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Magnesium interrelationships in monogastric and ruminant animals

T. Seenappa

To the Graduate Council:

I am submitting herewith a dissertation written by T. Seenappa entitled "Magnesium interrelationships in monogastric and ruminant animals." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Sam L. Hansard, Major Professor

We have read this dissertation and recommend its acceptance:

J.K. Bletner, C.C. Chamberlain, C.L. Cleland, G.M. Merriman

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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

July 21, 1971

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Lorin L Harvard
Major Professor

We have read this dissertation
and recommend its acceptance:

Charles L. Cleland

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MAGNESIUM INTERRELATIONSHIPS IN MONOGASTRIC AND RUMINANT ANIMALS

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

T. Seenappa

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ABSTRACT

Seventy-two albino rats, 5 sheep and 51 bovine blood samples were utilized in this study to investigate the relationship of dietary magnesium, potassium and nitrogen on hypomagnesemia. The rats were allotted to 12 synthetic diets composed of 9, 18 and 36% casein; 0.0, 0.6 and 1.3 g/kg ration of magnesium; and 0.0, 4.0 and 8.0 g/kg potassium ration reduced magnesium levels in plasma and tissues. An interaction between dietary potassium and magnesium appeared to be present. Four wethers were fed magnesium chloride, oxide, sulfate and nitrate to study the possibility of an interaction of these compounds with body minerals. Administration of each salt increased the plasma magnesium level and fecal and urinary excretion, but resulted in a negative balance of potassium and calcium. Dosing with citric acid and trans-aconitic acid had no apparent effect upon hypomagnesemia. Intravenously administered ^{28}Mg disappeared rapidly from the blood of sheep, and after two hours was followed by a slower expotential disappearance to 20 hours. Bovine blood samples were submitted by practicing Tennessee Veterinarians from cows which died of field cases of tetany. Sample analyses were compared with that from young and old cattle from the University of Tennessee Experimental Station herds during "grass tetany" season. Analyses indicated a lower than "normal" magnesium and calcium levels for Tennessee cattle.

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CHAPTER I

INTRODUCTION

Hypomagnesemic tetany, commonly known as grass tetany in cattle, has been a serious problem throughout most of the world, especially in the Western European countries. The disease has been reported to be a serious problem in certain sections of the United States; Texas, West Virginia, South Dakota and in California. Hjerpe (1964) reported that an estimated 4,000 to 6,000 head of beef cattle were lost in California from grass tetany during the winter of 1964-1965. The mortality rate in some herds reached as high as 20%.

The presence of grass tetany has been reported by several veterinarians in Tennessee. At present no statistics are available on the losses from the disease in the state. Although this malady is not a regular feature, sporadic cases are being continuously reported. Complaints from various counties of the State of Tennessee indicate that hypomagnesemic tetany is a problem to both dairy and beef farmers, and suggest that a closer look at this disease is necessary (Hansard, 1971).

The principal cause or causes of hypomagnesemia must be known in order that a sound method for its prevention might be found. Although extensive literature has been assembled, no complete description is available yet of the etiology. This study is an attempt to reconcile scientific data on the disease through the status of selected mineral interrelationships involved. The use of laboratory animals for obtaining

basic information while keeping bonafide species differences always in mind is desirable, considering the practical difficulties posed by experimenting with large animals. With these considerations, part of the work reported in this study was conducted with the white rat. The rats were fed twelve different diets so that the interrelationships of calcium, magnesium, potassium and protein nitrogen might be studied.

A cattle survey was made during the winter and spring quarter of 1971. Blood serum samples submitted by various veterinarians from different parts of the State of Tennessee were studied to determine the blood levels of calcium, magnesium and potassium, and also to provide diagnostic information for the veterinarians of suspected tetany cases. Normal values were also determined from samples collected and sent to the laboratory from cattle located at different University of Tennessee experimental stations throughout the State.

To study the availability of magnesium to the animal from different inorganic salts of magnesium, sheep which were maintained on a magnesium deficient diet were fed several inorganic salts of magnesium. By studying the concentration of magnesium in plasma, urine and feces, availability to the animal was measured.

CHAPTER II

LITERATURE REVIEW

Leroy (1926) first reported on the necessity of magnesium for normal animal growth. Since then many investigations have been conducted to study its physiological and nutritional functions. Many aspects associated with the role of the element are still not completely understood, particularly the interactions of magnesium with other ions that are used to induce deficiencies of magnesium in animals. At present it is generally accepted that magnesium is one of the major cation constituents of both animals and plants and that it is essential for life in all species (Gilbert, 1948).

The term "grass tetany" has long been familiar to animal scientists and livestock producers. It has been called "grass paralysis" (Morris and O'Dell, 1961). First reports dealing with this disease came from the Netherlands about 100 years ago. However, detailed reports on the disorder have come from many countries, especially from England and western European countries, following Sjollema's publication (Sjollema, 1932). In the past, the disease has also been referred to as lactation tetany, grass staggers, spring tetany, wheat pasture poisoning and atypical milk fever. It can occur in both lactating cows and pregnant heifers, generally on grass, but occasionally in stall-fed females. Grass tetany also occurs in sheep and occasionally in horses (Rook and Storry, 1962). Stewart (1954), in Scotland, suggested that the disorder be called "hypomagnesemic tetany: since hypomagnesemia was common to all cases.

Distribution of Magnesium in the Body

The distribution of magnesium in the body may be discussed in three general areas: (1) bone, (2) soft tissue and (3) extracellular fluid. Hypomagnesemia deals with blood serum or plasma. Shohl (1939) stated that the animal body contained about 0.05% by weight of magnesium, nearly 60% in the skeleton, 40% in the cells and the soft tissue, and 1.0% in the extracellular fluids. Rook and Storry (1962) reported values for the concentration of magnesium in plasma or serum for normal cattle, swine, sheep and goats to range from about 1.2 to 3.8 mg/100 ml. O'Dell (1960) has given similar ranges of values for laboratory animals, except guinea pigs which tend to have somewhat higher values. Wilson (1964) reported that magnesium in plasma exists in two forms, ultrafiltrable and non-filtrable. Of the total magnesium concentration in plasma, about 67% was located in the ultrafiltrable fraction, and the remaining was protein bound.

Magnesium in Bone

Wilson (1964) reported that more than 50% of the body magnesium was found in bone and between 0.4 and 0.7% in bone ash. It has been reported by Forbes (1966) in rats, Blaxter *et al.* (1954) in calves and Brant *et al.* (1958) in dogs, that the bone magnesium in the magnesium depleted animals dropped about 30 to 60%. These findings suggested bone to be the reservoir of magnesium, and that this fraction was highly labile. In cases of normal or excess magnesium in the diet, bone played little or no role in the regulation of plasma magnesium. But in cases of deficiency,

bone became the supply depot for plasma. However, the mobility of bone magnesium depended on various factors such as age of animal, stage of growth, renal function, acid base balance and the state of magnesium storage in the body. On the other hand, Field (1960) found the exchange of magnesium between plasma and bone to be very slow and suggested that bone was not a sufficient reservoir for maintaining plasma magnesium.

Magnesium in Soft Tissues

The concentration of magnesium in soft tissues varies according to the level of water, fat or fibrous material in the tissue. However, the concentration of the intracellular fluid was about 36 mg/100 ml in all tissues of sheep (Wilson, 1964). Since the extracellular fluid concentration was approximately 2.5 mg/100 ml it was easily understood that there was a great concentration gradient across the cell wall.

McIntyre and Davisdon (1958) reported the magnesium concentrations of liver, kidney and skeletal muscle of the rat as 81.7, 82.7 and 102.2 mg/100 ml, respectively, of fat-free dry matter.

Morris and O'Dell (1961) reported that the magnesium concentrations of fat-free dry matter of kidney, heart and skeletal muscle of guinea pigs to be 91.9, 105.0 and 121.0 mg/100 ml, respectively.

Experiments with radioactive magnesium indicated that the rate of exchange of magnesium between tissues and plasma of dogs was highest in heart, kidney and liver, and was slower in ovaries, thyroid, skeletal muscle and adrenal glands, in that order (Brant et al., 1958).

After intraventravenous injection of radio-magnesium, the concentration of ^{28}Mg in heart muscle was found to be much higher than in

skeletal muscle. To study the effect of contraction on the magnesium content of muscle, skeletal muscle was stimulated to contract at the same rate as the heart muscle. There was no difference in the ^{28}Mg concentration between stimulated and resting skeletal muscles.

Experiments of Care (1960) with ^{28}Mg 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 may be considered an indication that magnesium in soft tissue acts as a useful reserve for the extracellular magnesium.

Magnesium in Extracellular Fluid

Plasma or serum represents the extracellular fluid of the body for the study of magnesium. The concentration of magnesium in serum or plasma showed considerable variation, not only among species, but also among individuals within a species. However, the normal levels of magnesium in serum or plasma for all species was 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). Wilson (1964) reported that magnesium in plasma was found both as free ions and bound to plasma proteins. The ionic magnesium was the physiologically active form in the plasma and was ultra-filtrable. This form constituted about 70% of the total plasma magnesium. Wilson (1964) further reported that ionic magnesium in plasma ranged from 60 to 80% with an average value of 67%.

Magnesium Absorption in the Body

Reports have indicated that magnesium is absorbed primarily from the small intestine (Wilson, 1964) and secondarily from the fore-stomach and large intestine (Stewart, 1954). The source of magnesium absorbed by the intestine may be or two origins: (1) alimentary or exogenous and (2) endogenous. The latter is found in the digestive secretory organs. This may mean that any interference with the absorption of alimentary magnesium results in a loss of magnesium from the body fluids. Due to different methods, feeds and species of animals, there have been contradictory reports in the estimation of magnesium absorption.

Garner (1960) reported that 65 to 86% of the magnesium of fresh grass was retained by the guinea pig and retention was not dependent upon the magnesium content or the calcium to phosphorus ratio, or the crude fiber content of the feed.

Experimenting with sheep, Care (1960) obtained values for the availability of magnesium of hay as 23.3%. On the other hand, Kemp and T'Hart (1961) reported that for cows the availabilities of magnesium in pasture grasses cows were 10, 16 and 20% at the three stages: namely, early growth, prebloom and after bloom on the same grass.

Simeson et al. (1962) obtained magnesium availability data of 44.5 and 58.6% from a diet of milk in two experiments with calves, and digestibility values of 16.9 and 37.3% from a diet of hay and grain in experiments with cows.

Care and Ross (1962) reported that when magnesium was introduced into the rumen of sheep in ionic form, uptake was 10% with a grass and 16.6% with a hay diet.

Van't Klooster (1965) estimated minerals in the gut content of sheep by suspension of cellophane bags into the small and large intestine. A shift of ration from hay to grass resulted in a decrease in soluble calcium and magnesium concentrations in large intestine from 24.0 to 7.4% for calcium and 43 and 24% for magnesium.

Ferrando et al. (1965) reported that two sheep absorbed approximately 26% of the 2.0 g of magnesium contained in a daily feed supply of alfalfa over a period of eight days. From the ninth to seventeenth day, in addition to the regular supply of alfalfa, 2.0 g of magnesium as magnesium sulfate was administered, of which 39.6 to 70.0% was assimilated.

Absorption of Magnesium from Different Salts

Huffman et al. (1941) stated that magnesium as the carbonate, chloride or phosphate was better absorbed by cows or calves than sulfate, citrate, silicate or metallic magnesium, and that magnesium in natural feeds was utilized more efficiently than that in magnesium salts.

Steward and Moodie (1956) reported that the oral administration of magnesium as nitrate increased serum values in mature wethers from 2.5 to 9.7 and 13.0 mg/100 ml at 2.5 and 4.0 hours after dosing, respectively. When administered as the sulfate, the increase was less; it changed from 2.5 to 4.0 and 6.6 mg/100 ml serum magnesium for the same time intervals.

Thomas (1959) used three different magnesium salts (carbonate, sulfate and acetate) to supplement milk and feed for calves. He observed no difference in serum magnesium values. The availability of magnesium when fed as the sulfate, carbonate or acetate was approximately the same. If the amount of magnesium excreted in the urine was taken as an index of absorption, a number of soluble magnesium salts, citrate (Bogert and McKittrick, 1922), lactate (Carswell and Winter, 1931), chloride (Taylor and Winter, 1929) and sulfate (Hart and Steenback, 1913) would seem to be readily absorbed.

Meyer and Grund (1963) investigated the excretion of urine in two bull calves after supplemental feeding of magnesium carbonate, sulfate, oxide, chloride and magnesium acetate to study a comparison of magnesium absorption from these compounds. The most favorable absorption rate was found for magnesium oxide and magnesium chloride, which showed an increase in magnesium excretion of 15 and 11% and 14 and 12% for the two bulls, respectively. Magnesium as magnesium carbonate was not absorbed.

Brochart and Larvor (1961) reported marked individual differences between animals, and in the same animal from time to time, in the values obtained for the availability of magnesium in any given diet.

Field (1962) reported that an abrupt change of diet can cause a transient marked change of magnesium excretion in the urine, which has tentatively been attributed to a temporary change of absorption.

Storry and Rook (1963) used urinary excretion of magnesium in cows as a measure of absorption. The authors estimated the availability

of the various salts of magnesium and found that oxide, nitrate, acetate and lactate gave similar availability values, in that order, while sulfate and silicate were lower.

Gerken and Fontenot (1967) conducted metabolism trials to study the utilization of supplemental magnesium in steers when supplied as dolomitic limestone and magnesium oxide. The basal ration supplied 4.85 g of magnesium per day. Total daily magnesium intakes were 14.4 and 13.5 g for the ration supplemented with dolomitic limestone and magnesium oxide, respectively. Magnesium absorption, expressed as percentage of dietary magnesium, was 53, 27 and 52% for the basal, dolomitic limestone, and magnesium oxide rations, respectivley. The availability of supplemental magnesium, calculated by difference, was much higher for magnesium oxide than for dolomitic limestone. There was a significant difference in the change of blood serum magnesium value between steers fed dolomitic limestone and those fed for magnesium oxide. Values were higher for the magnesium oxide supplemented steers.

Ammerman and Chicco (1968) fed a basal diet to lambs containing 250 ppm magnesium, 0.2% phosphorus and 0.25% calcium for a seven day preliminary period. The lambs diets were then supplemented with four sources of magnesium, including magnesium carbonate, magnesium oxide, magnesium sulfate, and magnesite in the basal ration for five days. The supplemented diet contained 800 ppm magnesium. Total urinary and fecal collections were made for seven days. The apparent absorption of dietary magnesium carbonate, magnesium oxide, magnesium sulfate and magnesite was 56.4, 52.0, 52.0, and 9.4%, respectively. The apparent absorption values,

calculated to include intake and excreta data obtained during the basal period, were 73.3, 72.9, 77.6, and 14.2%, respectively, when presented in the same order as shown above. Net retention of magnesium for the various diets followed the same trend as that shown for the absorption data.

Factors Affecting Magnesium Absorption

Thatcher and Radike (1947) reported potassium administered by stomach tube, such as potassium chloride, potassium citrate, potassium acetate and potassium bicarbonate, to be toxic to rats. They found that toxicity of the chloride, acetate and citrate was not essentially different, while there was a lower tolerance for potassium bicarbonate.

Kunkel et al. (1953) reported that 5.0% of potassium, such as bicarbonate in cottonseed hulls, ground milo and soybean oil meal diet, resulted in a significant lowering of the magnesium content of the serum in ewes. This hypomagnesemia was without clinical symptoms. Similar results were reported by Eaton and Avampato (1952).

Head and Rook (1955) reported a correlation between high ruminal ammonia concentrations during the first few days of lush grass feeding and a decrease in serum magnesium concentration. There was also a decrease in the urinary excretion of magnesium, which was suggested to reflect a reduction in its intestinal absorption. Subsequently, the addition of ammonium acetate or ammonium carbonate to the rumen of cows fed on a diet of hay and concentrates, was shown to produce ruminal ammonia levels similar to those observed on a grass diet. A decrease

in urinary magnesium excretion occurred and a moderate reduction in serum magnesium concentration was observed. They suggested the hypomagnesemia to be due largely to a reduced availability of the magnesium in the lush grass.

Kemp et al. (1966) found that the addition of animal fat to winter rations of milking cows increased the percentage of magnesium in the feces, thus decreasing its apparent availability to the animal. Nitrogen fertilization of grassland was found to have an unfavorable influence on the supply of magnesium to the cattle due to formation of higher fatty acids in the pasture.

McWard (1969) observed that addition of 4.0% phytic acid to an isolated soybean protein-glucose diet increased the chick requirement for magnesium. Addition of 500 ppm of ethylenediaminetetraacetic acid (EDTA) with or without 4.0% phytic acid to the diet had no effect on the availability of magnesium to the chick.

Newton et al. (1969) conducted an experiment with wethers to determine the effects of feeding high levels of potassium on the metabolism of calcium, magnesium and potassium. Wethers were fed a basal ration containing 0.5% potassium and 0.1% magnesium to which was added 5.0% potassium bicarbonate for eight days. Feeding of high potassium levels resulted in a significant depression in magnesium absorption, with decreases varying from 34 to 61%. Blood calcium was not altered by high feeding of potassium. Blood potassium and sodium absorption were higher.

Stout et al. (1967) reported that more than 1.0% trans-aconitic acid was present on a dry-weight basis in early season forage grasses

during seasonal outbreaks of grass tetany. Of the two aconitate steroisomers, the level of cis-aconitate was comparatively lower than the trans-aconitate in all grass species.

Bohman et al. (1969) investigated the effect of trans-aconitic acid and related compounds on the magnesium content of bovine plasma. Citric acid plus potassium chloride lowered plasma magnesium in six days. The organic acids mentioned above had no effect on the magnesium content of bovine plasma. The potassium chloride gradually decreased plasma magnesium and feed consumption.

Scotto et al. (1971) reported that oral administration of potassium chloride increased the plasma concentration of calcium, magnesium and sodium during early intervals following post-administration. Cattle receiving high levels of potassium chloride had higher levels of plasma magnesium at one hour, and lower levels of plasma magnesium after eight and 24 hours.

The Role of Magnesium in Biochemical Processes

It was previously reported that magnesium activates many important enzymes in the body and is involved in protein synthesis. All enzymes which are required for transferring phosphate group(s) from ATP are activated by magnesium (Wacker and Valee, 1964). This property of magnesium makes the element necessary for the normal processes of glycolysis and in both anaerobic and aerobic metabolism. Magnesium is also required for the synthesis of DNA and RNA in the body (MacIntyre, 1963).

Factors Affecting Excretion of Magnesium

Kemp and T'Hart (1961) were of the opinion that excretion of magnesium from the body was through urine, feces and milk. Fecal magnesium constituted about 83% of the dietary magnesium and was mainly unabsorbed magnesium.

Stevenson and Wilson (1963) stated that endogenous magnesium was also present in the feces, and increased with the increase in the endogenous secretion into the intestines or with the decrease in the adsorption of magnesium.

It has been generally accepted that the kidney regulated the magnesium content of the body Kemp and T'Hart, 1961; Stevenson and Wilson, 1963). Storry and Rook (1963) showed that the withdrawal of dietary magnesium supplement from the ration resulted in a rapid fall in urinary magnesium in cows, from a value of 1.0 to 2.0 g per day to zero within four days. The decrease in serum magnesium was from 2.7 mg/100 ml down to 1.0 to 1.5 mg/100 ml and coincided with the urinary fall of magnesium but at a slower rate. Supplementation of magnesium in the diet resulted in a rapid increase in the urinary magnesium excretion.

Wilson (1964) suggested the excretion of magnesium to be principally a filtration-resorption mechanism, and that interactions with other ions might influence this mechanism. Such interactions have been reported between magnesium and calcium and magnesium and potassium (Peterson, 1963; Womersley, 1958).

Interrelationship

The idea of physiological antagonism in nutrition between minerals originated with Oscar Loew in 1891, who evolved this theory through experiments in plant nutrition, and later extended it to include the nutrition of animals. The basis for his belief, as applied to animal nutrition, lay not only in his own work, but in the experiments of others.

Mendel and Benedict (1909) studied the excretion of magnesium and calcium in dogs, cats and rabbits after injection of various salts of these metals. They used about 0.4% solution of calcium chloride, and found that in dogs 20% was eliminated during the first three hours. There was also an increased excretion of magnesium following injection of calcium. Excretion of calcium and magnesium increased after injection of magnesium chloride. Schiff (1920) found that the subcutaneous injection of magnesium sulfate in infants greatly increased urinary calcium excretion and also increased fecal calcium somewhat.

Whelan (1925) injected solutions of the chloride of calcium and magnesium and potassium into female dogs. An increase in urinary calcium followed the injection of magnesium chloride. However, the effect of calcium chloride on magnesium excretion was variable.

Clark and Geoffroy (1958) have shown that subcutaneous administration of 0.43 m•equivalent of magnesium sulfate per 100 g of body weight in a rat maintained on a calcium-free diet, resulted in a rapid increase of two to three fold in the rate of urinary excretion of radiocalcium.

Stronsky (1915) observed changes in the blood calcium and magnesium ratio after injecting narcotic doses of magnesium sulfate. There was an increase in the level of blood magnesium and a definite lowering in the calcium level. They attributed the observed narcosis in the animal to the lowering of calcium to magnesium ratio in the blood.

Richter-Quittner (1925) made an interesting observation that, whereas, normally only 50 to 60% of the calcium salts in the blood is ultra-filtrable, but after injection of magnesium, 89% was ultra-filterable. Thus, a change in the chemical combination of calcium in the blood seemed to be affected by the magnesium salts, which in this way produced the "washing out" effect of calcium in the urine after injection of magnesium salts. The accumulated evidence left little doubt that injection of magnesium salts resulted in a loss of calcium from the body.

The effects of magnesium injection on calcium retention is less clear. Early studies by Malcolm (1905) showed that doubling the magnesium intake of dogs increased urinary calcium slightly, and resulted in a more negative calcium balance.

Haag and Palmer (1928) indicated that high levels of dietary magnesium depressed the growth rate of rats fed diets low in phosphorus, regardless of the calcium level. When both calcium and phosphorus were low, on the other hand, there was little detrimental effect from a high magnesium intake.

In balance studies with rats by Allcock and MacIntyre (1962) it was shown that, in a magnesium deficient rat, the fecal excretion of

calcium was significantly lower than that of rats receiving normal amounts of calcium and magnesium. It was also shown that from the first day of magnesium deficiency, urinary excretion of calcium was diminished despite the presence of hypercalcemia. No change in the glomerular filtration rate was detected, and the decreased urinary excretion of calcium may have been due to an increase in absorption of filtered calcium by the renal tubular cells.

Experiments, in vitro, with intestinal loops from the rat, rabbit and guinea pig conducted by Schachter and Rosen (1959) have shown that calcium ions can be actively transported from the mucosal to serosal surface of everted gut sacs, and that the presence of magnesium ions depressed the transport of calcium, suggesting competition between the two.

Hart and Steenback (1913) showed earlier that when the magnesium intake of swine was increased there was a marked increase in urinary calcium and a negative calcium balance resulted. The addition of soluble phosphates largely counteracted the effect of magnesium resulting in a decreased loss of calcium. This effect did not appear to be due to a change in the pathway of elimination of calcium, since the fecal calcium showed only a slight increase during the same period. These results were strikingly corroborated by the work of Palmer et al. (1928) with cattle. The daily ingestion of magnesium sulfate caused a marked lowering of the calcium balance in cattle maintained on a phosphorus deficient ration. As in the case of swine, soluble phosphate overcame the effect of the magnesium salts fed along with calcium phosphate or bone meal for as long as a year, resulting in no ill effects.

The influence of calcium on magnesium metabolism has been studied less extensively. As cited earlier, Malcolm (1905) found that although a large intake of magnesium increased the urinary excretion of calcium in dogs, high calcium intake did not increase magnesium excretion. However, Heller and Haddad (1936) found that high calcium intake in rats reduced magnesium retention, and even caused loss of magnesium from the body.

Tuft and Greenburg (1938) were the first to report that a high content of calcium in the diet increased the magnesium deficiency symptoms in rats and in guinea pigs.

Feeding varying dietary levels of either magnesium and potassium, magnesium and sodium or all three elements, Forbes (1966) reported that increasing magnesium stimulated growth in weanling rats even at low levels of the other elements.

Increased intake of potassium, but not of sodium had some effect when the magnesium content of the diet was the least. Magnesium deficiency was associated with an increase of calcium and a decrease of magnesium concentration in all of the tissue examined. It was concluded that a small supplement of magnesium or potassium prevented the tissue changes associated with the deficient diets.

Investigations with adult rabbits fed magnesium deficient diets indicated decreased total and free magnesium in plasma, but the percentage of free magnesium was unaltered (Woodward, 1969).

Kinsella et al. (1967) found that high levels of dietary potassium consistently lowered plasma magnesium in rats. Additions of high levels

of sodium to the diet somewhat alleviated this condition. A significant interaction was found between dietary levels of potassium and sodium and plasma magnesium levels. Excess dietary potassium also lowered plasma magnesium levels in lambs. Balance studies with lambs showed that high potassium in the diet reduced the apparent digestibility of dietary magnesium. High levels of dietary sodium also reduced plasma magnesium levels.

Kiesel et al. (1969) reported a decrease in serum magnesium and serum potassium between the second and sixteenth weeks of feeding of lambs with magnesium deficient diets. However, there was no change in the serum calcium.

Six (1969) found that diets containing 120 ppm magnesium were barely sufficient for rats when dietary calcium was 0.2%. Magnesium reserves in bone were lower than for rats which had diets richer in magnesium, but plasma magnesium remained near normal.

When dietary calcium was 1.5% and magnesium 120 ppm there were signs of magnesium deficiency. Magnesium in bone, plasma and total body was lowered, although levels of magnesium and calcium in soft tissue were normal. Thus an increase in calcium in the diet resulted in an increase in magnesium absorption and a decline in magnesium and in the blood plasma. When vitamin E was added to the diet the antagonistic effect of calcium on magnesium was counteracted.

Investigations with guinea pigs fed magnesium and potassium deficient diets by Grace and O'Dell (1970) showed that growth rate was stimulated by an increase of potassium in the diet from 0.4 to 1.6% or from 0.8 to 1.6%, when magnesium was 0.5 and 0.1%, respectively.

Addition of potassium also reduced mortality when amounts of magnesium were limited. Low magnesium in the diet caused high sodium and calcium in skeletal and cardiac muscle. Addition of excess potassium to diets deficient in magnesium reduced calcification. Sodium in cardiac muscle was decreased by increased levels of dietary potassium when magnesium was low. Magnesium in plasma was decreased by deprivation of magnesium, but was not affected by potassium in the diet.

Feeding various levels of calcium, magnesium, and potassium to rats, Colby and Frye (1951a) observed that magnesium deficiency significantly depressed growth and caused hyperexcitability. Both high calcium and high potassium hastened the onset of the condition and increased the severity of deficiency signs. High potassium had the greater inhibitory effect on growth. High calcium produced 22% mortality, and blood analyses showed that severity of magnesium deficiency was increased by high calcium and potassium in the diet.

In later work with rats, Colby and Frye (1951b) found that high protein, low magnesium and high calcium diets each depressed growth. Both high protecin and high calcium diets increased the severity of magnesium deficiency, but the combination of high calcium with high protein was less detrimental. Depression of blood magnesium was less severe with high calcium plus high protein than with either alone. Low calcium was without detrimental effect, and when combined with high protein appeared to protect against low blood magnesium.

CHAPTER III

GENERAL EXPERIMENTAL PROCEDURE

This study was conducted in three parts. The first part of the investigation was designed to study the effects and interactions of dietary calcium, magnesium, and potassium with dietary protein nitrogen in young and old, white Spraque-Dawley rats. The study was made in two phases, each phase lasting for about three weeks. There were six treatments in each phase and the rats were grouped according to treatment (Table I). Rats were fed and watered ad libitum during the experimental period. At the end of each phase, the experimental rats were sacrificed and selected organs and tissue samples collected for chemical analyses. All samples were ashed 24 hours at 550°C, and both fresh and ash weights were recorded. The ash was taken into solution with 2 to 3 ml 6 N HCl, transferred to graduated tubes and made to volume with deionized water for total calcium, magnesium and potassium analyses. An atomic spectrophotometer, Perkin Elmer 303, was used for the determination of calcium, magnesium and potassium in all blood plasma and tissue samples collected.

Atomic absorption spectroscopy was based on the theory that the atoms of a particular element absorb light at a certain wave length which coincides with the spectral beam of that element. The sample to be analyzed was aspirated through a capillary tube into the atomizer, mixed with air and acetylene in the chamber and subsequently in the

TABLE I

SUMMARY PLAN OF EXPERIMENTAL RATION FED TO RATS

Treatment and rations[a]	Rat Age	
	Weanling	Adult
Phase I		
(Control) normal protein - normal Mg - normal K	3	3
Normal protein - normal Mg - high K	3	3
Low protein - low Mg - normal K	3	3
Low protein - high Mg - normal K	3	3
High protein - low Mg - normal K	3	3
High protein - high Mg - normal K	3	3
Phase II		
(Control) normal protein - normal Mg - normal K	3	3
Normal protein - high Mg - normal K	3	3
Low protein - normal Mg - low K	3	3
Low protein - normal Mg - high K	3	3
High protein - normal Mg - low K	3	3
High protein - normal Mg - high K	3	3

[a]The average calcium analysis for the ration was 12.81 mg/g.

flame. The pressures of air and acetylene introduced into the spray chamber for the flame were 25 and 4 pounds per square inch, respectively. The activated particles of the element absorb some of the light beam passing through the flame. The intensity of the remaining light beam was measured by the spectrophotometer (Willis, 1960).

The second part of the study involved sheep. Magnesium deficient diets were fed individually for several weeks, until plasma magnesium was reduced below the normal level. Rations containing different magnesium salts, such as, magnesium chloride, magnesium oxide, magnesium sulfate and magnesium nitrate were then superimposed. Relative availability to the animal was determined by 24 hours balance studies, using the concentration of magnesium in blood, urine and feces as criteria of difference. Mineral determination was made by ashing sample aliquots for 24 hours at 550°C. Ashed samples were taken into solution with 2.0 to 3.0 ml 6 N HCl, transferred to graduated tubes and made to volume with deionized water for total calcium, magnesium and potassium as described earlier.

Two western mature wethers and one ewe, maintained in a metabolism unit on magnesium deficient diets, were dosed with radioactive ^{28}Mg, as the chloride into the jugular vein, and the rate of absorption into the circulating blood and elimination of ^{28}Mg in urine and feces studied.

Blood, urine and feces were collected at frequent intervals for the study. Representative samples from total urine and feces were collected for radio-magnesium distribution and for chemistry. The

samples were ashed for 24 hours at 550°C, put into solution with 2 to 3 ml of 6 N HCl, transferred to graduated tubes, and made to volume with deionized water previous to the determination for total calcium. magnesium and potassium by routine spectrophotomic methods. The radioactivity was measured in a Nuclear Chicago automatic gamma counter unit.

The third part of the study involved a cattle survey from areas of the State of Tennessee. The objective of this survey was to analyze cow blood serum samples submitted by veterinarians from different parts of the state in order to determine the blood concentrations of calcium, magnesium and potassium. This provided diagnostic information to the veterinarians for suspected cases of grass tetany. Mineral values were also determined on blood samples from young and old animals collected from young and old cattle located in different experimental stations in the state.

Procedure with Rats

In phase I eighteen Spraque-Dawley rats from each of two age groups were used in this study. The rats in the first group were one old, and those of the second group were one year of age. Three young and three old rats from each group were subjected to each treatment (Table I).

There were six different diets prepared for the respective treatments. Magnesium, potassium and protein nitrogen were the three variables in the diets. The lowest level of magnesium recommended by the National Research Council (1962) was 0.4 g/kg, which was approximately three times that of the control diet. An increase in the protein

level has been reported to decrease blood magnesium concentration (Colby and Frye, 1951b). To check the validity of this theory and investigate the possible interactions with potassium and magnesium, levels of 9, 18 and 36% casein were fed in addition to the various dietary treatments with potassium and magnesium. A level of 1.8 g/kg of the diet, the lowest level of potassium in this sutdy, was recommended by the National Research Council (1962) as a normal level for growing rats. The level of 8 g/kg of the diet was approximately four and a half times that of the recommended level.

Each group of rats was maintained separately in individual galvanized iron cages. Diets and deionized water were provided ad libitum. The rations were added to stainless steel feeder cups, the water was provided in glass bottles fitted with stainless steel nipples. Weekly animal weight gains and feed consumption data were recorded.

Preparation of the diets. The composition of the various diets prepared and mineral mixtures added are shown in Tables II and III. The basal mineral mixture was prepared separately and the variable minerals were added according to the levels designated for each treatment. The diet for each treatment was mixed separately in an electric mixer for 30 minutes and stored in wide mouthed mayonnaise jars.

After two weeks, rats from each group were weighed and sacrificed. At sacrifice the following samples were taken from each rat: whole blood, heart, spleen, liver, kidney, muscle and whole femur. All samples were weighed into tared crucibles and ashed 24 hours at 550°C. The ash was put into solution with 2.0 to 3.0 ml 6 N HCl, transferred to graduated tubes and made to volume with deionized water for total

TABLE II

EXPERIMENTAL BASAL DIET FED RATS

Ingredients	g/kg
Cerelose[a]	702.9
Casein[b]	180.0
Cellulose[c]	30.0
D - L Methionine[d]	3.0
Corn Oil	40.0
Salt Mixture[e]	34.1
Vitamin Mixture[f]	10.0
	1000.0

[a]Cerelose, Corn Industrial International Inc., Englewood Cliff, New Jersey.

[b]Casein (vitamin-free), Nutritional Biochemicals Corporation, Cleveland, Ohio.

[c]Alphacel, Non-nutritive bulk, Nutritional Bilchemicals Corporation, Cleveland, Ohio.

[d]D. L. Methionine, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[e]Salt mixture (See Table III).

[f]Vitamin Diet Fortification Mixture in Dextrose, Nutritional Biochemicals Corporation, Cleveland, Ohio.

TABLE III

COMPOSITION OF THE SALT MIXTURE SUPPLIED TO RATS[a]

Ingredients	g/kg	Percent of Total
Common salts:		
Calcium carbonate	7.49	25.4
Calcium hypo-phosphate	20.35	68.7
Sodium Chloride	1.27	4.29
Magnesium sulfate	0.13	0.46
Ferrous sulfate	0.12	0.42
Cuprous sulfate	0.19	0.66
Zinc carbonate	0.23	0.47
Iodine pentoxide[b]		
	29.59	100.0

[a]The above salts were weighed out and mixed. Variable levels of magnesium and potassium (MgO_2 or K_2SO_4) were added to diets for each treatment, and made up to 34.1 g with cerelose.

[b]Iodine pentoxide added at the rate of 0.0004 g/kg to the above mixture.

calcium, magnesium and potassium analyses. An atomic absorption spectrophotometer, Perkin Elmer 303, was used for the determination of calcium, magnesium and potassium in the plasma and tissue samples. Conversions to mg/g of sample were made by the formula

$$\frac{\text{(dilution)(sample absorption)(concentration of standard)}}{\text{(fresh weight)(standard absorption)}}$$

In phase II, eighteen 30-day-old and eighteen one-year-old Spraque-Dawley rats were selected and randomly assigned to the above six treatments. Of the eighteen one-year-old rats, three males were assigned to each of the above six treatments. However, for the eighteen 30-day-old rats, two males and one female were chosen for each treatment.

Six different diets, varying in magnesium, potassium and protein nitrogen, were prepared. They were added to the basal diet, vide Table II (page 26), according to treatments (Table I, page 22). All rats were fed and watered ad libitum. Weekly weight gains and feed consumption data were recorded as in Phase I. Two weeks later the 36 rats were sacrificed and samples collected and processed as in Phase I.

Procedure with Sheep

Availability studies with magnesium salts. Two western wethers were maintained on magnesium deficient diets, vide Tables IV and V, and selected for the study. They were initially weighed and pre-dosing blood collections were made for blood chemistry. They were then placed in a sheep crate equipped for the separate collection of urine and feces, for a 24-hour balance study. At the end of the preliminary

TABLE IV

CHEMICAL COMPOSITION OF MAGNESIUM DEFICIENT RATION FED SHEEP

Ingredient	Percent
Cerelose[a]	29.7
Ground corn	10.0
Casein[b]	4.0
Urea[c]	4.0
Sodium chloride	1.0
Corn oil	1.0
Calcium phosphate	0.3
Vitamin mixture[d]	+
Corn cobs	50.0
	100.0

[a]Cerelose, Corn Inudstrial International Inc., Englewood Cliff, New Jersey.

[b]Casein (vitamin-free), Nutritional Biochemicals Corporation, Cleveland, Ohio.

[c]Urea (feed grade) 45% Nitrogen, Mississippi Chemical Corporation, Mississippi.

[d]Vitamin Diet Fortification Mixture in Dextrose, Nutritional Biochemical Corporation, Cleveland, Ohio.

TABLE V

MINERAL AND ENERGY COMPOSITION OF MAGNESIUM DEFICIENT DIET FED SHEEP[a]

Diet	Concentration, mg/g			Energy, K cal/g
	Ca	Mg	K	
Concentrate	6.46	0.52	1.89	4.05
Corn cob	0.17	0.33	3.51	5.36

[a]45.4 kg sheep consumed 0.82 kg/day of the ration.

period, two solutions of 50 ml of magnesium chloride were prepared. The magnesium chloride content of these solutions was 3.0 and 6.0 g. One wether was given 3.0 g of the magnesium chloride and the other was quantitatively administered 6.0 g of the solution by stomach tube. Blood samples were taken at frequent intervals from each animal, from the time of dosing until 72 hours, to determine the relative rate of blood absorption and feces-urainary elimination of magnesium chloride in these animals. Periodic urinary and fecal collections were made for the study of concentration of calcium, magnesium and potassium.

Dosing with magnesium oxide. In the second trial the wethers were dosed with magnesium oxide following similar preliminary preparations. One wether received 6.0 g and the other wether received 3.0 g via capsule administration into the esophagus by means of a balling gun. Pre- and post-collections of blood were made at frequent intervals. Blood samples were then centrifuged and the plasma diluted 1:10 and 1:100 for ionic concentration determinations. Ashed aliquots of feces and urine samples were diluted with deionized water and made to volume.

Dosing with magnesium sulfate and magnesium oxide. To compare the relative rate of absorption and the elimination of magnesium as the sulfate and oxide, the same wethers maintained on magnesium deficient diets were used in the third trial. Following a 24-hour balance study, one wether was dosed with 3.0 g of magnesium oxide by means of gelatin capsule. Likewise, the other was dosed with 3.0 g of magnesium sulfate. After dosing, blood and excretory samples were taken at regular intervals

throughout a 72-hour period. Blood samples were centrifuged and the plasma calcium, magnesium and potassium concentration was measured spectrophotometrically. Representative samples of urine and feces were taken, ashed, diluted and ionic concentration determined.

Dosing with magnesium nitrate. In the fourth trial, the two wethers maintained on the magnesium deficient diet were dosed with magnesium nitrate after a one-day preliminary balance study. One wether received 3.0 g and the other was dosed with 6.0 g of the salt by gelatin capsule administration into the esophagus by means of a balling gun. Post collections of blood, feces and urine were made at frequent intervals. Blood samples were centrifuged and the plasma samples were analyzed for calcium, magnesium and potassium in an atomic absorption spectrophotometer. One month later, the animals were dosed with 63.3 and 31.6 g of magnesium nitrate. The experiment resulted in the death of the animals.

Oral dosing with trans-aconitic acid and citric acid. One ewe and one wether weighting 50 and 60 kg, respectively, were selected for this study. They were maintained on an experimental diet deficient in magnesium (Table IV, page 29) until blood plasma magnesium decreased to 1.24 mg/100 ml. After a 24-hour balance study, each was dosed with 50 g of citric acid, orally. Pre- and post-dosing blood, feces and urine samples were collected for the determination of total calcium, magnesium and potassium. After a 6-hour period, they were again dosed. The wether received 50 g trans-aconitic acid and the ewe received 50 g of

citric acid orally in capsules. Samples of blood, urine and feces were collected over a 24-hour period. Representative samples were taken in tared crucibles and dried at 100°C for 12 hours prior to ashing for 24 hours at 550°C. Ashed samples were taken into solution with 2 to 3 ml of concentrated HCl, transferred to graduated tubes, and made to appropriate dilutions with deionized water for determination of total calcium, magnesium and potassium.

^{28}Magnesium studies with sheep. In order to investigate the absorption and excretion rate and patterns of ^{28}Mg in sheep, one ewe weighting 60 kg was maintained on a magnesium deficient diet, vide Table IV (page 29). After feeding the animals for four weeks, the plasma magnesium level was below 1.2 mg/100 ml. The ^{28}Mg was obtained from Bookhaven National Laboratory as magnesium chloride in concentrated hydrochloric acid, with a half-life of 20 hours. After collection of blood samples prior to dosing, the ewe was quantitatively dosed intraveneously intraveneously into the jugular vein with 1.0 ml containing 36.6 μc of ^{28}Mg as the chloride. A Bardahl containing catheter was inserted previous to dosing for the continuous sampling of urine from the bladder. Concurrent short-term blood and urine samples (5 minutes to 1 hour) were collected during the first 12-hour post-dosing, and thereafter at 12-hour intervals. Measured quantities of the collected samples were placed in tared flat-bottom tubes for ^{28}Mg measurements the same day. Total urine and feces collected from the animals were recorded and a representative sample taken, dried at 100°C for 12 hours prior to ashing for 24 hours at 550°C. The ashed samples were taken into solution with 2 to 5 ml concentrated HCl, transferred to calibrated

tubes, made to appropriate dilutions with deionized water for total mineral analyses.

All blood samples were centrifuged, 2 ml plasma collected in each tube and immediately measured in the scintillation counter. Plasma samples were then precipitated by adding an equal amount of 10% trichloroacetic acid, centrifuged and 2 ml of supernatant pipetted for subsequent radiomagnesium measurement. After several weeks time the same ewe, maintained on a magnesium deficient diet, was again dosed similarly with ^{28}Mg after preliminary preparation and catherization. After dosing, blood samples, feces and urine were collected at short intervals (5 minutes to 1 hour) during the first 12-hour period and plasma, plasma supernatant, urine and fecal samples were measured as in the first trial.

Procedure with Cattle, a Survey

During the winter of 1969-70, the Tennessee Veterinary Medical Association expressed urgent concern for research done in Tennessee on grass tetany. One of their requests was for a diagnostic center where veterinarians in private practice could submit blood samples for mineral analyses. They also requested that similar analyses by made of blood from apparently normal cattle located in different areas of the state, in order that some idea of the mineral content of the blood of normal cattle might be obtained. These requests were in addition to an appeal for research into the basic phenomenon associated with grass tetany in cattle.

In order to supply diagnostic service and to help establish normal values for selected minerals in the blood of Tennessee cattle, the Department of Animal Husbandry-Veterinary Science at the University of Tennessee at Knoxville, began a "survey" of cattle blood samples collected by private veterinarians from various locations in the state.

Blood samples from cattle were collected by veterinarians throughout the state during the winter and spring of 1971, and samples were forwarded for analysis. While the majority of these samples were collected from living cows with suspected cases of tetany, samples were also collected from cows with no evidence of tetany, and from cows dead with suspected tetany or unknown causes. Accordingly, there were 32 serum samples analyzed from cows having clinical evidence of tetany, 16 samples from cows having no evidence of tetany and 10 samples from cows dead from suspected tetany. Each sample of blood was accompanied by a form upon which the veterinarians indicated the age, breed of cow, and season in which the sample was taken. The clinical status, the interval to parturition and the current type of feeding regime in practice was reported. The determinations for ionic concentrations were made in the same way as the blood from the University of Tennessee herds.

The University's herd samples for this study included blood from 25 young and 30 old cows, in herds from north-central, west, and eastern parts of the state. Aliquots of each serum sample were diluted 1:10 and the ionic concentrations were determined on the Perkin Elmer 303, spectrophotometer. These results were sent to respective veterinarians as diagnostic information, and a seasonal consolidated report was prepared.

CHAPTER IV

RESULTS AND DISCUSSION

Growth and Feed Conversion in Rats

Phase I. The data on the weekly weight gains and feed conversion efficiencies for rats are given in Table VI and Table VII. The data indicates that growth of young rats was depressed by the low magnesium diets (IIA and IIIA) regardless of the protein level. Table VI shows that rats on diets IIA and IIIA gained 4.0 and 6.3 g (7 and 11%) during the first week and 1.0 and 2.6 g (8 and 16%) during the second week, respectively. These growth rates were much less than that for rats on diets with "high" or "normal" magnesium during this phase of the experiment. Feed required per unit gain was much higher for the low magnesium rats than for those on the other diets. During the first week, treatments IIA and IIIA consumed 9.6 and 7.9 g of feed for a gain of 1.0 g, as compared to those on diets IA, IB, IIB and IIIB which consumed 2.9 g feed per gram gain, the lowest feed to gain ratio of all the treatments. Lower feed efficiency by rats on the low magnesium diets may be partly due to the lower feed consumption by these groups.

Weight gains and feed conversion efficiencies of the low magnesium diet groups were markedly lower during the second week, as compared with the first week. The data indicates a decrease in feed utilization with age and a cumulative deleterious effect of continued dietary magnesium deficiency. McAleese and Forbes (1961) reported daily weight

TABLE VI

EFFECT OF DIFFERENT DIETARY LEVELS OF PROTEIN, MAGNESIUM AND POTASSIUM ON BODY WEIGHTS OF YOUNG RATS, PHASE I

Treatment and Ration	IA	IB	IIA	IIB	IIIA	IIIB
		First Week				
Average initial weight, g	55.0	56.6	56.3	49.3	54.0	50.0
Average feed consumption, g	49.7	55.0	38.6	49.0	50.0	41.3
Net gain in weight, g	12.3	12.3	4.0	10.0	6.3	14.0
Feed requirement/unit gain, g	4.1	4.4	9.6	4.9	7.9	2.9
		Second Week				
Average feed consumption, g	58.3	56.3	44.6	55.3	47.0	52.0
Net gain in weight, g	15.3	11.6	3.0	8.0	2.6	9.0
Feed requirement/unit gain, g	3.8	4.8	44.6	6.9	18.0	5.7

Diet IA. Normal protein, normal potassium and normal magnesium. IB. Normal protein, normal magnesium and high potassium. IIA. Low protein, normal potassium and low magnesium. IIB. Low protein, normal potassium and high magnesium. IIIA. High protein, normal potassium and low magnesium. IIIB. High protein, normal potassium and high magnesium.

TABLE VII

SUMMARY EFFECT OF DIFFERENT DIETARY LEVELS OF PROTEIN, MAGNESIUM AND POTASSIUM ON YOUNG RATS, PHASE II

Treatment and Ration	IA	IB	IIA	IIB	IIIA	IIIB
		First Week				
Average initial weight, g	65.0	55.0	58.0	65.0	59.0	67.0
Average feed consumption, g	59.3	57.6	40.6	58.6	52.6	59.6
Net gain in weight, g	3.3	5.0	- 3.0	8.0	- 3.0	6.3
Feed requirement/unit gain, g	17.9	8.2	-13.5	7.3	-17.5	9.4
		Second Week				
Average feed consumption, g	54.6	56.6	31.0	54.3	48.3	56.0
Net gain in weight, g	17.0	17.0	- 6.0	18.0	- 4.6	17.3
Feed requirmenet/unit gain, g	3.2	3.3	-51.6	3.0	-10.5	3.2

Diet IA. Normal protein, normal potassium and normal magnesium. IB. Normal protein, normal potassium and high magnesium. IIA. Low protein, normal magnesium and low potassium. IIB. Low protein, normal magnesium and high potassium. IIIA. High protein, normal magnesium and low potassium. IIIB. High protein, normal magnesium and high potassium.

gains of rats were markedly increased by elevating the dietary magnesium up to 118.0 ppm. Further increase of magnesium in the diet in this work did not change daily weight gains and are in agreement with this report.

Phase II. Feeding high levels of potassium (8.0 g/kg in the diet) resulted in increased rate of gain for the two-week period. Table VII shows that the young rats on diets IIB and IIIB, both high in potassium, gained 8.0 and 6.3 g during the first week and 18.0 and 17.3 g during the second week. This was much higher than gains in other treatments. The increased weekly weight gains due to potassium were accompanied by increases in feed efficiency for the two-week period. At the end of the two-week period, feed conversion efficiencies decreased for lots IIB and IIIB, but weekly weight gains did not change. These results are in agreement with the works of Morris and O'Dell (1970) who obtained increased growth rate in guinea pigs by feeding high levels of potassium in the diet.

Young rats on the low potassium - normal magnesium diets IIA and IIIA lost weight during the first and second weeks on each level of protein. The loss of weight during the first week was -3.0 g for treatments IIA and IIIA, increasing during the second week to -6.0 g and -4.6 g for the treatments. Data indicated that the feeding of low potassium diets to rats results in a decrease in the feed consumption as well as the daily body weight gains, and that these effects are more severe with low potassium diets than with low magnesium diets. Old rats, in both phases of the study, had low feed consumption ranging from 5.0 to 15.0% of the ration fed. During this adaptation period, the group

of old rats lost weight ranging from 20 to 40%. This might have been due to ration change or to palatability of the feed in the treatments involved. For convenience of presentation the results of old rats are not included in this table. Results with the young rats are in agreement with the works of Tuft and Greenberg (1938) and Martin and Wilson (1960).

It has been reported that with high levels of potassium in the diet, the weight gains of sheep (Kunkel et al., 1953) and rats (Colby and Frye, 1951a; Forbes, 1966) were decreased. However, much higher concentrations of potassium (5 and 8%) were used by these authors than in the present study.

Tissue analyses. Calcium, magnesium and potassium content of heart, liver, kidney and whole femur was determined on young and old rats on the fresh weight basis. The mean values of determination for the different variables are given in Tables VIII and IX. In both studies, the feeding of high potassium (8 g/kg) in the diet markedly decreased the tissue level of magnesium in the heart, liver, kidney and femur. There was a 34% reduction in the level of magnesium in the heart, 29% in the liver, 41% in the kidney and 34% in the femur bone. Feeding of high protein (36%) to rats (young or old) with low potassium (4 g/kg) increased the calcium level in the heart, kidney, liver and femur and markedly increased the magnesium level. Feeding of high magnesium (1.3 g/kg) in the diet had no apparent effect on the level of calcium and potassium. However, there was a slight increase in magnesium level of heart, kidney and femur. Feeding low protein (95%) and low magnesium in the diet resulted in reduction in the magnesium level in all the

TABLE VIII

MEAN CONCENTRATION OF CALCIUM, MAGNESIUM AND POTASSIUM IN TISSUES OF YOUNG AND OLD RATS FED DIFFERENT DIETARY LEVELS OF PROTEIN, POTASSIUM AND MAGNESIUM, PHASE I

Lot*	Animal Age	Heart, mg/g			Liver, mg/g			Kidney, mg/g			Femur, mg/g		
		Ca	Mg	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K
IA	Young	0.68	0.59	11.22	0.60	0.66	15.48	10.74	0.64	14.08	462.00	0.62	0.43
	Old	0.76	0.76	13.09	0.81	0.80	15.50	12.44	0.68	16.33	598.56	0.80	0.60
IB	Young	0.79	0.33	12.86	0.61	0.35	14.30	9.04	0.36	14.04	585.54	0.40	0.50
	Old	0.87	0.51	14.22	0.87	0.46	15.33	10.82	0.45	15.31	646.15	0.38	0.44
IIA	Young	0.74	0.50	13.02	0.58	0.61	14.42	11.88	0.59	14.16	499.20	0.37	0.44
	Old	0.84	0.71	13.02	0.72	0.68	14.10	13.26	0.65	14.48	605.85	0.47	0.48
IIB	Young	0.72	0.60	12.91	0.61	0.60	14.02	10.02	0.49	13.56	445.05	0.68	0.41
	Old	0.78	0.74	14.29	0.70	0.62	14.16	13.06	0.68	15.12	713.39	0.80	0.57
IIIA	Young	0.69	0.48	13.04	0.64	0.50	14.81	12.12	0.31	13.24	414.09	0.26	0.47
	Old	0.88	0.51	15.29	0.86	0.61	15.80	15.58	0.43	15.64	498.15	0.42	0.52
IIIB	Young	0.68	0.40	14.05	0.74	0.44	14.30	13.52	0.31	13.47	451.27	0.39	0.44
	Old	0.77	0.42	14.70	0.84	0.54	15.81	17.28	0.48	16.13	715.16	0.43	0.54

*IA. Normal protein, normal magnesium and normal potassium. IB. Normal protein, normal magnesium and high potassium. IIA. Low protein, low magnesium and normal potassium. IIB. Low protein, high magnesium and normal potassium. IIIA. High protein, low magnesium and normal potassium. IIIB. High protein, high magnesium and normal potassium.

TABLE IX

MEAN CONCENTRATION OF CALCIUM, MAGNESIUM AND POTASSIUM IN THE TISSUES OF YOUNG AND OLD RATS FED DIFFERENT DIETARY LEVELS OF PROTEIN, POTASSIUM AND MAGNESIUM FOR THREE WEEKS, PHASE II

Lot*	Animal Age	Heart, Mg/g			Liver, mg/g			Kidney, mg/g			Femur, mg/g		
		Ca	Mg	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K
IA	Young	0.64	0.59	12.52	0.57	0.69	14.78	11.10	0.58	12.08	431.87	0.63	0.40
	Old	0.84	0.74	13.33	0.80	0.76	16.95	14.40	0.79	14.24	587.48	0.86	0.53
IB	Young	0.58	0.61	10.72	0.58	0.65	14.68	11.59	0.65	10.36	432.32	0.60	0.44
	Old	0.83	0.78	14.03	0.76	0.77	15.60	14.51	0.86	12.34	541.07	0.82	0.43
IIA	Young	0.51	0.53	11.68	0.55	0.62	13.88	11.95	0.44	11.26	411.05	0.52	0.42
	Old	0.58	0.58	13.54	0.68	0.76	14.59	13.90	0.68	14.14	575.68	0.60	0.55
IIB	Young	0.65	0.25	10.63	0.60	0.20	13.29	14.94	0.21	11.31	468.06	0.21	0.42
	Old	0.96	0.49	12.30	0.92	0.36	14.57	16.37	0.38	14.32	597.00	0.30	0.60
IIIA	Young	0.69	9.45	13.86	0.67	0.34	14.51	12.06	0.54	13.06	419.39	0.49	0.41
	Old	0.92	0.66	15.30	0.89	0.65	14.446	14.88	0.62	14.87	608.46	0.61	0.55
IIIB	Young	0.67	0.20	11.46	0.66	0.20	13.21	12.00	0.27	11.44	497.42	0.19	0.42
	Old	0.86	0.45	15.26	0.87	0.46	14.04	14.82	0.38	13.55	592.31	0.34	0.54

* Diet IA. Normal protein, normal magnesium and normal potassium. IB. Normal protein, high magnesium, normal potassium. IIA. Low protein, normal magnesium and low potassium. IIB. Low protein, normal magnesium and high potassium. IIIA. High protein, normal magnesium and low potassium. IIIB. High protein, normal magnesium and high potassium.

tissues and markedly increased the calcium level in heart, kidney, liver and femur. High protein (36%) and high dietary potassium (8 g/kg) markedly decreased tissue magnesium level. Magnesium level was markedly reduced below that in controls by 52% in the heart, 55% in the liver, and by 64% in the femur of young and old rats. The increase of calcium level averaged 23% heart, 43% liver, 52% for kidney and 60% for femur. These results were in agreement with those of Forbes (1966) and Morris and O'Dell (1961). However, these concentrations of calcium, magnesium and potassium were lower compared to the work of MacIntyre and Davidson (1958) and Forbes (1966).

McAleese and Forbes (1961) found that the magnesium content of femur in rats decreased from 0.56 to 0.15% with low magnesium diets. Similar results were obtained in the present study. The results of Morris and O'Dell (1961) in the guinea pig and Smith (1959) in calves, and our present study suggest the mobility of bone magnesium to be an important factor in magnesium nutrition. If mobilized bone magnesium is used directly to compensate serum magnesium deficiency, it may delay the onset of hypomagnesemic conditions for a considerable time. However, the magnesium balance experiments by Kemp et al. (1961) suggested that the rate of mobilization of stored bone magnesium was insufficient to prevent hypomagnesemia in mature cows. It was concluded that the dietary supply of available magnesium played a major role in magnesium nutrition and hypomagnesemia. Differences in all the means reported herein may suggest that mineral exchange in heart muscle may be very high and largely dependent on the dietary variables involved. Brant et al. (1958) reported that after intravenous administration of ^{28}Mg

into dogs, higher tissue concentration of ^{28}Mg was found in the heart muscle. Results presented also suggest higher rates of mineral exchange in the heart tissue of young rats.

Blood serum analyses. Concentrations of magnesium, calcium and potassium in blood serum were determined for young and old rats sacrificed and mean values of these analyses are shown in Tables X and XI. Young rats had an average weight of 80 g and old rats had an average weight of 342 g at sacrifice. There were no differences in the blood levels of calcium and potassium among the various treatments. However, there were differences in magnesium levels. When standard protein content rations were fed, magnesium level in the serum was reduced by magnesium deficiency in the diet. The addition of potassium caused a further lowering of the serum magnesium. High protein plus high dietary potassium produced considerable reductions in serum magnesium. This indicated the combination of high protein and potassium fed at high levels to be more severe by depressing magnesium than the feeding of either alone. These results were in agreement with the previous by Colby and Frye (1951b) for rats, and cows (Kemp et al., 1961; and Welt, 1964). The hypomagnesemic effect of increased potassium intake has also been reported for rats by Forbes (1966) and in sheep by Kunkel et al. (1953).

Table XI shows that when high protein levels were fed there was a small increase in the serum calcium levels. This increase was not great, but it indicated a trend in both phases of study. This may mean that feeding of higher levels of protein caused higher blood protein levels which tied up more calcium. High levels of protein in the ration

TABLE X

SERUM ELECTROLYTE VALUES OF YOUNG AND OLD RATS FED DIFFERENT DIETARY LEVELS OF POTASSIUM, PROTEIN AND MAGNESIUM[a], PHASE I

Treatment and Ration	IA	IB	IIA	IIB	IIIA	IIIB
Average Age	Control	Control + high K	Low protein + low Mg	Low protein + high Mg	High protein + low Mg	High protein + high Mg
Serum Calcium, mg percent						
Young	10.98	7.64	7.46	9.22	11.11	11.15
Old	9.19	7.41	7.02	7.89	10.60	10.19
Serum Magnesium, mg percent						
Young	1.76	1.15	1.22	2.04	1.22	1.09
Old	2.33	1.43	1.46	1.94	1.25	1.17
Serum Potassium, mg percent						
Young	15.91	16.22	15.80	15.43	15.54	15.15
Old	14.74	16.43	15.39	14.94	15.09	14.52

[a]Three weanling and three one-year-old rats in each treatment.

Diet IA. Normal protein, normal magnesium and normal potassium. IB. Normal protein, normal magnesium and high potassium. IIA. Low protein, low magnesium and normal potassium. IIB. Low protein, high magnesium and normal potassium. IIIA. High protein, low magnesium and normal potassium. IIIB. High protein, high magnesium and normal potassium.

TABLE XI

SERUM ELECTROLYTE VALUES OF YOUNG AND OLD RATS FED DIFFERENT LEVELS OF DIETARY POTASSIUM, PROTEIN AND MAGNESIUM[a], PHASE II

Treatment and Ration	IA	IB	IIA	IIB	IIIA	IIIB
Average Age	Control	Control + high Mg	Control + low K	Control + high K	High protein + low K	High protein + high K
Serum Calcium, mg percent						
Young	10.77	9.80	9.46	10.87	11.46	11.45
Old	10.20	9.36	10.54	10.20	11.49	10.62
Serum Magnesium, mg percent						
Young	2.02	1.98	1.97	0.80	0.86	0.50
Old	2.08	1.91	2.03	0.93	0.99	0.60
Serum Potassium, gm percent						
Young	16.27	15.39	14.22	14.91	14.43	14.47
Old	15.13	13.73	31.75	14.33	41.31	14.45

[a]Three weanlings and three on-year-old rats in each treatment.

Diet IA. Normal protein, normal magnesium and normal potassium. IB. Normal protein, high magnesium and normal potassium. IIA. Low protein, normal magnesium and low potassium. IIB. Low protein, normal magnesium and high potassium. IIIA. High protein, normal magnesium and low potassium. IIIB. High protein, normal magnesium and high potassium.

caused a marked depression in the serum magnesium. This finding was in agreement with Colby and Frye (1951b) and Welt (1964).

Ender et al. (1957), however, found that fertilization of pastures with potassium sulfate resulted in a more pronounced hypomagnesemia in sheep than the pasture top-dressed with sulfur-free nitrogen and potassium fertilizers. The magnesium balance experiments with cattle by Kemp et al. (1961) and with guinea pigs by Garner (1950) have indicated that the availability of magnesium in the diet is an important factor in hypomagnesemia. Since the increasing levels of nitrogen and potassium in the ration decrease the serum magnesium, the interference for magnesium utilization may have taken place in the digestive tract. The works of Allcroft and Green (1934) and Butler (1963) indicate that the onset of hypomagnesemia on pastures, which have adequate levels of magnesium, supports this assumption. Van't Klooster (1965) reported that the solubilities of calcium and magnesium in the large intestine of the sheep are 24 to 43% from hay, but only 7.4 and 24.0% from grass. In the present study the higher levels of protein (nitrogen) and potassium in the diets may have decreased the absorption of magnesium either by mass action or through lowering the solubility of magnesium.

Excess Citric Acid Effect Upon Sheep

In order to show the effect of added dietary citric acid on sheep, a mature ewe was administered 50 g of citric acid in a gelatin capsule by means of a balling gun. The mineral composition of the

pre- and post-collection of blood samples is given in Table XII. Following a seven-hour interval, she was given a second 50 g dosage.

Blood samples, drawn every four hours, indicated no change in calcium, magnesium, or potassium during the first 20 hours. Samples drawn after this time, however, indicated a slight decrease in plasma magnesium from 1.8 to 1.3 mg/100 ml. These samples showed no change in either calcium or potassium, and findings suggest that the decrease in magnesium may be due to the interference of citric acid in normal gut absorption of magnesium. Although these tests indicate slight hypomagnesemia, there were no other clinical symptoms of tetany. Results with this one animal were in agreement with the work of Burt and Thomson (1962).

Bohman et al., (1969) fed Hereford heifers daily with 200 g of citric acid, plus 225 g of potassium chloride for six days. They found that citric acid plus KCl significantly decreased plasma magnesium, but had no significant effect on plasma calcium. Citric acid dosing also decreased feed consumption. In our study only 100 g of citric acid was fed for 24 hours and no effect on appetite was observed.

Excess Trans-Aconitic Acid Effects

A western wether weighing 52 kg, was fed a magnesium deficient diet (Table IV, page 29) and administered a 50 g dosage of trans-aconitic acid* by means of gelatin capsules. Blood samples were collected

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* Trans-aconitic acid (Grade II) 85-95%. Sigma Chemical Company, St. Louis, Missouri.

TABLE XII

ADDED DIETARY CITRIC ACID EFFECTS ON PLASMA ELECTROLYTES OF SHEEP

Time of Sampling H.A.D.[a]	Plasma Concentrations		
	Ca	Mg	K
0 hours[b]	6.8	1.87	15.01
2 hours	7.6	1.92	15.71
4 hours	7.1	1.84	15.46
7 hours	7.0	1.87	15.98
24 hours	7.1	1.48	15.01
26 hours	7.2	1.32	15.71
30 hours	7.3	1.32	15.89

[a] H.A.D. is hours after dosing by gelatin capsule.

[b] 50 g citric acid given orally at 0 and 7 hours.

from the jugular vein of the animal prior to dosing and two hours after dosing. After a six-hour interval, the wether was given a second 50 g dose trans-aconitic acid and blood samples were collected. After five minutes, the animal collapsed and died. An autopsy was performed immediately after the death of the animal, and tissue samples were collected from various organs. The samples were dried at 100°C for 24 hours and then ashed at 550°C for 12 hours. These ashed samples were diluted with deionized water 1:10, 1:1000 and mineral concentrations were determined by the Perkin Elmer 303 Atomic Absorption Spectrophotometer. Mineral concentrations of blood plasma and the organs are shown in Table XIII. The data indicated that plasma calcium level increased from 11.84 to 15.51 mg per 100 ml within six hours from the time of dosing. Plasma potassium decreased by 1.0 mg from pre- to post-collection period. The plasma magnesium level was unchanged.

Bohman et al. (1969) observed congestion of the ventral wall of rumen and small intestine and noted autolysis of the kidneys in steers receiving 157 g of trans-aconitic acid daily for a seven-day period. Since this study was done for only six hours, such lesions were not observed.

Magnesium Salts and Plasma Calcium, Magnesium and Potassium

The effect of supplementation with 3.0 and 6.0 g of dietary magnesium salts and the mean concentration of plasma calcium, magnesium and potassium in sheep is shown in tables XIV and XV. For the 3.0 g dose the supplementation of magnesium was 0.7 g, 1.8 g, 0.3 g, 0.6 g, respectively for chloride, oxide, nitrate and sulfate. For the 6.0 g

TABLE XIII

ADDED DIETARY TRANS-ACONITIC ACID EFFECTS ON CATION LEVELS OF VARIOUS TISSUES AND ORGANS OF SHEEP FED A MAGNESIUM DEFICIENT DIET

	Organ Weight, g	Concentration					
		Ca		Mg		K	
		mg/100 ml					
Plasma pre-dosing		11.84		1.24		24.92	
Plasma 2 hours after dosing		10.64		1.22		23.32	
Plasma 6 hours after dosing		15.51		1.24		24.74	
		Mg/g					
Heart	244	0.04	(9.76)[a]	0.15	(36.60)	3.65	(890.60)
Liver	614	0.12	(73.68)	0.31	(190.34)	0.06	(36.84)
Spleen	102	0.01	(1.02)	0.11	(11.22)	1.81	(184.62)
Kidney	152	0.16	(24.32)	0.22	(33.44)	1.88	(285.76)
Sternum		51.86		0.46		0.66	
Bile		0.25		0.07		1.85	
Muscle		0.01		0.24		3.0	

[a]Weight of organ X mg/g cation.

TABLE XIV

DIETARY SUPPLEMENTATION EFFECTS OF A THREE-GRAM DOSE OF MAGNESIUM SALTS ON SHEEP PLASMA CALCIUM, MAGNESIUM AND POTASSIUM LEVELS[a]

Hours After Dosing	Dietary Source of Magnesium[b]											
	Chloride Mg %			Oxide Mg %			Nitrate Mg %			Sulfate Mg %		
	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K
0 hours	5.3	0.6	16.1	5.3	1.3	17.5	7.1	1.2	12.6	6.4	1.0	19.0
3 hours	5.1	1.3	12.7	5.5	1.3	13.2	818	2.1	16.2	6.2	1.2	13.7
6 hours	5.2	1.7	17.1	5.3	1.4	16.5	8.9	2.1	12.9	5.3	1.1	14.8
12 hours	5.9	1.9	21.4	4.5	1.5	17.3	8.8	2.2	11.5	6.5	1.4	15.5
24 hours	7.0	2.0	19.0	5.8	1.8	16.4	8.4	2.2	12.6	6.4	1.2	20.1
48 hours	7.3	1.5	18.2	5.1	2.2	17.2	8.6	1.8	11.4	5.6	1.2	11.1

[a]Two sheep per treatment in balance studies.

[b]Oral dose admistered as 3.0 g of magnesium salt as chloride, oxide, nitrate or sulfate containing 25, 60, 9, and 20% magnesium, respectively.

TABLE XV

DIETARY SUPPLEMENTATION EFFECTS OF A SIX-GRAM DOSE OF MAGNESIUM SALTS ON SHEEP PLASMA CALCIUM, MAGNESIUM AND POTASSIUM LEVELS[a]

Hours After Dosing	Dietary Source of Magnesium[b]								
	Chloride Mg %			Oxide Mg %			Nitrate Mg %		
	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K
o hours	6.0	1.4	20.6	6.5	1.6	18.1	6.3	1.2	20.1
3 hours	6.9	1.9	16.0	6.2	1.9	13.4	8.8	2.2	23.7
6 hours	6.4	2.0	18.0	5.3	2.2	13.4	6.6	2.3	24.2
12 hours	6.1	2.2	21.7	5.7	3.3	14.8	6.7	1.9	14.8
24 hours	5.5	2.4	18.0	6.5	3.3	15.2	6.7	1.8	14.3
48 hours	7.3	1.5	18.2	6.4	2.3	14.8	6.7	2.0	13.4

[a]Two sheep per treatment in balance studies.

[b]Oral dose admistered as 6.0 g of magnesium salt as chloride, oxide or nitrate, containing 25, 60 and 9%, respectively.

dose the supplementation of magnesium was 1.5 g, 3.6 g, 0.5 g and 1.2 g, respectively for chloride, oxide, nitrate and sulfate. An increase in supplemental magnesium was correlated with an increase of plasma magnesium. At all times, added magnesium chloride produced the greatest percentage increase within two hours. Following a 3.0 g dosage of magnesium chloride, plasma magnesium level increased sharply from 0.65 mg/100 ml to 1.3 mg/100 ml. Magnesium levels continued to increase gradually to 24 hours after dosing, and thereafter declined.

Percentage increase was 115% at three hours, 192% at six hours and 207% at 24 hours. Oral admistration of 3.0 g magnesium oxide gradually increased plasma magnesium levels from six hours until 48 hours, then after 72 hours, there was a decline. (These findings suggested that there was no renal clearance.) Similarly, 6.0 g of magnesium oxide increased blood magnesium level at 18% at three hours, 37% at six hours and 106% at 24 hours.

Administration of magnesium nitrate to sheep produced approximately one-half the increase as that observed for magnesium chloride. The plasma magnesium increased from 1.2 mg/100 ml to 2.1 mg/100 ml after three hours, remained constant until 24 hours, and then declined gradually until 72 hours. These findings suggest that the salt was absorbed quickly into the body, but that excretion was slow because of slow renal clearance. Dosage with magnesium sulfate showed a slight increase in levels of plasma magnesium until 12 hours, but from 24 to 48 hours the 3.0 g added to magnesium sulfate resulted in less increase than did the magnesium oxide.

Magnesium nitrate at the 6.0 g level caused the greatest increase of plamsa magnesium up to six hours after dosage. From 12 to 24 hours, however, the magnesium oxide showed a higher increase than either the magnesium nitrate or the magnesium chloride. In comparing dosage levels, the 6.0 g dose caused a greater average plasma magnesium increase from all sources than did the 3.0 g dose. The values for the concentration of calcium, magnesium and potassium obtained are in agreement with those found in the literature (Allcroft and Green, 1934; Duncan et al., 1938; and Fisher, 1960).

Magnesium Salts and Fecal and Urinary Calcium, Magnesium and Potassium Balance

The effects of different levels and sources of magnesium fed to sheep on the balance of calcium, magnesium and potassium are shown in Table XVI and the mean level of intake and mean quantities excreted in feces and urine are illustrated graphically in Figure 1. When an average of 1.71 g of the different sources of magnesium was fed to magnesium deficient wethers, there was an excretion rate of 0.82 g of calcium in feces and 0.52 g in the urine for a 24-hour period. Whereas, excretion of magnesium was 0.55 g in the feces and 0.16 g in the urine. In case of potassium, there was an excretion rate of 0.72 g in feces and 1.04 g in the urine per day.

When an average of 2.52 g of different sources of magnesium were fed to the same wethers, there was an excretion rate of 1.14 g of calcium in feces and 0.42 g in the urine, whereas, the excretion rate of the

TABLE XVI

DIETARY LEVEL AND SOURCE EFFECTS OF FED MAGNESIUM ON CALCIUM, MAGNESIUM AND POTASSIUM BALANCE IN MAGNESIUM DEFICIENT SHEEP[a]

Source	Added Amount, g	Intake, g			Feces, g			Urine, g			Balance, g			Percent		
		Ca	Mg	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K
Control sheep[b]		5.60	1.60	3.00	1.17	0.18	0.48	0.30	0.13	1.30	4.13	1.49	1.22	73	82	40
Mg nitrate[c]	3.0	3.80	1.06	1.81	0.87	0.24	1.06	1.16	0.07	0.70	1.77	0.75	0.55	46	29	2
Mg chloride[d]	3.0	2.28	1.65	0.34	0.73	0.54	0.69	0.13	0.24	1.29	1.42	0.87	-1.64	62	52	-482
Mg oxide[e]	3.0	2.85	2.44	2.46	0.83	0.89	0.47	0.28	0.17	1.13	1.74	1.38	0.86	61	56	34
Total		8.93	5.15	4.61	2.43	1.67	2.22	1.57	0.48	3.12	4.93	3.00	-0.73	169	137	-446
Mean		2.97	1.71	1.53	0.82	0.55	0.72	0.52	0.16	1.04	1.64	1.00	-0.24	56	45	148

TABLE XVI (Continued)

Source	Added Amount, g	Intake, g			Feces, g			Urine, g			Balance, g			Percent		
		Ca	Mg	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K
Mg nitrate[f]	6.0	3.06	1.04	1.88	1.62	0.23	0.71	0.26	0.17	1.18	1.18	0.64	-0.01	38	61	-0.5
Mg chloride[g]	6.0	0.41	2.35	0.28	0.99	0.19	0.61	0.72	0.53	0.20	-1.30	1.63	-1.53	-317	69	-189
Mg oxide[h]	6.0	2.85	4.17	2.46	0.83	0.89	0.47	0.28	0.17	1.13	1.74	3.11	0.86	61	74	34
Total		6.32	7.56	4.62	3.44	1.31	1.79	1.26	0.87	2.51	1.62	5.38	0.32	-218	204	-155.5
Mean		2.10	2.52	1.52	1.14	0.43	0.59	0.42	0.29	0.83	0.54	1.79	0.10	72	68	51.0

[a]Mean blood levels of magnesium for deficient sheep were 1.2 mg/100 ml.

[b]Control sheep consumed 1.6 kg basal ration for 24 hours.

[c]Magnesium deficient sheep consumed 1.2 kg basal ration for 24 hours.

[d]Magnesium deficient sheep consumed 1.1 kg basal ration for 24 hours.

[e]Magnesium deficient sheep consumed 0.9 kg basal ration for 24 hours.

[f]Magnesium deficient sheep consumed 1.0 kg basal ration for 24 hours.

[g]Magnesium deficient sheep consumed 1.1 kg basal ration for 24 hours.

[h]Magnesium deficient sheep consumed 1.2 kg basal ration for 24 hours.

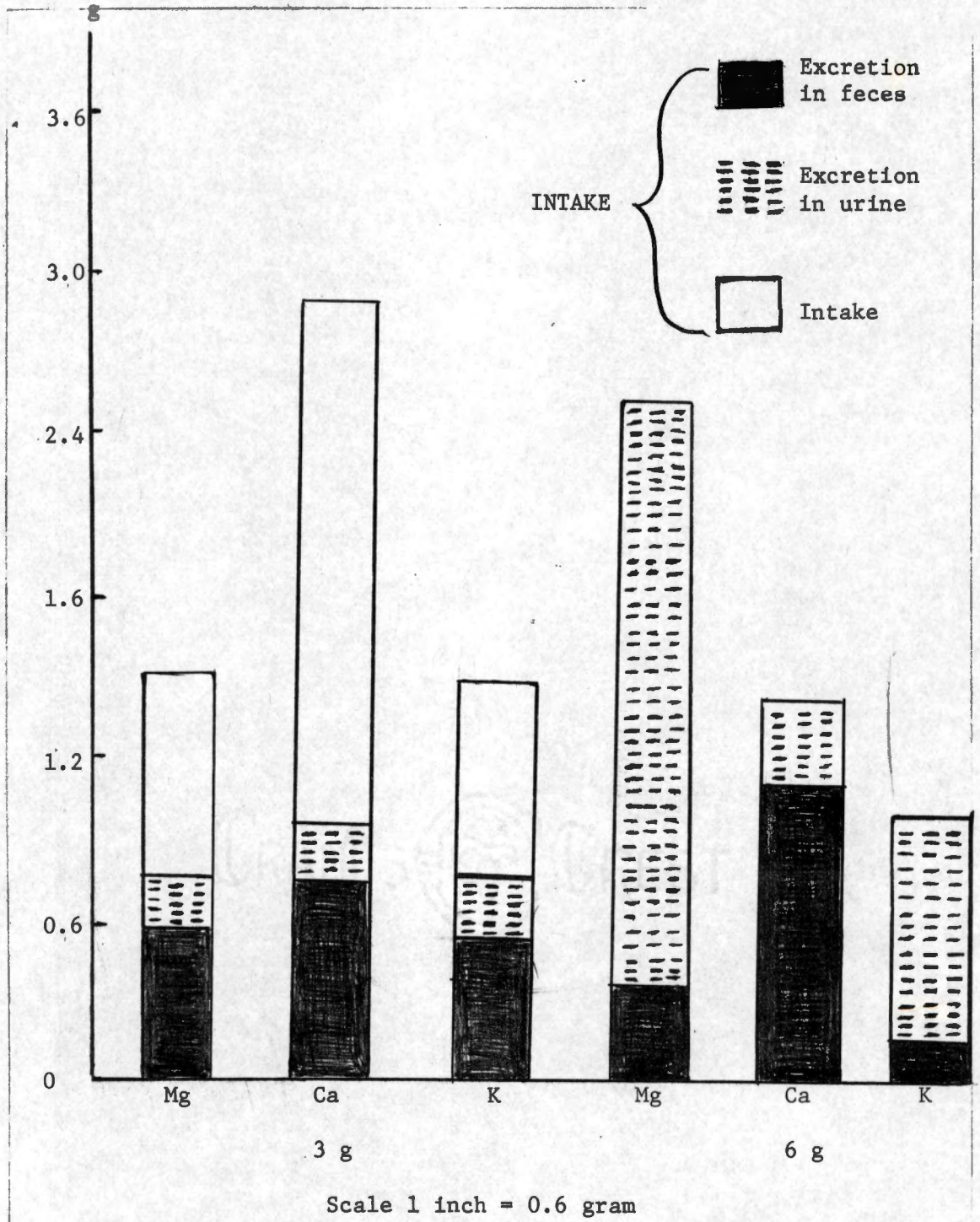

Figure 1. Mean daily levels of intake (g) and excretion (g) in feces and urine of calcium, magnesium and potassium in magnesium deficient sheep.

magnesium was 0.43 g in the feces and 0.29 g in the urine, for a 24-hour period. Excretion rates of potassium were 0.59 g in the feces and 0.83 g in the urine per day.

With both levels of magnesium dosing, the percentage of magnesium accumulated was highest with magnesium oxide, which was lower than the percentage retention in the control. Results suggest that there was more antagonism with potassium when using magnesium chloride. The antagonism was very high at the 6.0 g level (-189%). At the 6.0 g level, magnesium chloride was also antagonistic with calcium retention (-317%). At the 3.0 g level there was no apparent magnesium antagonistic effect with calcium. However, magnesium chloride and magnesium oxide slightly lowered calcium retention. At the 3.0 g level, magnesium was antagonistic to potassium (-482%). The accumulated evidence in the balance experiment shows that excess magnesium had a detrimental effect upon the potassium utilization. Supplemented dietary magnesium had a detrimental effect upon potassium and calcium utilization. The fecal and urinary excretion criteria demonstrated a lesser utilization of dietary potassium when the level of magnesium in the diet was increased. The negative balance of calcium and potassium indicated depletion of these salts from the body tissue of the animal when the diet was supplemented with magnesium.

Malcolm (1905) first showed that doubling the magnesium intake of dogs increased urinary fecal calcium and potassium only slightly, but resulted in a more negative calcium balance.

Hart and Steenback (1913) showed that when magnesium intake of swine was increased, there was a marked increase in fecal calcium and

potassium and a negative balance of calcium resulted. Whelan (1925) injected 6.0% magnesium chloride solution, and found an increase in urinary calcium. Clark and Geoffroy (1958) showed that following the subcutaneous administration of 0.43 m•equivalent of magnesium sulfate per 100 g of body weight to rats there was a rapid increase of two to three fold in the rate of urinary calcium.

In the present study, there was a negative balance of potassium at both 3.0 and 6.0 g dosage levels (Table XVI, page 56). There was a higher depletion of potassium than calcium. Results are in agreement with those of Malcolm (1905) and Hart and Steenback (1913).

Added Magnesium Nitrate on Sheep

One mature Suffolk ewe and a wether maintained on a magnesium deficient diet were selected for the study. The ewe was administered 63.3 g of magnesium nitrate containing 6.0 g magnesium, and a wether was dosed with 31.6 g magnesium nitrate containing 3.0 g magnesium by means of gelatin capsules. Blood samples were collected from the animals prior to dosing and after administration of the salt. Although the levels were supposedly below toxic levels, the ewe collapsed and died within one hour of dosing and the wether died six hours after dosing. Both animals prior to death showed weakness, collapse, dyspnea, cyanosis and rapid pulse. Post mortem examination of the animals revealed no froth nor obstruction in the respiratory passage, and it was presumed that the animals did not suffocate. Blood in both animals was dark. Discoloration of the blood was seen as it flowed across the

tissues. Blood-stained fluid was seen in the pericardial sac of both animals. The animals presumably died of nitrate poisoning. Table XVII shows plasma changes in the mineral composition at pre- and post-dosing with magnesium nitrate. Plasma calcium level was slightly increased in both of the animals from the pre- to post-dosing period. Plasma magnesium level of the wether was increased by 0.6 mg/100 ml and in the ewe by 0.4 mg/100 ml from the pre- to post-dosing period. Plasma potassium was unchanged in both animals from pre- to post dosing.

A short clinical test was done as described by Coles (1967) by boiling the blood samples of the deceased animals along with normal ovine blood samples for 45 minutes in the hot-water bath. The blood samples of the deceased animals were slightly pink in color, while the normal blood samples remained bright red. This was a positive test for nitrate poisoning according to Coles (1967) who reported the change to pink coloration upon boiling the suspected blood samples to be a positive indication of nitrate poisoning. The total organ weight and concentration of calcium, magnesium and potassium in the tissues of the dead ewe and wether are shown in Tables XVIII and XIX, respectively. Jubb and Kennedy (1963) reported that nitrate poisoning depended upon the oxidation of hemoglobin to methemoglobin. There are iron-containing respiratory enzymes in addition to hemoglobin which may be oxidized; nitrates are used therapeutically to dilate blood vessels, and in nitrate poisoning, a serious drop in blood pressure is to be anticipated. The blood vessels which are most sensitive to the dilatory effects of nitrate include those of the head, brain and meninges, the coronary vessels and then the visceral vessels, being slightly less

TABLE XVII

ELECTROLYTE CHANGES IN PLASMA COMPOSITION OF SHEEP DOSED WITH MAGNESIUM NITRATE

Animal		Plasma Concentration, mg/percent		
		Ca	Mg	K
Suffolk ewe	pre-dosing	6.4	1.4	20.2
	post-dosing	6.2	1.8	21.6
Wether	pre-dosing	6.4	1.0	20.2
	post-dosing	6.8	1.6	18.6

TABLE XVIII

MINERAL COMPOSITION OF SEVERAL ORGANS OF EWE[a] PRESUMABLY DYING OF NITRATE POISONING

Tissue	Total Weight, g	Mineral Concentrations[b], mg/g					
		Ca		Mg		K	
Heart	308	0.17	(52.36)[c]	0.05	(15.40)	1.18	(363.44)
Liver	810	0.12	(97.20)	0.04	(32.40)	0.97	(785.70)
Spleen	144	0.15	(21.60)	0.11	(15.84)	8.62	(1241.28)
Kidney	75	0.28	(21.00)	0.21	(15.75)	1.10	(82.50)
Bile	-	0.17		0.02		0.14	
Femur End	-	180.30		2.44		0.61	
Femur Shaft	-	128.16		1.46		0.42	
Muscle		0.04		0.11		1.13	

[a]Suffolk ewe weighed 53.6 Kg at time of dosing.

[b]The ewe died one hour after oral dosing.

[c]Figures in parenthesis refer to the total cation concentration in organ.

TABLE XIX

MINERAL COMPOSITION OF SEVERAL ORGANS OF WETHER PRESUMABLY DYING OF NITRATE POISONING[a]

Tissue	Total Weight, g	Mineral Concentrations[b], mg/g					
		Ca		Mg		K	
Heart	89	0.11	(9.79)[c]	0.03	(2.67)	2.73	(242.97)
Liver	518	0.08	(41.44)	0.03	(15.54)	1.21	(626.78)
Spleen	46	0.04	(1.84)	0.03	(1.38)	5.04	(231.84)
Kidney	50	0.39	(19.50)	0.29	(14.50)	1.12	(56.00)
Bile	-	2.06		0.04		0.16	
Femur End	-	287.30		7.31		0.70	
Femur Shaft	-	137.58		2.42		0.15	
Muscle	-	0.05		0.14		0.98	

[a]Western wether weighed 30.4 kg at time of dosing.

[b]The wether died six hours after oral dosing.

[c]Figures in parenthesis refer to the total cation concentration in organ.

sensitive. It is reasonable that the rapid collapse during nitrate poisoning was due to hemodynamic disturbances as much as to the inability of the oxidized hemoglobin to transport oxygen.

Magnesium^{28} studies in sheep. An experiment was designed to use ^{28}Mg for the studies of Mg uptake, behavior, rate of clearance from the blood, and the excretion patterns for magnesium in sheep. Two western wethers, each weighing approximately 50 kg and one ewe weighing 60 kg received a single 1.0 ml carrier-free dose, containing 36 μc ^{28}Mg. The isotope was administered intravenously by a nylon catheter previously inserted into the jugular vein. The comparative concentrations of ^{28}Mg in blood, plasma, plasma supernatant, feces and urine as a function of time following intravenous administration are illustrated graphically in Figures 2 and 3. A somewhat similar pattern was observed in previous experiments when ^{28}Mg was administered to wethers. Immediately after dosing the disappearance curve, the shape of the curve for blood, plasma and supernatant declined sharply. This indicated the radio-magnesium to be actively removed. About 14 hours after the injection of ^{28}Mg, equilibrium between the RBC (red blood cells) and plasma appeared to have been established. The occurrence of lower levels of ^{28}Mg in the deficient animals was possibly due to the more rapid equilibrium with the deficient tissue, since percentage dietary absorption was much greater in the magnesium deficient animals. The rapid rate of disappearance from plasma of ^{28}Mg in these animals was probably due principally to increased tissue uptake.

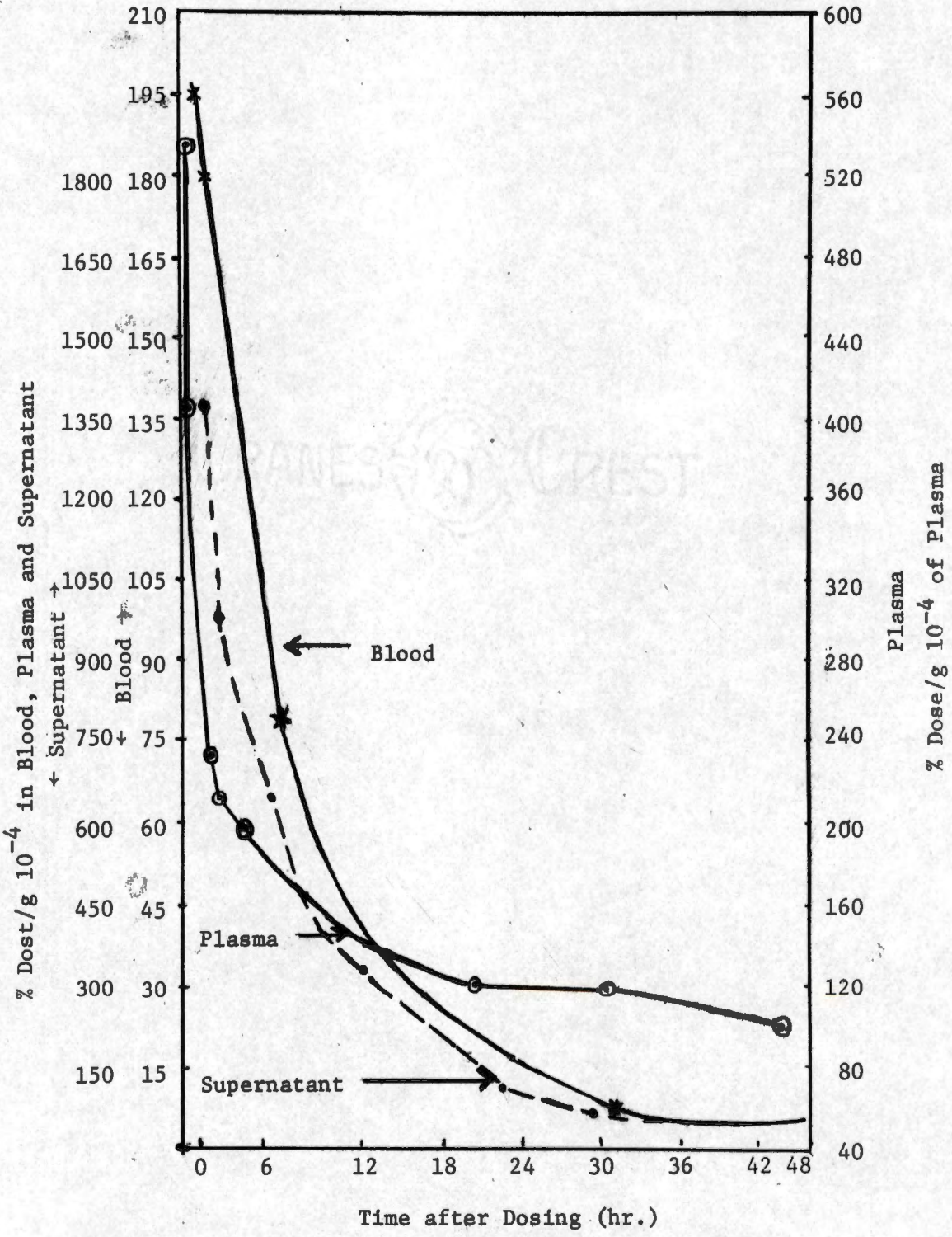

Figure 2. Comparative concentration of ^{28}Mg in blood, plasma and supernatant of sheep.

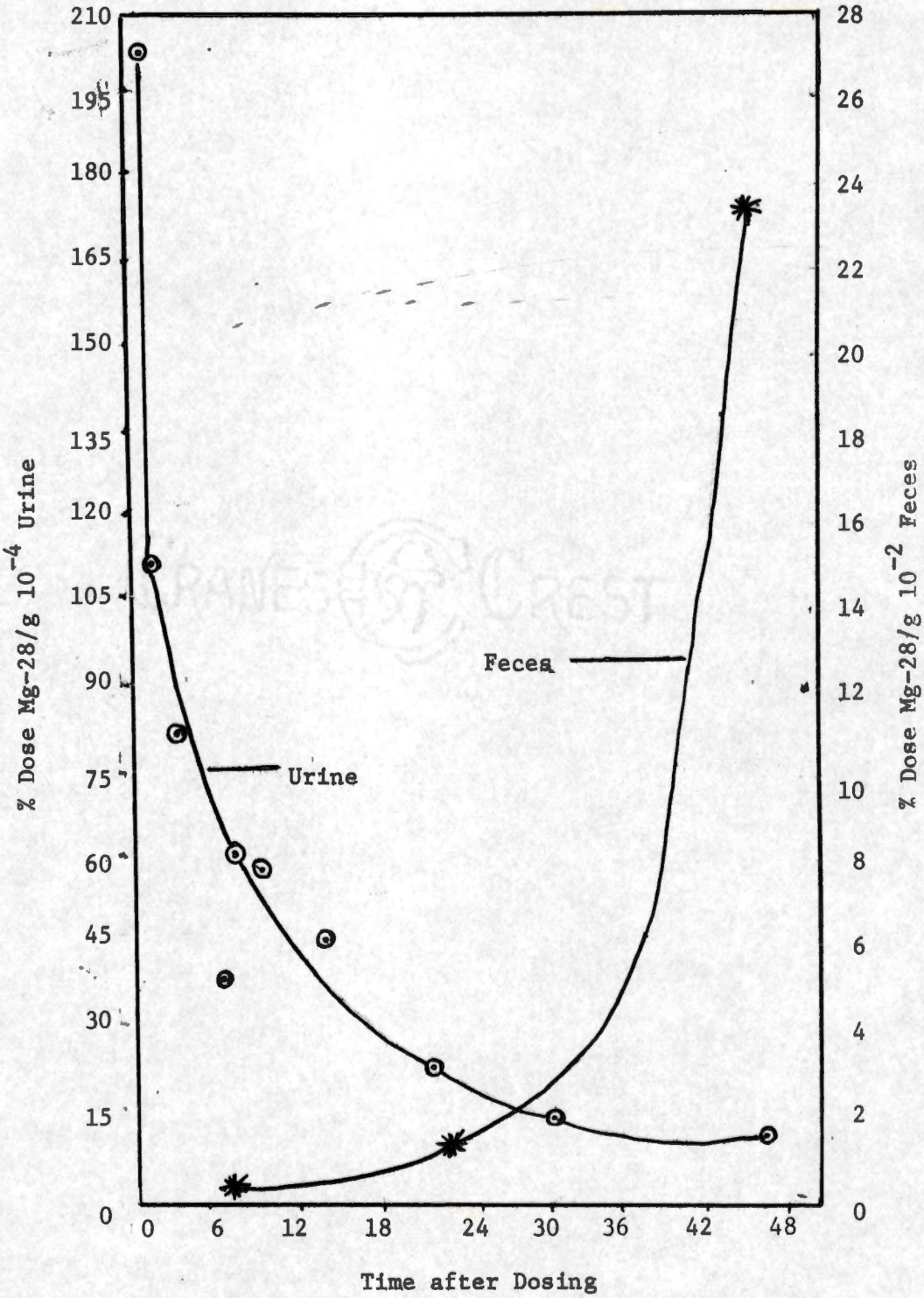

Figure 3. Comparative concentration of ^{28}Mg in feces and urine of sheep.

Only a small percentage of the radio-magnesium was eliminated in the urine and the feces (Figure 3). Less than 0.2% of the intravenous radio-magnesium had been excreted within 35 hours via urine, with highest concentration being observed two hours after dose administration. Less than 2% of the ^{28}Mg dose was excreted in the feces during a 48-hour post-dosing period. These results were similar to those reported by McAleese et al. (1961).

Endogeneous fecal excretion. The endogenous fecal loss of magnesium was derived from the various unabsorbed digestive secretions and the desquamated epithelial cells of the digestive tract (Storry and Rook, 1962). Endogenous fecal magnesium was measured in two wethers and one ewe dosed with 36 μc of carrier-free magnesium, and the total fecal endogenous ^{28}Mg after 36 hours was calculated from the following formula:

$$\frac{\text{Specific activity of feces}}{\text{Specific activity of plasma}} \times \text{daily fecal magnesium loss} \times 100$$

The daily endogenous fecal loss of magnesium averaged 1.03 mg/kg body weight for the ewe and averaged 1.58 mg/kg for the wethers. These results are comparatively lower than those reported by Field et al. (1958) and MacDonald et al. (1959) who reported 4.0 to 5.0 mg/kg body weight in sheep. Blaxter et al. (1954) obtained 5.0 to 6.0 mg/kg body weight in cattle. Care (1960) reported that during periods of magnesium deficiency sheep may be able to conserve magnesium for body utilization by reducing endogeneous fecal magnesium. He also suggested that the concentration of magnesium in the bile decreased during the development of hypomagnesemia. Therefore, the low endogeneous fecal value

noted in this study may have resulted from more body magnesium being utilized, which resulted in the excretion of lesser amounts of endogenous fecal magnesium (Table XX).

Survey of Tennessee cattle. This survey was designed to study selected cation concentrations in random blood samples taken from cows in the state, either having clinical cases of tetany, no evidence of tetany or dead cows suspected of tetany. In nearly all cases, the serum was separated before submission to the laboratory. Table XXI shows the results of the chemical analyses of serum collected from 8 Holstein, 20 Hereford, 12 Jersey and 11 Angus cattle during the winter and spring of 1971. It should be noted that an unknown number of practitioners interpreted "no evidence of tetany" to mean a sick or prostrate animal showing no tremors or convulsions.

Serum samples from 27 non-parous heifers and 32 parous cows were obtained from the Greenville, Highland Rim and the Ames Plantation Experimental Stations to allow a comparison of the serum values of cows obtained from field cases with normal cows (See Appendix Table XXIII, page 88).

The serum calcium values of cows with clinical evidence of tetany ranged from 2.7 mg to 11.84 mg/100 ml with an average value of 6.1 mg/100 ml. The serum magnesium values ranged from 0.24 to 2.45 mg/100 ml with an average of 1.25 mg/100 ml and serum potassium values ranged from 6.9 mg to 36.84 mg/100 ml with an average value of 15.94/100 ml.

The serum calcium values of cows dead from suspected tetany ranged from 3.6 mg to 22.83 mg/100 ml, with an average of 8.98 mg/100 ml.

TABLE XX

FECAL ENDOGENOUS EXCRETION OF MAGNESIUM FROM SHEEP DOSED WITH ^{28}Mg

Sheep Number	Weight, kg	Specific Activity in Plasma	Specific Activity in Feces[a]	Daily Fecal Loss og Mg	Daily Grams of Feces	Endogenous Magnesium, percent[b]	Endogenous Magnesium, Mg/day[c]	Magnesium, mg/kg
1	2	3	4	5	6	7	8	9
Ewe 1	60	1.98	0.60	86.63	170	30.30	51.51	0.86
Ewe 2	60	2.20	0.78	63.89	204	35.45	72.31	1.20
Wether 45	50	2.00	0.65	92.99	301	32.50	97.82	1.95
Wether 51	50	1.50	0.68	68.87	134	45.33	60.74	1.21

[a] S.A. calculated 12 hours after dosing.

[b] Endogenous Mg calculated as $\frac{\text{(specific activity in feces)}}{\text{(specific activity in plasma)}} \times$ daily fecal loss $\times$ 100.

[c] Endogenous magnesium mg/day calculated as loss per 24 hours.

TABLE XXI

SUMMARY SHEET OF BLOOD SAMPLES COLLECTED FROM TENNESSEE CATTLE

Classification	Number of Animals	Calcium, mg/100 ml		Magnesium, mg/100 ml		Potassium, mg/100 ml	
		Mean	Range	Mean	Range	Mean	Range
Clinical evidence of tetany	32	6.10	2.70 to 11.84	1.25	0.24 to 2.45	15.94	6.90 to 46.84
Dead of suspected tetany	10	8.98	3.60 to 22.83	1.35	0.24 to 1.45	41.10	8.30 to 64.80
No observed evidence of tetany	16	7.43	3.70 to 20.60	1.50	0.24 to 2.11	11.26	7.85 to 18.90
Normal young cows	27	7.37	5.80 to 12.10	1.37	0.70 to 2.00	20.21	15.20 to 25.20
Normal old cows	32	7.04	4.20 to 10.50	1.31	0.81 to 1.90	18.64	12.70 to 24.80

The serum magnesium values ranged from 0.24 to 1.45 mg/100 ml, with an average value of 1.35 mg/100 ml, and the serum potassium values ranged from 8.30 mg to 64.80 mg, with an average of 41.10 mg/100 ml. It will be noted in Appendix Table XXIV (page 89) that samples C-1 and C-168 had abnormally high values for calcium. If these two samples are omitted from the average of this group, the average, in milligrams/100 ml, will be 6.32 for calcium, 1.17 for magnesium and 37.41 for potassium.

The serum calcium level of cows with no evidence of tetany ranged from 3.70 mg to 20.60 mg/100 ml with an average of 7.43 mg/100 ml. The serum magnesium values ranged from 0.24 to 2.11 mg/100 ml with an average of 1.50 mg/100 ml and the serum potassium level ranged from 7.85 to 18.90 mg/100 ml with an average of 11.26 mg/100 ml.

The serum levels for cows from herds at the University of Tennessee Experimental Station ranged for calcium from 4.20 to 10.9 mg/100 ml with an average of 7.04 mg/100 ml. The serum magnesium levels of cows ranged from 0.81 to 1.90 mg/100 ml with an average of 1.31 mg/100 ml and the serum potassium ranged from 12.70 to 24.80 mg/100 ml with an average of 18.64 mg/100 ml.

The serum calcium levels for year heifers from the University of Tennessee Experimental Station ranged from 5.8 to 12.1 mg/100 ml, averaging 7.37 mg/100 ml. The serum magnesium levels of the heifers ranged from 0.70 to 2.0 mg/100 ml with the mean value being 1.37 mg/100 ml and the serum potassium level ranged from 15.20 to 25.20 mg/100 ml with an average of 20.21 mg/100 ml.

In Table XXI, page 70, 32 analyses are shwon for the serum samples received. Out of these, the veterinarians had not marked their chart in

some cases as to whether there was clinical evidence of tetany. Hence, the assumption was made that the cows were suffering from tetany or the veterinarian would not have sent the analyses there. The calcium on these samples was low, averaging 6.1 mg/100 ml. The magnesium, however, was not as low as one might suspect, averaging 1.25 mg/100 ml.

Out of the 16 serum samples received from cows with no evidence of tetany, 15 samples averaged low for calcium, except C-176, which had an abnormally high calcium level. The magnesium levels, however, for this group, averaged 1.5 mg/100 ml before adjustment and 1.3 mg afterwards. This was lower than the 2.3 mg/100 ml level cited for normal cows by Storry and Rook (1962). The serum potassium levels averaged 11.2 mg/100 ml before adjustment for this cow.

The serum samples received from the Greenville, Highland Rim Plateau and Ames Plantation Experimental Station averaged low in serum calcium values, while the magnesium levels averaged about the same or slightly higher than the blood from the cows in the clinical cases. These were cattle without observed clinical evidence of tetany. The young cows were yearlings and had not been bred or had just recently been placed with the breeding group.

The lower serum magnesium values for the Experimental Station cattle may have been due to seasonal effect or pasture conditions. Various studies have reported such effects (Kemp et al., 1961). Moreover, the blood samples were collected during grass tetany season. An additional consideration may be that the blood samples were collected from these cattle only one time. Analyses of additional blood samples

from these animals at different seasons would be valuable in establishing both normal and seasonal blood mineral levels.

The low serum magnesium level for those cows with clinical evidence of tetany, and for cows with no observed evidence of tetany was perhaps due to the fact that the samples were collected during the period when grass tetany is not prevelant. Butler (1963) has indicated that tetany prone pastures were significantly lower in magnesium than normal pastures. This report supported the theory that when cows are turned to pasture in the spring, the cows may not consume enough dry matter due to high moisture in the fresh grass, resulting in a low intake of total magnesium.

Moreover, 80% of the blood samples received from the veterinarians were from cows which had recently calved. It had been reported by Wilson (1964) that blood magnesium levels of these cows would be lower than normal. Hence, the low level of magnesium observed in our experiment was in agreement with those reported by Wilson (1964).

Rook and Storry (1962) have stated that hypomagnesemia was not a result of a simple deficiency, but was an anomaly of a complex nature. A number of interacting factors have been shown to be responsible for the disorder, and may be evident in this study.

CHAPTER V

SUMMARY AND CONCLUSIONS

Experiments using rats and sheep and the results of a survey using serum from beef and dairy cows from various areas of Tennessee were used to study the various interrelationships of hypomagnesemia. In the rat experiment, 72 albino rats received 12 synthetic dietary treatments, consisting of three levels of protein (nitrogen), magnesium and potassium. In the sheep study, four Western wethers on magnesium deficient diets received 3.0 and 6.0 g doses of magnesium chloride, oxide, sulfate or nitrate which furnished 0.7, 1.8, 0.6, and 0.3 g of magnesium (3 g dose) and 1.5, 3.6, 1.2 and 0.6 g of magnesium (6 g dose). Two wethers and one ewe, also on magnesium deficient diets, received intravenous dosages of ^{28}Mg. In the survey with beef and dairy cattle, blood serum analyses were performed on 51 cattle of various breeds during the winter and spring of 1971. The results for all experiments are summarized as follows.

The decrease in magnesium and increase in protein and potassium in the diet of rats resulted in lower plasma magnesium concentrations. The decrease in dietary magnesium reduced the plasma magnesium from 2.0 to 1.2 mg/100 ml. The increase in dietary nitrogen and potassium, and the decrease of dietary magnesium reduced the plasma magnesium level of the heart, liver, kidney and femur.

Dietary supplementation of 3.0 and 6.0 g of magnesium chloride, oxide, sulfate and nitrate to wethers on magnesium deficient diets

resulted in an increased plasma level of magnesium, increased fecal and urinary excretion, and a resulting negative balance of potassium and calcium.

Feeding of 45.0 and 25.0 g of magnesium nitrate of an ewe weighing 60 kg and a wether weighing 50 kg, respectively, resulted in the deaths of these animals. These deaths may have been caused by nitrate poisoning.

Studies with sheep dosed intravenously with ^{28}Mg indicated blood, blood plasma and plasma supernatant disappearances to be very rapid during the first two hours after dosing, followed by a slower expotential disappearance rate until approximately 20 hours when most of the blood activity had disappeared. A small quantity of ^{28}Mg was excreted in the feces and urine. Endogenous fecal excretion studies indicated that sheep on magnesium deficient diets excrete smaller amounts of endogenouf fecal magnesium compared to normal animals. These results are believed to be due to body conservation of more magnesium by the deficiency animal.

Short term supplementation of the diet of wethers with citric acid and trans-aconitic acid resulted in no apparent effect on the plasma electrolytes, nor did it induce clinical tetany.

Serum samples from the survey cattle were classified as clinical evidence of tetany, no observed evidence of tetany and cows dead of suspected tetany. Serum analyses showed that all the cows sampled had lower serum magnesium levels than those reported in the literature as normal. Since the samples were collected during the tetany season, the outcome may have resulted from seasonal factors, or the use of single samples.

It may be concluded that the hypomagnesemic condition in animals was not a simple deficiency or a simple interference of another factor, but instead a complex one. The experiments reported in this dissertation showed that increasing dietary nitrogen and potassium as well as decreasing dietary magnesium concentrations would decrease the magnesium levels in the blood of animals. The interferences of nitrogen and potassium in magnesium metabolism may have taken place during the absorption in the digestive system and/or at the cellular level. Since the magnesium ion appeared to be involved in numerous biological processes in the body, it may be expected that any disruptions in these biological processes in turn may affect magnesium metabolism. This conclusion was confirmed by a recent report that trans-aconitic acid, an inhibitor of the tricarboxylic acid cycle, was suggested to be partially responsible for hypomagnesemia in cattle in early spring.

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APPENDIX

TABLE XXII

CALCIUM MAGNESIUM AND POTASSIUM SERUM LEVELS IN COWS WITH CLINICAL EVIDENCE OF TETANY

Number	County	Breed	Age, years	Estimated Days to Parturition	Herd Size	Serum Level, Mg/100 ml		
						Ca	Mg	K
C-3	McMinn	Angus	5	240	40	10.0	0.60	18.60
C-5	Cumberland	Angus	4	-	20	8.8	1.50	32.00
C-9	Rutherford	Hereford	6	30	10	5.2	0.51	22.80
C-63	Haywood	Holstein	5	90	84	6.4	1.70	8.80
C-64	Haywood	Holstein	5	-	84	7.1	1.90	11.30
C-65	Haywood	Holstein	5	-	84	7.0	1.87	11.20
C-67	McMinn	Hereford	?	30	25	7.7	1.40	13.00
C-68	McMinn	Hereford	?	30	50	4.6	0.78	11.10
C-69	McMinn	Hereford	?	30	69	5.4	0.1	11.00
C-70	McMinn	Angus	?	30	12	6.7	0.81	9.00
C-77	McMinn	Jersey	7	150	-	2.9	1.17	8.30
C-82	McMinn	Holstein	8	180	75	4.6	1.49	7.90
C-85	McMinn	Hereford	4	30	25	3.7	0.24	7.70
C-86	McMinn	Hereford	4	14	25	3.3	0.34	18.40
C-87	Rutherford	Hereford	8	30	-	7.9	1.20	11.50
C-90	Rutherford	Angus	7	10	88	2.7	1.50	6.90
C-115	Rutherford	Herefore	9	270	140	8.2	1.43	11.80
C-136	Cumberland	Angus	10	14	40	4.5	0.42	36.84
C-137	Cumberland	Angus	5	14	-	3.4	0.29	27.72
C-138	Rutherford	Hereford	8	42	-	6.6	0.42	22.00

TABLE XXII (Continued)

Number	County	Breed	Age, years	Estimated Days to Parturition	Herd Size	Serum Level, Mg/100 ml		
						Ca	Mg	K
C-170	Hamilton	Hereford	10	30	-	11.8	2.20	14.20
C-171	Chester	Jersey	5	42	-	8.52	2.39	18.73
C-172	Rutherford	?	4	7	-	4.59	2.45	14.15
C-173	Rutherford	?	?	-	-	7.40	0.50	18.70
C-174	Knox	Jersey	?	180	-	6.50	2.40	19.60
C-175	Knox	Jersey	?	180	-	6.50	2.40	19.60
C-177	McMinn	Jersey	5	-	-	3.33	1.53	20.60
C-182	McMinn	Holstein	6	-	-	3.00	1.57	19.20
C-178	McMinn	Holstein	10	-	-	9.12	1.80	25.00
C-179	Roane	Angus	?	-	-	6.70	0.80	10.80
C-180	Roane	?	?	-	-	7.70	1.33	9.20
C-181	Roane		?	-	-	3.60	1.23	12.50
Average						6.10	1.25	15.94

TABLE XXIII

CALCIUM, MAGNESIUM AND POTASSIUM SERUM LEVELS IN COWS WITH "NO EVIDENCE OF TETANY"

Number	County	Breed	Age, years	Estimated days to Parturition	Herd Size	Serum Levels, mg/100 ml		
						Ca	Mg	K
C-2	McMinn	Angus	3	14	10	8.9	2.40	17.1
C-10	Moscow	Holstein	3	90	-	10.6	1.85	15.4
C-71	Coffee	Hereford	8	30	100	5.9	1.41	9.3
C-72	Rutherford	Hereford	10	150	30	6.1	0.67	8.1
C-73	Rutherford	Jersey	8	45	40	3.0	0.37	7.8
C-74	Wilson	Hereford	10	21	80	6.9	1.16	9.2
C-75	Rutherford	Hereford	6	30	36	8.4	1.40	12.12
C-76	Rutherford	St. Horn	12	-	10	6.4	1.33	10.35
C-78	McMinn	Jersey	7	Full Term	-	5.4	1.12	11.62
C-79	McMinn	Jersey	5	3	15	4.7	2.11	10.57
C-80	McMinn	Jersey	5	10	100	5.7	1.37	9.88
C-84	McMinn	Jersey	6	2	15	3.7	0.24	7.90
C-88	Rutherford	Jersey	10	60	-	8.3	1.10	9.00
C-89	Lincoln	Holstein	6	30	100	8.5	2.00	5.70
C-169	Rutherford	Hereford	10	240	160	8.8	1.46	16.22
C-176	McMinn	Jersey	8	30	50	17.5	4.14	18.90
Average						7.43	1.50	11.26

TABLE XXIV

CALCIUM, MAGNESIUM AND POTASSIUM SERUM LEVELS IN COWS DEAD OF SUSPECTED TETANY

Number	County	Breed	Age, years	Estimated Days to Parturition	Herd Size	Serum Levels, mg/100 ml		
						Ca	Mg	K
C-1	Blount	-	13	30	-	16.4	3.6	64.8
C-4	Cumberland	Angus	4	21	-	7.3	0.24	15.2
C-6	Henderson	Hereford	5	60	75	9.1	1.39	54.1
C-7	Henderson	Hereford	-	-	75	9.1	1.39	54.1
C-8	Henderson	Hereford	-	-	75	5.1	1.39	55.0
C-11	Bledsoe	Angus	3	30	-	4.5	1.06	17.6
C-66	Henderson	(calf)	5	-	-	4.4	0.79	27.5
C-81	McMinn	Hereford	6	30	-	3.6	1.45	8.3
C-168	Sequatchie	Hereford	10	60	15	22.83	0.45	46.98
C-183	Cumberland	Angus	2(mo.)	-	210	7.4	1.88	62.00
Average						8.98	1.35	41.10

VITA

T. Seenappa was born in Mysor State, India on April 6, 1928. He was graduated from a Government High School in 1945. He attended the University of Bombay and received a Bachelor of Veterinary Science Degree in 1953. From 1953 until 1960, he worked as a Veterinarian in Mysore State. He served as a lecturer in physiology in Veterinary School, Bangalore from 1960 to 1963.

In September 1963, he entered graduate school at the University of Tennessee. He received a Master of Science Degree in Animal Husbandry in December 1964. Upon returning to India, he served for three years as Assistant Professor of Animal Husbandry in Bangalore Veterinary School. He obtained a Scholarship in September 1968 to work on an advanced Degree in Animal Nutrition. In August 1971, he was graduated with a Doctor of Philosophy Degree.

He is married to the former R. Jayalakshmi of Bangalore and they have five children.