluid, on the other hand, is much more important in that it maintains homeostasis of the central nervous system (Care, 1967). The reserves of magnesium in the muscle and other soft tissues comprise nearly 40 percent of the total body magnesium and also represents an important reservoir for magnesium and magnesium homeostasis.

Environmental Factors

Allcroft (1954) has reported that tetany often occurs on cold, wet mornings after an animal has been shifted from a winter diet onto a rapidly growing spring pasture. Forages in cool weather have a lower concentration of magnesium and a higher concentration of potassium compared to forages grown during warmer weather (Grunes et al., 1970). This condition of the forage could result in a depressed level of magnesium and subsequent hypomagnesemic tetany as previously discussed in the potassium section.

Other Factors

In addition to those factors already discussed, Aikawa (1971) in his review mentioned several factors which may interfere with magnesium availability. Apparently fertilization of pastures with nitrogen has generally resulted in lowered blood serum magnesium in ruminants grazing those pastures (Fontenot, 1972; Kemp, 1958; McLaren et al., 1975), possibly because of an increased rumen pH due to a high ammonia level.

High levels of certain Krebs cycle organic acids, citric and transaconitic acid, in conjunction with high potassium in the forage

have been mentioned as factors in the development of hypomagnesemia and grass tetany possibly through the formation of chelates with magnesium (Bohman et al., 1969; Grunes et al., 1970; House and Van Campen, 1971).

A general conclusion appears to be that hypomagnesemia rarely occurs when the magnesium content of the forage exceeds 0.20 percent of the forage dry matter (Aikawa, 1971).

IV. FUNCTION OF MAGNESIUM

Magnesium is fourth in order of cation abundance in the body, and second only to potassium within the cell (Care, 1967). It is primarily an intracellular ion and is an important co-factor in the activity of many enzyme systems, particularly those involving adenosine triphosphate (ATP) (Wilson, 1964). These systems are concerned with the utilization of energy for many processes, particularly synthesis and transport of material (phosphate groups) in and out of cells. Specifically, magnesium is necessary for protein synthesis, gluconeogensis, glycolysis and practically all systems involved in catabolism and anabolism.

Magnesium in the extracellular fluid is important in nerve conduction, transmission at myoneural functions, muscular contraction, and cellular adhesion (Aikawa, 1971). The effect of a low concentration of magnesium in the extracellular fluid is an increase in muscular irritability by a summation of the above effects (Wilson, 1964). This process of excitation or irritability involves occupation of anionic

sites on the nerve membrane normally (resting) occupied by divalent cations (Ca^{++} and Mg^{++}) with monovalent cations (Na^{+} and K^{+}). Therefore, the ratio of calcium and magnesium to potassium and sodium is crucial in normal neural functions (Aikawa, 1971).

Recent contributions suggest that the one single fundamental role or mechanism of action of magnesium in all biological processes is that of a chelating agent.

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CHAPTER III

EXPERIMENTAL PROCEDURE

I. GENERAL DESIGN OF EXPERIMENT

This experiment was conducted to study the metabolism of magnesium, calcium and potassium and to evaluate the level of these minerals in bone, skeletal muscle, cerebrospinal fluid and plasma using yearling and mature wethers in separate magnesium depletion and repletion trials. Trial I (yearling wethers) and trial II (mature wethers) were each 27 days in duration. Biopsy samples were taken from control animals fed a magnesium supplemented diet and treatment animals fed first a low magnesium diet (depletion) then switched to a magnesium supplemented diet (repletion).

II. MANAGEMENT AND PROCEDURE

Animals

From a group of recently purchased yearling lambs located at Blount farms of The University of Tennessee, 15 crossbred wethers were selected according to weight and uniformity of appearance to be used in the first trial. The wethers were placed in dual-type metabolism stalls as described by Briggs and Gallup (1949) in order that fecal and urine collections and individual feed consumption could be recorded. The animals were given an adjustment period of 10 to 14 days at which

time the sheep were dewormed with .276 mg of levamisole hydrochloride (Tramisol)[1] because of generally low hematocrit readings and high fecal egg counts among the group. During this preliminary period, the yearling wethers were fed a corn cob based ration supplemented with all nutrients needed to maintain proper bodily function. Throughout the trials, the sheep were fed once a day and watered with distilled water twice a day.

The 15 mature crossbred wethers for the second trial were selected from a flock maintained at Blount farm using the same criteria as in trial I. These mature animals were handled identically to those in the first trial.

Treatments

From each group of 15 wethers in the adjustment period, 12 were selected for each trial, respectively. Four wethers were allotted to a control group, and eight to a treatment group according to weight, percent hematocrit, and plasma magnesium level (Table I). The wethers were then allotted into four surgical groups with one control and two treatment animals in each group.

The control animals received the basal corn cob ration supplemented with magnesium (.2 percent), while the treatment group was fed the basal ration with no added magnesium (.03 percent). The composition of the rations are shown in Tables II and III. The control wethers received the magnesium supplemented diet throughout the trial while the treatment animals were maintained on the low magnesium diet for the

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[1]American Cyanamid Company, Princeton, New Jersey.

TABLE I

FACTORS CONSIDERED IN ALLOTMENT OF TREATMENT GROUPS

Trial I - Yearling Wethers		
Factor	Control	Treatment
Average plasma Mg (mg/100ml)	2.28	2.26
Average hematocrit (%)	30.80	32.60
Average body weight (kg)	38.30	35.00

Trial II - Mature Wethers		
Factor	Control	Treatment
Average plasma Mg (mg/100ml)	2.20	2.13
Average hematocrit (%)	31.00	32.00
Average body weight (kg)	84.50	81.00

TABLE II

SHEEP RATION COMPOSITION

Ingredients	Composition (%)
Corn cobs, gr	60.0
Corn gluten meal, 60%	12.0
Dextrose	15.0
Starch	4.2
Corn oil	2.0
Urea	1.0
Mineral mix*	5.6
Vitamin mix	.2
	100.0

*Mineral mix	Kilograms per 1000 kg of ration
$CaHPO_4 .2H_2O$	7.1
$CaCO_3$	1.8
NaCL	5.0
KCL	42.2
KI	.00013
$CuSO_4. H_2O$	.014
$CoSO_4. 5H_2O$	.00042
$ZnCL_2$	.1043
$MnSO_4$	.0137
$FeSO_4. 7H_2O$	.2363
Se premix	.2
	56.669
Control mix with added MgO	3.33
	59.999

TABLE III

PERCENTAGE ANALYSIS OF RATION

Nutrient	Ration	
	Control	Treatment
Magnesium	.24	.031
Calcium	.42	.42
Potassium	3.24	3.20
Crude Protein	14.84	13.94

depletion phase or until the plasma magnesium level dropped below approximately 1.0 mg/100 ml. At this time, the ration was changed to the control or magnesium supplemented ration for the repletion phase.

Data Collected

The daily feed consumption of each animal as well as individual daily urine and fecal excretion rates were measured and recorded. Approximately 10 percent of the total daily samples of feed, urine and feces was collected and pooled into 3-day samples. The samples were stored at -4° Centigrade until they were thawed for analysis of magnesium, calcium and potassium.

Individual samples of blood, bone, skeletal muscle and cerebrospinal fluid were surgically removed from each wether four times during each trial. The first and second surgeries were at the beginning and at the depletion point, respectively, while the third and fourth surgeries followed 24 hours after repletion began and at the end of each trial, respectively. Blood was taken via jugular veinipuncture using heparinized syringes. The plasma was then frozen for subsequent analysis.

The four periods of surgery each lasted four days in order to complete twelve wethers. The biopsy procedure was performed on one surgery group (3 sheep) per day for these four periods during each trial. The interval between individual surgeries was seven days throughout the trials, while balance data are reported only for three-day collection periods to include days of surgery for each wether.

Proper veterinary surgical biopsy procedure was used throughout the trials.

Surgical Biopsy Procedure and Cerebrospinal Tap

The wethers were sedated with Rompun (xylazine)[2] using 1.1 milligrams per kilogram of body weight injected intramuscularly. After the Rompun had taken effect, the sheep were placed on a surgical table in right lateral recumbency and restrained using hemp rope to secure hooves and nylon rope tied across the animal's body. Xylocaine (lidocaine)[3] was injected subcutaneously at the site of the cerebrospinal tap and at the surgery site. Strict aseptic technique was used at all times.

The cerebrospinal tap was performed using an 18 gauge-4 inch cardiac needle with stylette. With the sheep's head flexed ventrally, a needle was carefully inserted through the skin, subcutaneous structures, and nuchal ligament to the base of the skull. The needle position was carefully adjusted until the atlanto-occipital aperture was located between the occipital bone and the dorsal arch of the atlas (Habel, 1975). The needle was then gently directed through the opening until the dura mater and the arachnoid were penetrated situating the needle in the sub-arachnoid space. A definite reflex response (jerk) usually indicated that the penetration of these meninges had been

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[2]Haver-Lockhart Laboratories, Shawnee, Kansas.

[3]Astra Pharmaceutical Products, Inc., Worcester, Massachusetts.

accomplished. One or two milliliters of fluid were withdrawn and immediately frozen for future analysis. The procedure was somewhat tedious and resulted in unsuccessful fluid withdrawal on several occasions.

The bone and skeletal muscle biopsies were taken from the thorax approximately at the center of the eighth rib. A three to four inch incision was made parallel to and directly above the eighth rib. A skeletal muscle sample weighing .2 to .6 grams was removed usually from the external intercostal muscle during the process of retracting the subcutaneous tissue to expose the rib shaft. The periosteum covering the rib was split and laid back, and then a bone sample weighing .1 to .2 grams was extracted from the dorsal surface of the rib using a Stryker Autopsy Saw.[4] The opening in the rib shaft was filled with gel-foam to retard hemorrhage, and the incision was closed using chromic catgut for suturing the subcutaneous tissues and veta-fil to close the skin. The samples were weighed and frozen for subsequent analysis. All wethers received, intramuscularly, a daily prophylactic dose of Combiotic[5] consisting of 800,000 units procaine penicillin G and dihydrostreptomycin sulfate equivalent to 1.0 gram dihydrostreptomycin base for four days post operation, and the sutures were removed after one week.

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[4]Stryker Corporation, Kalamazoo, Michigan.

[5]Pfizer Agricultural Division, New York, New York.

III. CHEMICAL ANALYSIS

Plasma

Plasma samples were diluted to a known volume for stable mineral analysis using a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer. Magnesium, calcium, and potassium analyses using this instrument were run for all samples collected during the trials, and the standard procedure for the preparation of different samples was observed.

Bone

Bone samples were first dried and weighed, and then ashed at 550°C for six to eight hours. The ash was put into solution using two milliliters of six normal hydrochloric acid (6NHCL) and then diluted to a known volume.

Skeletal Muscle

The skeletal muscle samples were dried in a vacuum oven at 100°C for five to seven hours. The dried samples were weighted and then ashed and diluted in the same manner as the bone. No attempt was made to remove the adipose tissue from the samples.

Cerebrospinal Fluid

The cerebrospinal fluid was treated identical to the plasma.

Feed and Feces

Aliquot samples (.003 percent) of feces and feed were taken and ashed at 550°C for six to eight hours and put into solution with five

milliliters of 6NHCL. The samples were then diluted to a known volume for stable mineral analysis.

Urine

Urine samples were stored in the cooler ($2^{o}C$) after hydrochloric acid had been added. Five milliliters of each urine sample was ashed at $550^{o}C$ for six to eight hours, put into solution with two milliliters of 6NHCL, and then diluted to a known volume.

As mentioned above, all samples were analyzed for stable magnesium, calcium, and potassium using the prescribed method. Lanthanum Trioxide (.1 percent) was added to all magnesium and calcium dilutions to prevent phosphate interference.

IV. STATISTICAL ANALYSIS

Due to some non-homogenous variances among certain samples, a t-test procedure which also calculates an approximate t-value for means with unequal variances was used to analyze the data in this experiment. Since the t-test can only test two means at a time, comparisons of individual means between treatment and control as well as comparisons of the means among each treatment were performed.

Statistical Analysis System (SAS-76), developed by Barr et al. (1976), was used for analysis of these data.

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CHAPTER IV

RESULTS AND DISCUSSION

I. FEED CONSUMPTION

The total 3-day feed consumption for trials I and II are shown in Table IV. Chicco et al. (1973) have shown that a diet low in magnesium can result in decreased feed intake by sheep. These present results show that the control wethers consumed more ($P < .01$) feed during collection periods 3 and 4 in trial I at which time the treatment wethers were being fed a magnesium supplemented diet. This was a possible carry-over effect from the wethers consuming a magnesium deficient diet during periods 1 and 2. The standard error of the mean ($\pm$ 125g) for the treatment group during period 2 in trial I indicates the larger variation in consumption (magnesium deficient diet) among the animals in this group. The control group consumed an average of 296 grams more than the treatment group during period 2, but this difference was not significant.

However, during period 3, the control group consumed an average of 241 grams more ($P < .01$) than the treatment group. This is also an indication of the large variation in consumption of the magnesium deficient diet by the treatment wethers at period 2.

TABLE IV

TOTAL 3-DAY FEED CONSUMPTION FOR SHEEP DURING COLLECTION PERIODS (GRAMS) (± S.E.M.)

Trial I		
Period	Control	Treatment
1	2633	2186
2	2955 ± 8	2659 ± 125
3[a]	2945 ± 41	2704 ± 30
4	2861 ± 36	2652 ± 22

Trial II		
Period	Control	Treatment
1	2522	2571
2	2781 ± 216	2921 ± 54
3[a]	3194 ± 44	2984 ± 120
4	3529 ± 145	3420 ± 105

[a]Magnesium repletion.

II. BALANCE DATA

Trial I

The average 3-day magnesium balance for each collection period in trial I (yearling wethers) is presented in Table V. The balance means for the control wethers are greater than the means of the treatment wethers at period 1 ($P < .01$) and period 2 ($P < .05$). There are no differences within the control group, but within the treatment group, the means for the respective collection periods were: period 1 less than 2, 3 ($P < .01$) and 4 ($P < .05$); and period 2 less than period 4 ($P < .05$). The control wethers remained in a consistent positive balance, while the magnesium depleted-repleted wethers started in a negative balance and ended in a positive balance higher than the controls. In both groups, the feces was the main route of magnesium excretion.

The average 3-day calcium balance is shown in Appendix Table VII. Differences among means were not significant. Both treatment and control wethers remained in a positive calcium balance throughout the trial, and the feces was the main excretory path for calcium.

Potassium balance data are shown in Appendix Table VIII. The only different means occurred within the control group when the potassium balance during period 4 was greater ($P < .05$) than during period 1. The balances for potassium remained very high for both control and treatment animals throughout trial I. The urine was the main route of excretion for potassium.

Trial II

The magnesium balance data for trial II (mature wethers) is presented in Table VI. There was a more negative magnesium balance

TABLE V

TOTAL 3-DAY MAGNESIUM BALANCE DURING COLLECTION PERIODS (GRAMS) (± S.E.M.), TRIAL I

Control				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	5.95 ± .70	3.17 ± .49	1.10 ± .43	1.68 ± .25
2	6.66 ± .11	4.44 ± .33	.94 ± .33	1.28 ± .39
3	6.48 ± .24	3.53 ± .30	1.37 ± .41	1.58 ± .61
4	6.32 ± .46	3.11 ± .32	1.75 ± .61	1.46 ± .44

Treatment				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	.77 ± .06	.77 ± .07	.26 ± .04	- .26 ± .06
2	.89 ± .05	.71 ± .08	.12 ± .03	.06 ± .05
3[a]	5.14 ± .50	2.92 ± .20	.96 ± .23	1.26 ± .24
4	5.89 ± .48	2.60 ± .33	1.10 ± .26	2.19 ± .51

[a]Magnesium repletion.

TABLE VI

TOTAL 3-DAY MAGNESIUM BALANCE DURING COLLECTION PERIODS (GRAMS) (± S.E.M.)
TRIAL II

Control				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	6.54 ± 1.50	4.99 ± 1.31	2.26 ± .33	- .71 ± .16
2	6.64 ± .67	3.65 ± .81	1.89 ± .36	1.10 ± .97
3	7.24 ± .75	5.52 ± .85	2.08 ± .27	- .36 ± .45
4	8.97 ± .83	-----	2.80 ± .05	-----

Treatment				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	.69 ± .13	1.40 ± .14	.49 ± .08	-1.20 ± .23
2	.78 ± .11	1.04 ± .17	.10 ± .02	- .36 ± .11
3[a]	6.52 ± 1.10	5.89 ± .92	1.58 ± .29	- .95 ± .49
4	9.11 ± 1.00	-----	2.40 ± .29	-----

[a]Magnesium repletion.

($P < .01$) at period 1 than period 2 within the treatment group. No fourth period balances could be calculated due to a sampling error in feces collection. Both the control and treatment wethers remained in a generally more negative magnesium balance throughout this trial. As in trial I, the feces was the main route of magnesium excretion.

The calcium and potassium balance data are presented in Appendix Tables IX and X, respectively. No significant differences for these minerals were found within or between the means of the experimental groups. The calcium balance for both control and treatment wethers was in a negative range throughout the trial, and the feces was the main excretory pathway for calcium. The potassium balance was high (positive) for both treatment and control groups during each period. The majority of potassium was excreted in the urine.

Discussion of Balance Data

The range of values for the balance data (intake, urine, feces and balance) for calcium and magnesium in both trials agrees with previous data for young and mature sheep (Chicco et al., 1973; Dutton and Fontenot, 1967; Field, 1962). With both calcium and magnesium, the more positive balances of the young sheep as compared to the mature sheep were a result of the higher requirement of these minerals for growth and development in yearling wethers. This growth was indicated by consistent weight gains of the young sheep throughout the trial. Both the control and treatment animals in trial II (mature wethers) lost an average of four kilograms from the beginning to the end of the trial.

In trial I, the lower magnesium balances for the treatment wethers during periods 1 and 2 are a result of the magnesium deficient ration fed this group until 24 hours before the third surgery in period 3. After this repletion of magnesium, the magnesium balance of the treatment group increased significantly through periods 3 and 4. This decrease and increase in the magnesium balance in sheep is in agreement with Field (1962) and Chicco et al. (1973).

In trial II, the treatment and control wethers remained essentially in a negative magnesium and calcium balance throughout the trial. This negative balance is typical for mature animals due to the fact they did not require these minerals for the rapid growth and development characteristic of young animals.

The potassium balance data for trial I of this study was not in general agreement with data previously reported (Fontenot et al., 1960; Suttle and Field, 1967). The values of this trial indicate that the average daily retention of potassium ranged from 11.0 grams to 14.0 grams per animal, a level considerably above that found in other young sheep. However, Fontenot et al. (1960) mentioned that young wether lambs receiving a 5 percent potassium diet appeared to retain approximately 8 grams of potassium. Marbury (1976) found that wethers receiving a magnesium deficient diet retained more potassium than those fed a magnesium supplemented diet. The results of these two trials do not show this trend. Field (1967) observed some fairly large potassium balances, although not constant, in young wethers. The

potassium balance values for the mature sheep (trial II) are within an elevated range (3 to 10 grams daily), but are more reasonable than those for young wethers.

III. PLASMA DATA

Trial I

The plasma magnesium, calcium, and potassium levels for the yearling wethers are presented in Figures 1, 2 and 3, respectively. The control plasma magnesium mean at surgery 2 is greater ($P < .01$) than the treatment mean at that surgery. Within the control animals, the mean at the time of surgery 4 is greater ($P < .05$) than mean at surgery 1. Within the treatment group, the means for the respective surgeries were: surgery 1 greater than surgery 2 ($P < .01$) and less than surgery 4 ($P < .05$); surgery 2 less ($P < .01$) than surgeries 3 and 4; and surgery 3 less than surgery 4 ($P < .01$).

The control plasma calcium level at surgery 4 is less ($P < .05$) than the treatment plasma calcium level at that same point. The control mean of plasma calcium at surgery 1 is less ($P < .05$) than the control mean at surgery 4. Within the treatment group for plasma calcium, the mean at surgery 4 is greater than the means at surgery 1 ($P < .05$), surgery 2 ($P < .01$) and surgery 3 ($P < .05$).

Within the treatment group for plasma potassium, the mean at surgery 2 is less than the means at surgeries 3 and 4 ($P < .05$). No differences were found between the means of the two groups (control and treatment) nor within the means of control wethers.

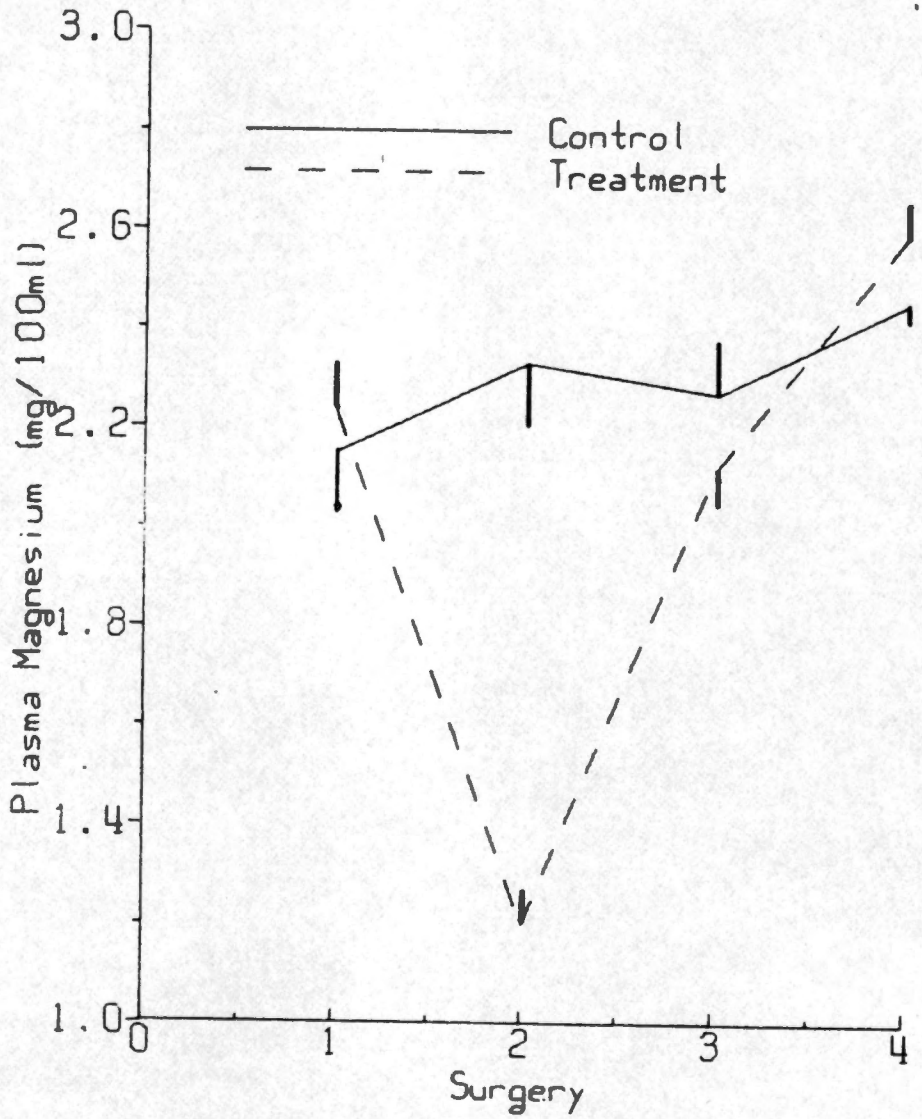


Figure 1. Average Plasma Magnesium of Control and Treatment Wethers in Trial I (± S.E.M.).

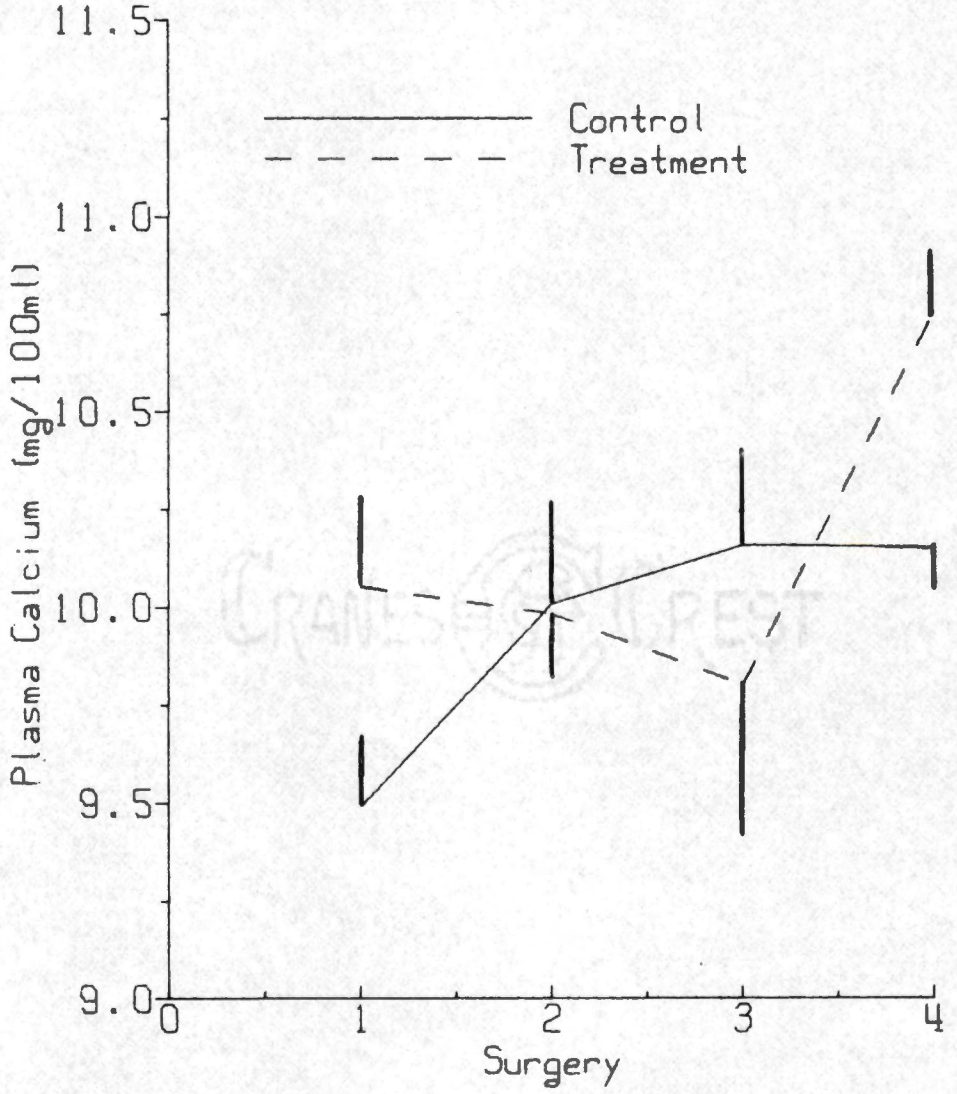


Figure 2. Average Plasma Calcium of Control and Treatment Wethers in Trial I (± S.E.M.).

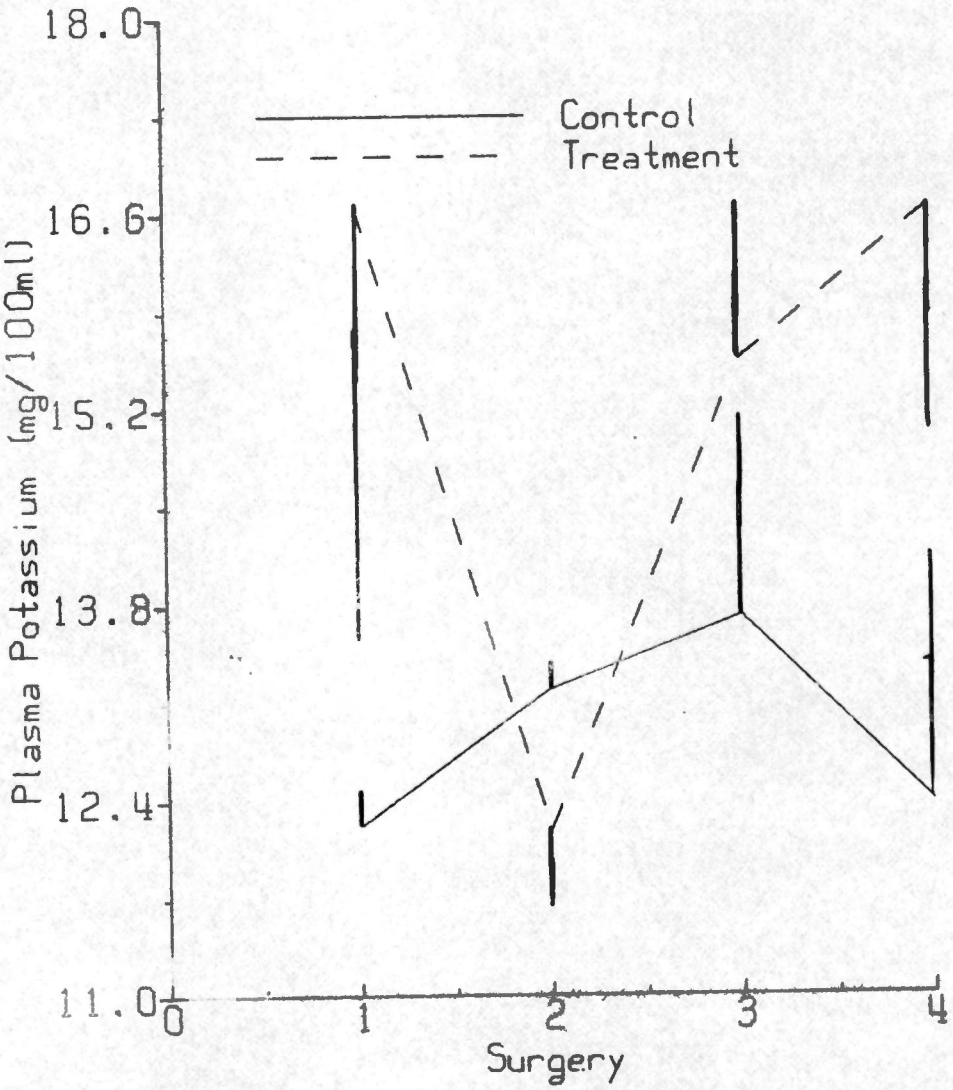


Figure 3. Average Plasma Potassium of Control and Treatment Wethers in Trial I (± S.E.M.).

Trial II

The plasma magnesium levels for trial II are presented in Figure 4. At the point of depletion (surgery 2), the control plasma magnesium level was greater ($P < .01$) than the treatment plasma magnesium level. No differences occurred among means within the control group, but within the treatment group, the plasma magnesium mean at surgery 1 is greater ($P < .01$) than the means at surgeries 2 and 3; the mean at surgery 2 is less ($P < .01$) than means at surgeries 3 and 4; and mean at surgery 3 is less ($P < .01$) than mean at surgery 4.

The plasma levels of calcium and potassium in trial II are shown in Figures 5 and 6, respectively. There were no statistically different means among or between any surgeries for these two minerals.

Discussion of Plasma Data

The plasma magnesium means at surgery 2 for the treatment groups in both trials represent the point of magnesium depletion for these groups. This depletion of plasma magnesium was essentially the result of the wethers consuming a magnesium deficient diet (Rook, 1961). No clinical or physical symptoms of hypomagnesemic tetany were observed in either trial, possibly because the average plasma magnesium value at the depletion point was approximately 1.2 mg/100 ml, and the symptoms of tetany usually do not occur unless the serum or plasma value is below 1.0 mg/100ml (Stewart, 1954; Bohman et al., 1969).

The increase of treatment plasma calcium values from surgery 1 to surgery 4 in trial I is perhaps due to the fact that in certain

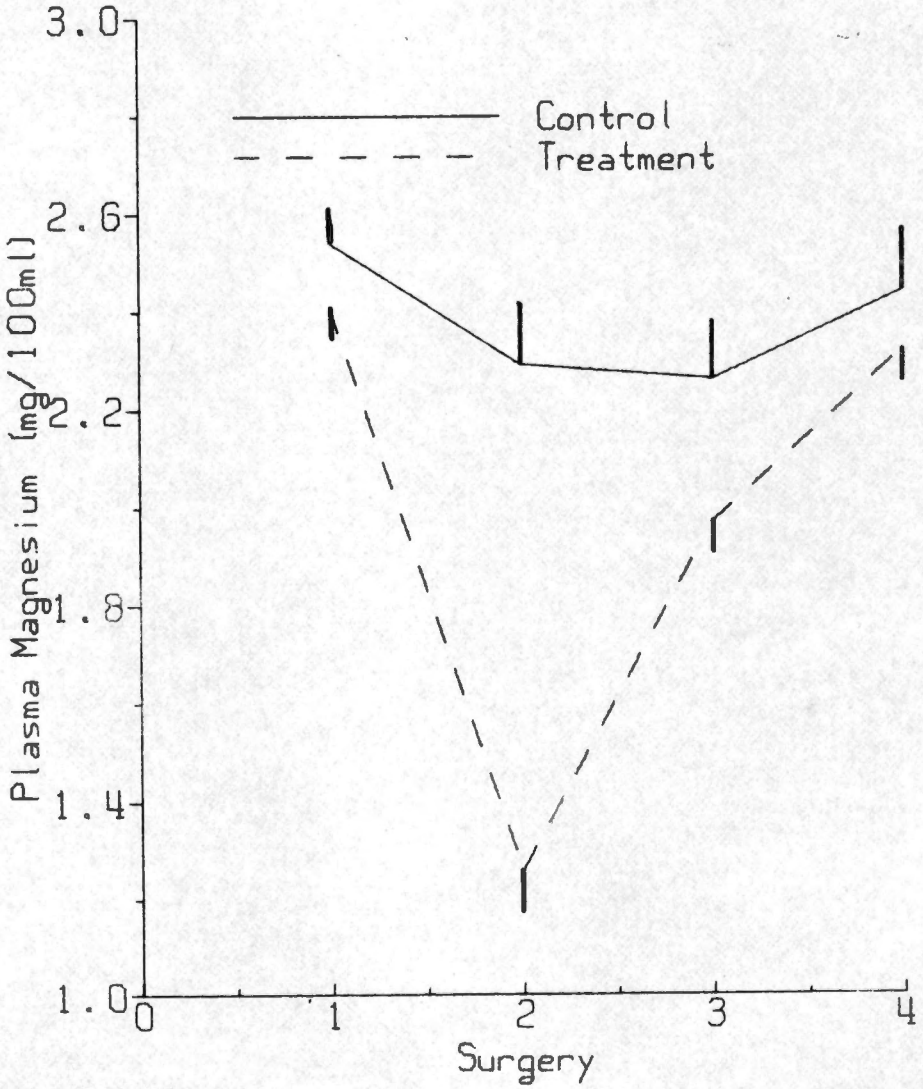


Figure 4. Average Plasma Magnesium of Control and Treatment Wethers in Trial II (± S.E.M.).

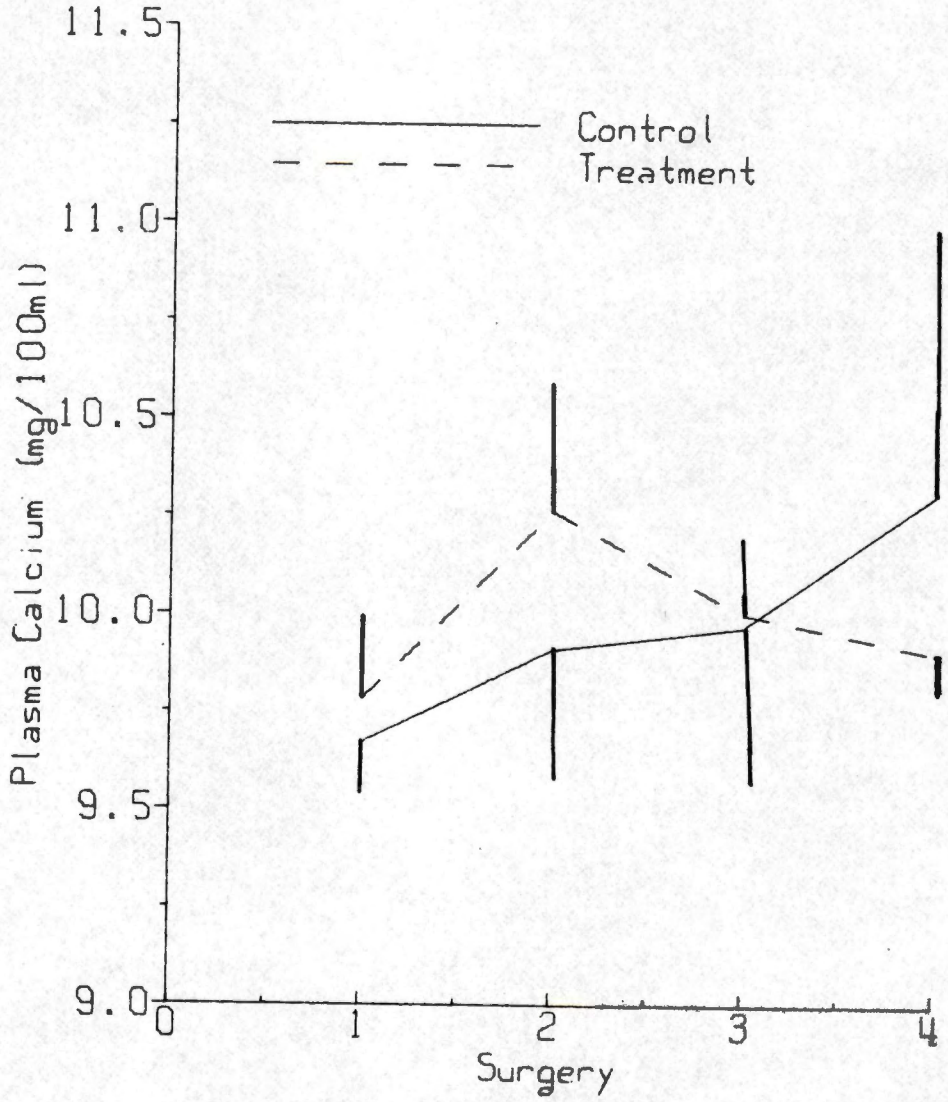


Figure 5. Average Plasma Calcium of Control and Treatment Wethers in Trial II (± S.E.M.).

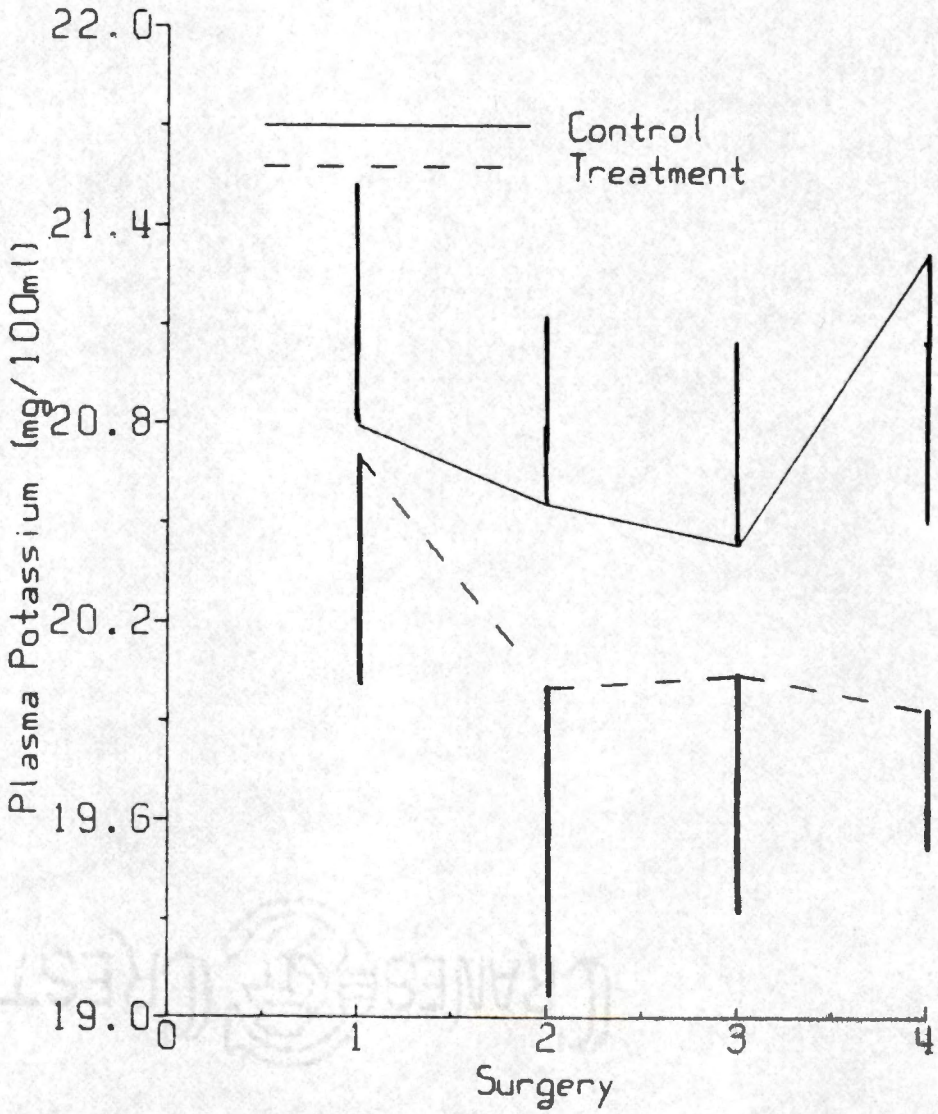


Figure 6. Average Plasma Potassium of Control and Treatment Wethers in Trial II (± S.E.M.).

species, hypocalcemia is sometimes associated with hypomagnesemia (Rook and Storry, 1962). If the plasma calcium values were subnormal during magnesium deficiency, the increase to higher plasma calcium values may be the result of magnesium repletion affecting the mobilization of calcium from the bone (Herd, 1966).

In the plasma potassium values for trial II, no statistically different means were found, but in trial I there was a considerable decrease in plasma potassium of the treatment wethers at surgery 2 followed by a similar increase when repletion occurred. No conclusion could be made concerning this drop in plasma potassium. The values for plasma potassium for the young wethers are lower than levels observed in the literature (Newton et al., 1972; Suttle and Field, 1967). However, Sanwal (1974) presented similar plasma potassium values for lambs subjected to varying temperatures. The mature wethers in trial II have plasma potassium levels which fall within an acceptable range.

IV. BONE DATA

Trial I

The average bone magnesium levels for trial I are shown in Figure 7. The average bone magnesium level for the control group at surgery 3 is greater ($P < .01$) than the treatment level at that same surgery. There were no different means within the control group, but within the treatment group the means for the respective surgeries were:

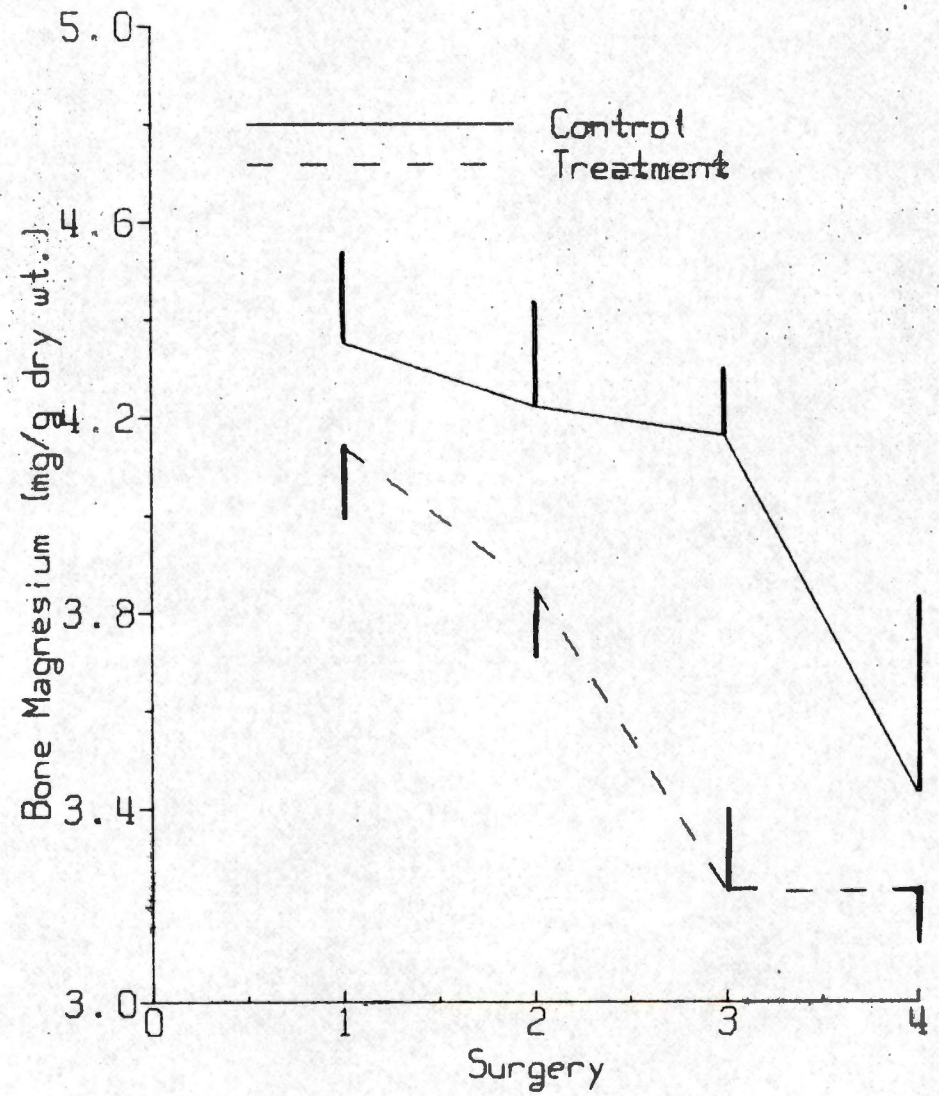


Figure 7. Average Bone Magnesium of Control and Treatment Wethers in Trial I (± S.E.M.).

surgery 1 greater ($P < .01$) than surgery 3 and surgery 4; and surgery 2 greater than surgeries 3 ($P < .05$) and 4 ($P < .01$).

The average calcium bone levels are presented in Appendix Figure 9. There were no differences between means of control and treatment animals nor within the means of the control group. Within the treatment group, the bone calcium mean at surgery 1 is greater than the mean at surgery 4 ($P < .05$); the mean at surgery 2 is greater than the mean at surgery 3 ($P < .05$); and the mean at surgery 2 is greater than mean at surgery 4 ($P < .05$).

The bone potassium data (Figure 10 in Appendix) shows the bone potassium level for the control group is greater ($P < .05$) than the level for the treatment group at surgery 1. Within the control group, the mean at surgery 1 is greater than means at surgeries 2 ($P < .05$) and 3 ($P < .01$). No differences were found within the means of the treatment group.

The percentage of ash in dry bone of the control group for surgeries 1 through 4, respectively, was 54.0 percent, 52.4 percent, 49.2 percent and 47.2 percent. For the treatment group, the bone ash percentages were 55.4 percent, 55.5 percent, 50.5 percent and 47.5 percent for surgeries 1 through 4, respectively.

Trial II

The bone levels for magnesium in trial II are presented in Figure 8. The corresponding calcium and potassium levels in bone are given in Appendix Figures 11 and 12, respectively. No differences

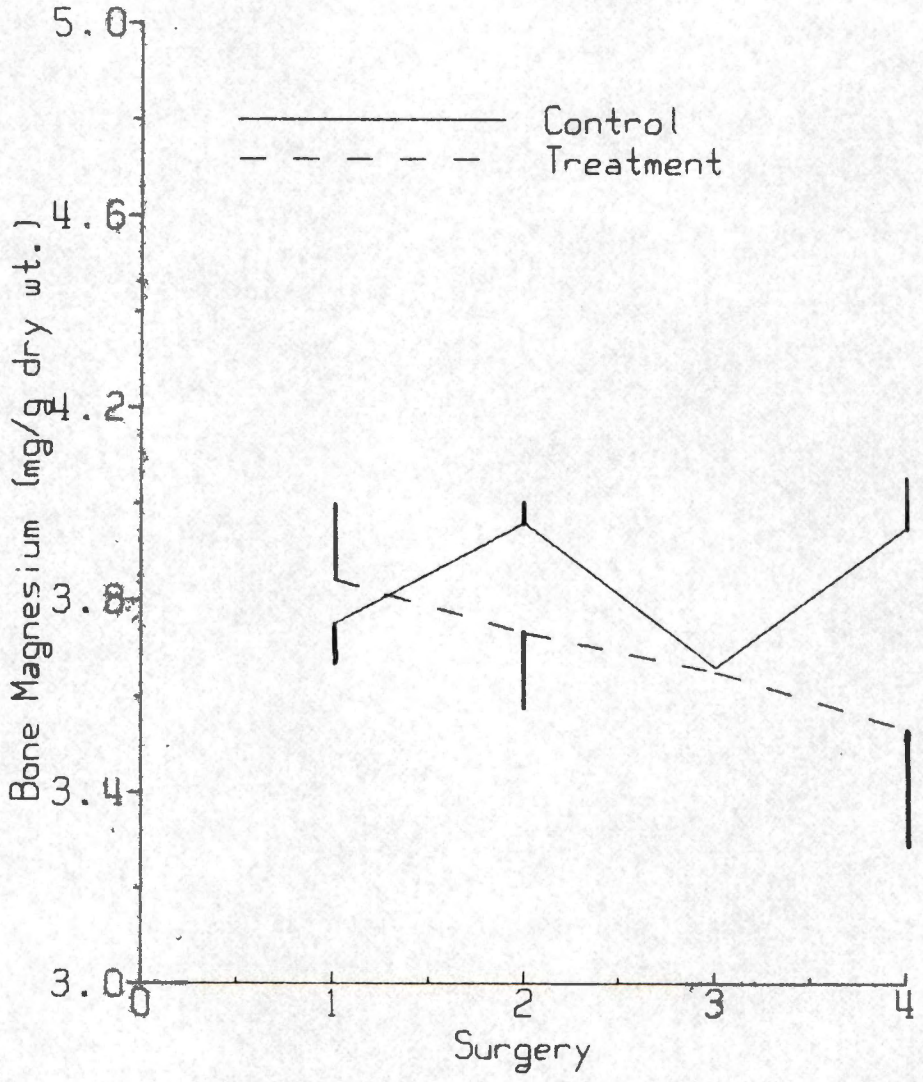


Figure 8. Average Bone Magnesium of Control and Treatment Wethers in Trial II (± S.E.M.).

were found in any comparisons for any of the minerals except at surgery 4 the control bone potassium level was greater ($P < .01$) than the treatment bone potassium level.

The percentage of ash in dry bone of the control wethers were 51.7 percent, 50.4 percent, 62.8 percent and 55.1 percent for surgeries 1 through 4, respectively. In the treatment group (surgeries 1 through 4, respectively), the bone ash percentages were 54.7 percent, 53.8 percent, 52.6 percent and 54.9 percent.

Discussion of Bone Data

In trial I, the depletion of magnesium at surgery 3 by the treatment wethers as opposed to the control group represents approximately a 22 percent decrease in magnesium in the bone sample. This is in general agreement with Blaxter et al. (1954) and Smith (1959) who obtained about a 30 percent decrease in the level of magnesium in the bones of young calves. Duckworth and Godden (1941) and MacIntyre and Davidsson (1958) reported a substantial decrease in the bone of rats consuming a low-magnesium diet. There is a definite decline of magnesium in the bone between surgeries 1 and 3 of the treatment animals. The sheep were switched to a magnesium repletion diet 24 hours before the third surgery, and received this repletion diet through the remainder of the trial. There was no immediate effect of the repletion of magnesium in the bone of these young sheep. This is in agreement with McAleese et al. (1961) who found that the control lambs had a greater uptake of ^{28}Mg in the long bone than the magnesium deficient

lambs. They reasoned that the deficient lambs have other magnesium priorities and that repletion may be a slow process in the bone of depleted animals due to competition of other tissues and possibly mechanical blocking.

The calcium content of the bone of both treatment and control wethers shows a decreasing trend. This same trend is found in the bone ash percentages which confirms the decrease in calcium. This is not in agreement with Smith (1959) who found no change in the ash or calcium values of bone of depleted calves. McAleese and Forbes (1961) found an accumulation of calcium in the bone of rats deficient in magnesium which also disagrees with the present data. Blaxter et al. (1954) found an increase in the calcium content of bones of magnesium deficient calves probably due to the replacement of magnesium ions by calcium ions. The decrease of calcium in the bones of both control and treatment sheep could not readily be explained.

No reasonable conclusions can be obtained from the potassium bone data in trial I.

The bone levels for trial II (mature wethers) are in a reasonable range. The magnesium content of bone in the treatment wethers showed a decreasing trend, but was not severe enough to be significant. Field (1960) showed that the uptake of ^{28}Mg by the skeleton of a 5-year-old sheep to be only about 2 percent of the total skeletal magnesium available for release to satisfy physiological needs. Experiments by Rook (1961) confirm that the skeletal reserves in the mature cow are small. The present data are in agreement with these workers in that

only a small amount, if any, of the skeletal magnesium is mobilized in a magnesium deficient state in the mature animal.

The calcium and potassium data indicate no significant explainable differences. Because these sheep are mature, the release of these minerals from the bone is a more difficult and slower process due to a progressively diminished blood supply to the bone (Rook and Storry, 1962).

V. SKELETAL MUSCLE DATA

Trial I

The skeletal muscle values for magnesium, calcium and potassium appear in Appendix Table XI. Within the control group, the skeletal muscle magnesium level at surgery 1 is greater ($P < .01$) than the level at surgery 4, and the level at surgery 3 is greater ($P < .05$) than the level at surgery 4. The muscle calcium values within the treatment group are greater ($P < .05$) at surgery 1 than surgery 3.

The potassium values in the skeletal muscle are statistically greater ($P < .01$) for the treatment group at surgery 1 than for the control group. There are no differences within the control group, but within the treatment group, the mean at surgery 1 is less ($P < .01$) than the means at surgeries 2 and 3.

Trial II

The mature wethers in trial II (Table XII in Appendix) showed no differences in the magnesium and potassium levels of the skeletal

muscle for either control or treatment wethers. The control muscle calcium level at surgery 4 is greater ($P < .05$) than the treatment calcium level. Within the control animals, the calcium level at surgery 3 is greater than the level at surgery 4 ($P < .05$).

Discussion of Skeletal Muscle Data

The levels of magnesium in the skeletal muscle in both trials is somewhat lower than the range described by Rook and Storry (1962) of .06 to .13 percent of the dry matter. Even though not significant, there was a trend for the treatment group in trial I which indicates a small depletion and repletion of magnesium throughout the trial.

Some workers (Blaxter and Rook, 1954; Blaxter et al., 1954) have found no measurable decline of magnesium concentration in the soft tissues of cattle under magnesium deprivation. These present results tend to support this theory. The differences found within the control group cannot be explained.

The calcium content of the skeletal muscle of these sheep (trial I) approximates the same trend as the magnesium, an insignificant depletion and repletion of muscle calcium. No calcification of the muscle had taken place as occasionally reported in previous research with soft tissue (Aikawa, 1971).

The results for potassium levels in the skeletal muscle are inconclusive. MacIntyre and Davidsson (1958) correlated the simultaneous decrease of magnesium and potassium in the skeletal muscle of rats. They concluded that the potassium deficiency in skeletal muscle seen

during magnesium deprivation is secondary to an effect of the magnesium deficiency on mitochondrial function. Rook and Storry (1962) attributed this decrease in muscle potassium to edema or a dilution effect.

The results in trial II for skeletal muscle mineral levels are in agreement with Rook and Storry (1962) and indicate no measurable or significant effect on skeletal muscle mineral levels in sheep under magnesium depletion.

VI. CEREBROSPINAL FLUID DATA

Trial I

The mineral levels of cerebrospinal fluid taken in trial I are shown in Appendix Table XIII. There are no significant changes in the magnesium or calcium levels of the cerebrospinal fluid between or within the two groups (control and treatment). The potassium levels of cerebrospinal fluid within the control group are greater ($P < .05$) at surgery 2 than at surgery 3, and less at surgery 3 than surgery 4 ($P < .05$).

Trial II

In trial II (Table XIV in Appendix), no differences were found for the cerebrospinal fluid magnesium or calcium levels within or between any groups. The potassium content of the cerebrospinal fluid within the treatment group is greater at surgery 1 than surgery 2 ($P < .01$), and less at surgery 2 than surgery 3 ($P < .05$).

Discussion of Cerebrospinal Fluid Data

The results from both trials I and II support the generally accepted theory that the mineral level in the cerebrospinal fluid remains essentially unchanged in the presence of a low plasma magnesium concentration (Care, 1967; Rook and Storry, 1962).

CHAPTER V

SUMMARY

Two separate trials using yearling and mature wethers were conducted to study the effect of magnesium depletion and repletion on the metabolism of magnesium, calcium and potassium and on the level of these minerals in bone, skeletal muscle, cerebrospinal fluid and plasma. Biopsy samples of these tissues and extracellular fluids were taken from these wethers during four different sampling periods throughout each trial.

Twelve crossbred yearling wether lambs in trial I and twelve crossbred mature wethers in trial II were divided, for each trial, respectively, into a control group of four wethers fed a .24 percent magnesium supplemented diet and a treatment group of eight wethers fed a .031 percent magnesium deficient diet (depletion phase), and then fed a magnesium supplemented diet (repletion phase). The point of magnesium depletion (ave. 1.2 mg magnesium/100 ml plasma) occurred at the second surgery, and magnesium repletion began 24 hours before the third surgery.

In trial I (yearling wethers), the bone magnesium level of the depleted wethers was significantly lower ($P < .01$) at surgery 3 than the bone level of the control wethers. Repletion of magnesium did not have an immediate effect on bone magnesium in the young wethers.

Although not significant, the bone magnesium level of the treatment wethers (trial II) showed a depletion trend throughout the trial.

The skeletal muscle magnesium levels of the treatment wethers in trial I showed a slight, but not significant, decreasing trend through surgeries 2 and 3. The mineral levels of the skeletal muscle in trial II indicated no measurable or significant effect due to magnesium depletion or repletion.

The plasma magnesium levels for the treatment wethers in both trials dropped significantly ($P < .01$) below that of the control wethers. This decrease of plasma magnesium had no effect on the mineral status of the cerebrospinal fluid in either young or mature sheep.

The young sheep maintained a more positive balance of calcium and magnesium than the mature sheep. The effect of magnesium depletion and repletion upon the magnesium balance of the treatment wethers was evident in trial I due to the low balance at collection periods 1 and 2, and the high balance at periods 3 and 4.

The effect of consuming a magnesium deficient ration resulted in a lower feed intake for the treatment wethers than for controls in trial I at period 3, and presumably a similar carry-over effect also reduced treatment consumption at period 4.

The results of this experiment show that in a magnesium deficient situation, the magnesium in the bone of yearling (growing) wethers is more labile than that of the mature wethers, and the bone

is not immediately repleted with magnesium when supplemental magnesium is fed. The plasma magnesium level drops rapidly in magnesium deprivation, but the skeletal muscle mineral levels as well as the cerebrospinal fluid mineral levels remain relatively constant in a magnesium deficient situation.

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APPENDIX

TABLE VII

TOTAL 3-DAY CALCIUM BALANCE DURING COLLECTION PERIODS (GRAMS) (± S.E.M.), TRIAL I

Control				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	10.20 ± .12	7.49 ± 1.07	1.25 ± .31	1.46 ± 1.20
2	11.40 ± .19	9.17 ± .85	1.47 ± .16	.76 ± .71
3	11.08 .41	7.40 .58	1.46 .33	2.26 .62
4	11.31 ± .65	7.01 ± .77	1.41 ± .48	2.89 ± .77

Treatment				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	9.90 ± .78	8.49 ± .84	.79 ± .12	.62 ± .81
2	11.87 ± .63	9.01 ± .90	1.13 ± .16	1.73 ± .31
3[a]	10.48 ± .65	7.25 ± .56	1.15 ± .13	2.08 ± .59
4	10.38 ± .85	6.56 ± .77	.91 ± .16	2.91 ± .76

[a]Magnesium repletion.

TABLE VIII

TOTAL 3-DAY POTASSIUM BALANCE DURING COLLECTION PERIODS (GRAMS) (± S.E.M.), TRIAL I

Control				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	87.6 ± 10.0	8.6 ± 1.9	44.0 ± 8.5	35.0 ± 2.1
2	98.1 ± 1.6	6.9 ± 1.8	54.5 ± 3.7	36.7 ± 2.8
3	95.3 ± 3.5	6.5 ± 1.5	49.2 ± 3.2	39.6 ± 2.0
4	93.1 ± 6.8	4.9 ± 1.4	72.4 ± 7.4	36.8 ± 2.0

Treatment				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	78.2 ± 6.2	8.9 ± 1.5	35.1 ± 4.1	34.2 ± 4.0
2	90.7 ± 5.0	9.0 ± 1.3	44.2 ± 2.3	37.0 ± 5.0
3[a]	90.1 ± 5.3	6.3 ± 1.0	49.6 ± 2.5	34.0 ± 3.2
4	86.8 ± 7.1	5.1 ± 1.0	47.9 ± 4.1	33.0 ± 2.4

[a]Magnesium repletion.

TABLE IX

TOTAL 3-DAY CALCIUM BALANCE DURING COLLECTION PERIODS (GRAMS) (± S.E.M.), TRIAL II

Control				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	11.4 ± 2.6	10.7 ± 2.4	2.0 ± .7	-1.3 ± .84
2	11.5 ± 1.2	10.1 ± .9	2.1 ± .4	- .6 ± .7
3	14.2 ± 1.4	12.9 ± 1.7	1.9 ± .3	- .4 ± 1.8
4	15.6 ± 1.4	-----	2.5 ± .3	-----

Treatment				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	10.1 ± 1.9	11.8 ± 1.0	1.9 ± .5	-3.6 ± 1.0
2	11.3 ± 1.7	12.2 ± 1.6	1.8 ± .3	-2.7 ± 1.3
3[a]	13.6 ± 2.1	14.1 ± 1.8	3.0 ± .6	-3.5 ± 1.0
4	15.8 ± 1.8	-----	2.1 ± .3	-----

[a]Magnesium repletion.

TABLE X

TOTAL 3-DAY POTASSIUM BALANCE DURING COLLECTION PERIODS (GRAMS) (± S.E.M.), TRIAL II

Control				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	79.8 ± 18.0	6.2 ± 1.7	60.1 ± 11.0	13.5 ± 12.0
2	81.0 ± 8.2	7.7 ± 2.2	54.0 ± 7.8	19.3 ± 2.5
3	100.0 ± 10.0	6.4 ± 1.2	61.4 ± 7.9	31.2 ± 5.8
4	109.0 ± 10.0	-----	72.5 ± 5.9	-----

Treatment				
		Excretion		
Period	Intake	Feces	Urine	Balance
1	73.2 ± 14.0	13.4 ± 2.1	49.2 ± 9.1	10.6 ± 3.9
2	81.7 ± 12.0	13.6 ± 1.9	62.6 ± 5.0	15.5 ± 3.5
3[a]	93.4 ± 15.8	8.0 ± 1.6	68.6 ± 10.0	16.8 ± 7.1
4	111.0 ± 12.4	-----	68.7 ± 10.0	-----

[a]Magnesium repletion.

TABLE XI

AVERAGE SKELETAL MUSCLE MINERAL LEVELS OF CONTROL AND TREATMENT WETHERS (mg/g dry wt.) (± S.E.M.), TRIAL I

Surgery		Magnesium	Calcium	Potassium
1	control	.39 ± .03	.29 ± .06	13.1 ± 1.70
	treatment	.37 ± .06	.48 ± .07	7.6 ± .89
2	control	.39 ± .05	.41 ± .07	14.5 ± .94
	treatment	.34 ± .04	.36 ± .05	13.3 ± 1.23
3[a]	control	.35 ± .03	.47 ± .13	14.0 ± .91
	treatment	.24 ± .05	.27 ± .05	10.7 ± 1.62
4	control	.25 ± .02	.20 ± .01	12.9 ± .84
	treatment	.32 ± .05	.31 ± .05	14.4 ± 1.10

[a]Magnesium repletion.

TABLE XII

AVERAGE SKELETAL MUSCLE MINERAL LEVELS OF CONTROL AND TREATMENT WETHERS (mg/g dry wt.) (± S.E.M.), TRIAL II

Surgery		Magnesium	Calcium	Potassium
1	control	.30 ± .07	1.01 ± .52	2.34 ± 1.01
	treatment	.33 ± .06	.70 ± .32	2.00 ± .58
2	control	.50 ± .06	.31 ± .07	2.94 ± .65
	treatment	.36 ± .09	.39 ± .07	3.86 ± 1.03
3[a]	control	.44 ± .06	.42 ± .04	2.51 ± .38
	treatment	.37 ± .07	.59 ± .23	2.86 ± .63
4	control	.31 ± .07	.24 ± .04	3.62 ± 1.50
	treatment	.36 ± .06	1.11 ± .36	2.27 ± .34

[a]Magnesium repletion.

TABLE XIII

AVERAGE MINERAL LEVELS IN CEREBROSPINAL FLUID OF CONTROL AND TREATMENT WETHERS (mg/100ml) (± S.E.M.), TRIAL I

Surgery		Magnesium	Calcium	Potassium
1	control	2.03 ± .15	4.69 ± .07	18.70 ± 1.80
	treatment	2.17 ± .09	4.60 ± .24	20.21 ± .75
2	control	2.31 ± .04	4.92 ± .12	21.23 ± .87
	treatment	2.22 ± .07	5.10 ± .07	20.31 ± 1.10
3[a]	control	2.23 ± .10	5.13 ± .50	16.94 ± 1.42
	treatment	2.38 ± .09	5.51 ± .39	19.63 ± 1.40
4	control	2.19 ± .06	4.79 ± .17	21.86 ± .49
	treatment	2.22 ± .04	5.16 ± .09	20.02 ± .79

[a]Magnesium repletion.

TABLE XIV

AVERAGE MINERAL LEVELS IN CEREBROSPINAL FLUID OF CONTROL AND TREATMENT WETHERS (mg/100ml) (± S.E.M.), TRIAL II

Surgery		Magnesium	Calcium	Potassium
1	control	3.18 ± .44	4.92 ± .11	16.77 ± .06
	treatment	2.91 ± .21	5.35 ± .31	15.91 ± .43
2	control	3.68 ± .33	5.21 ± .19	17.34 ± .44
	treatment	2.92 ± .24	4.80 ± .30	17.10 ± .28
3[a]	control	3.46 ± .18	5.16 ± .68	16.97 ± .23
	treatment	3.29 ± .51	4.84 ± .57	17.85 ± 1.00
4	control	3.42 ± .42	5.69 ± .61	16.04 ± .63
	treatment	2.92 ± .21	5.01 ± .52	15.58 ± .49

[a]Magnesium repletion.

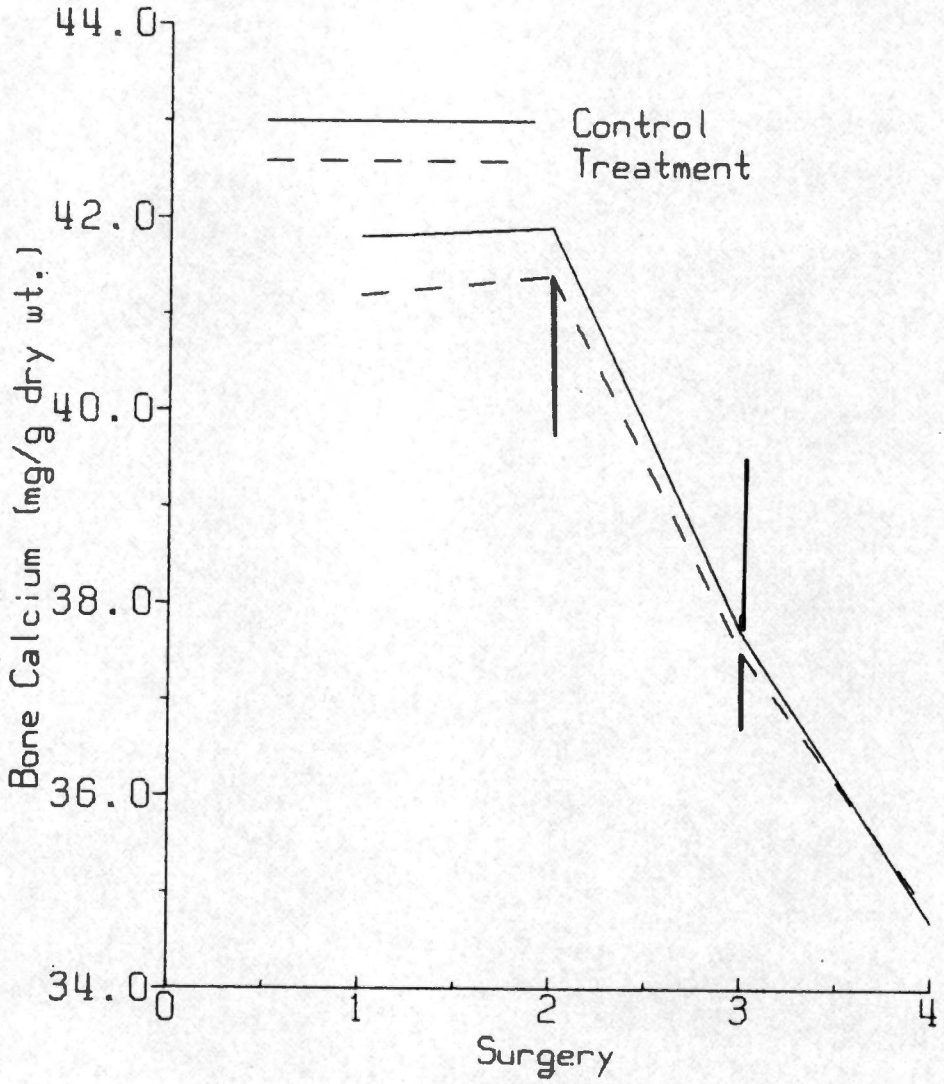


Figure 9. Average Bone Calcium of Control and Treatment Wethers in Trial I (± S.E.M.).

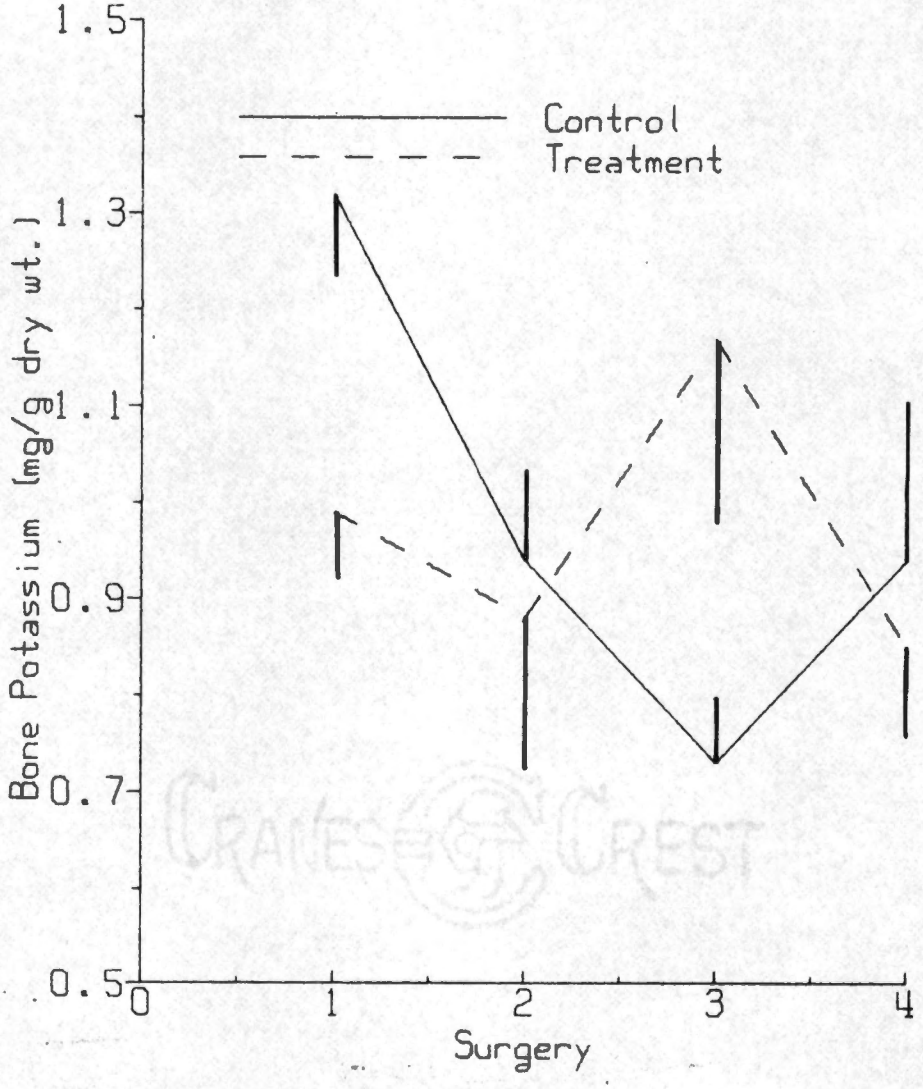


Figure 10. Average Bone Potassium of Control and Treatment Wethers in Trial I (± S.E.M.).

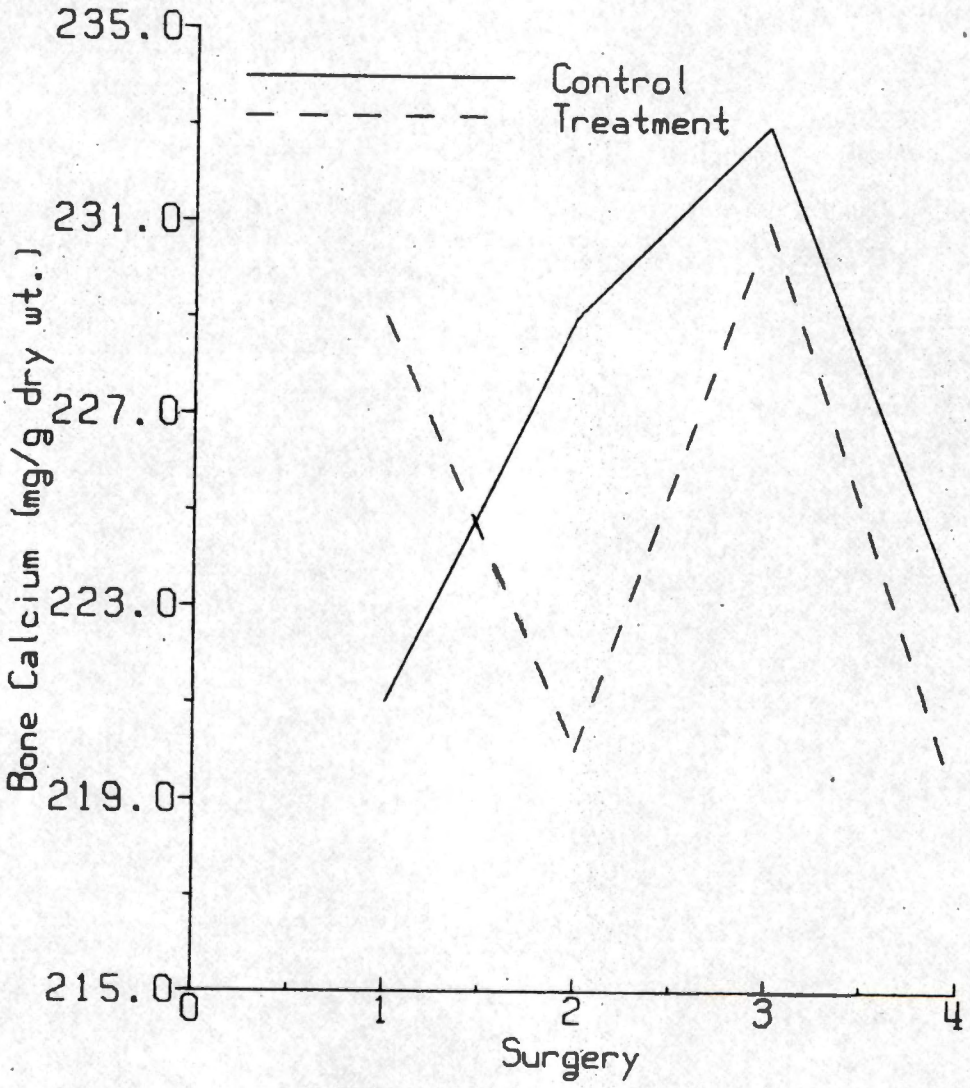


Figure 11. Average Bone Calcium of Control and Treatment Wethers in Trial II.

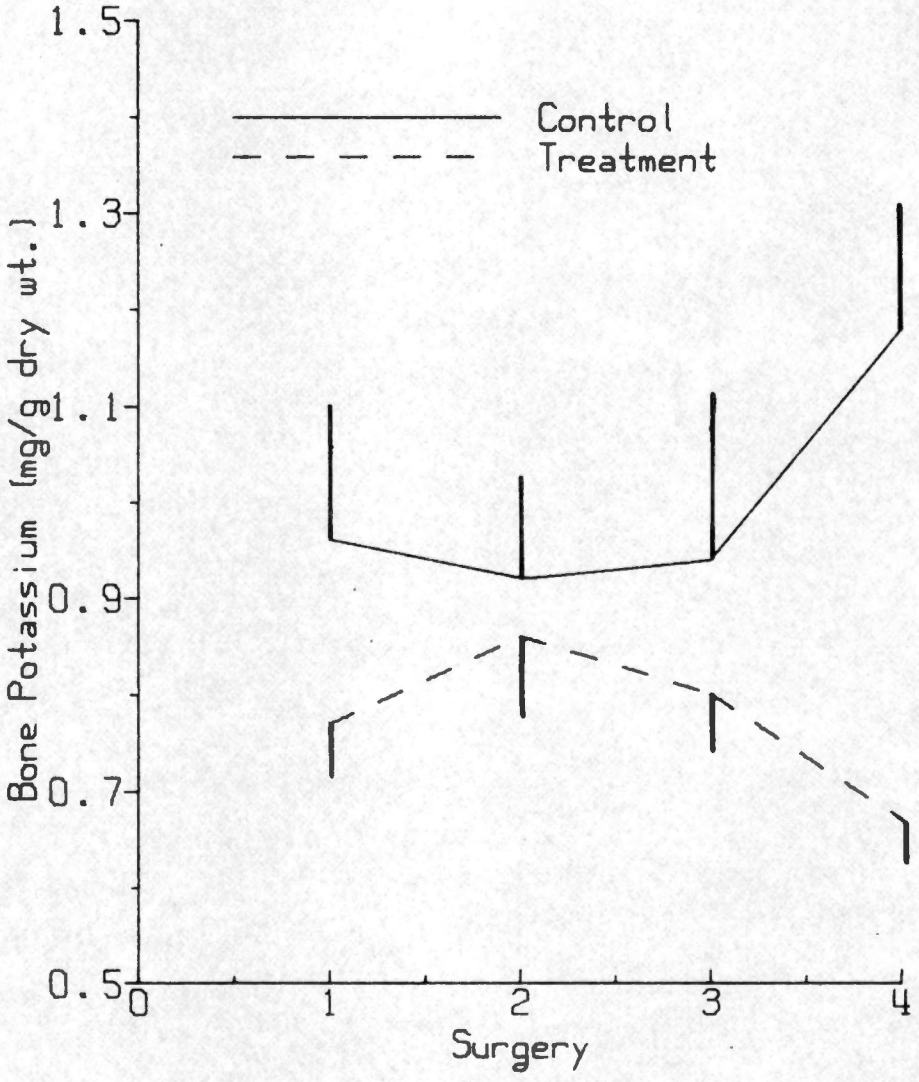


Figure 12. Average Bone Potassium of Control and Treatment Wethers in Trial II (± S.E.M.).

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VITA

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