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I am submitting herewith a thesis written by Christopher K. Reynolds entitled "A Study of Magnesium Metabolism, Depletion and Repletion in Young Calves." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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A STUDY OF MAGNESIUM METABOLISM, DEPLETION AND REPLETION IN YOUNG CALVES

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Christopher K. Reynolds

December 1981

3055554

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. M. C. Bell for his advice and guidance in conducting the research and preparation of this manuscript. The author wishes to thank Dr. M. H. Sims for his help in conducting the research, serving on the graduate committee and reviewing the manuscript. The author is grateful to Dr. J. P. Hitchcock for serving on the graduate committee and reviewing the manuscript. The author also wishes to thank Dr. J. B. McLaren for advice and help in conducting the statistical analysis.

The author is grateful to Gayle Lambert, Debra Saul, David Hodge and all fellow graduate students for assistance in collection of data and advice during this research.

The author is also grateful to The University of Tennessee Computer Center for the use of that facility for analyses of these data.

Deep appreciation is also expressed to his parents, Darlene and Richard S. Reynolds, Jr., and all his family and friends who have provided support and encouragement throughout his college career.

ABSTRACT

The objectives of this experiment were: (1) to produce hypomagnesemia in young male calves; (2) to study the effects of magnesium (Mg) deficiency and excess dietary potassium (K) on mineral metabolism in hypomagnesemic and normal calves; and (3) to study the effects of $MgCl_2 \cdot 6H_2O$ enemas on plasma, cerebrospinal fluid (CSF) and red blood cell (RBC) mineral levels. In this study, calves were used as models for adult, lactating cows.

Preliminary studies were initiated to establish procedures and determine the effectiveness of a semi-purified, low Mg, dry diet. Calves did poorly on the initial diet used in 2 preliminary trials involving 2 calves each. The composition of the diet was altered and a calf was maintained on this diet alone for 7 weeks. After the calf became severely hypomagnesemic, plasma and CSF Mg level were repleted using an enema of $MgCl_2 \cdot 6H_2O$ solution. Procedures for administering the enema and taking samples of blood and CSF were refined and finalized with this calf.

Two trials were run involving 8 calves each which were fed 4 different diets. The diets were: Mg deficient basal diet, basal diet plus K, basal diet plus Mg and basal diet plus Mg and K. For the balance study, only samples collected from 9 calves were used for statistical analysis because of problems with diarrhea and anorexia. Samples of feces were collected using canvas collection bags and aliquots of urine were collected daily for a 5 day balance trial.

All 16 calves were used in the repletion study which was initiated immediately after the balance study for both trials. Each calf was given an enema of $MgCl_2 \cdot 6H_2O$ solution while under general anesthesia. The enema was held in for 5 minutes and samples of blood and CSF were taken at 5, 15, 30, 60, 120 and 180 minutes after administration of the enema.

For calves fed the Mg supplemented diets, the intake, fecal excretion and balance of Mg were greater ($P < .05$) than calves fed deficient diets. Calf urinary excretion of Mg was not different among treatments. Supplemental K appeared to have no effect on Mg excretion or balance.

Dietary K appeared to have no effect on plasma Mg level. The $MgCl_2 \cdot 6H_2O$ enemas caused a rapid increase in plasma Mg level. A significant increase in CSF Mg level was also observed in calves on the Mg deficient diets which had subnormal levels of CSF Mg. The $MgCl_2 \cdot 6H_2O$ enema appeared to have no effect on plasma calcium (Ca), and K, RBC minerals and packed cell volume (PCV). One calf in trial 1 had 4 episodes of tetany and was treated successfully 3 times with a $MgCl_2 \cdot 6H_2O$ enema. CSF samples taken during tetany had subnormal levels of Mg. Young dairy calves appeared to be good models for Mg studies in adult cows.

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CHAPTER I

INTRODUCTION

Mg is an essential mineral element in ruminant nutrition. Much of the Mg found in the body is stored in bone and the availability of this stored Mg has been shown to decrease with age. Mg found in the soft tissues is involved in numerous enzymatic reactions and is essential for normal neuromuscular function (Rook and Storry, 1962). Hypomagnesemia and grass tetany is a metabolic disorder which occurs in ruminants world-wide. The disorder occurs primarily in older, lactating females grazing lush, rapidly growing spring forage. Since 1928, the disease has been known to be related to low serum levels of Mg (Grunes et al., 1970).

Numerous factors have been shown to affect the metabolism of Mg and play a role in the etiology of grass tetany. Most of these factors either cause reduced intake of Mg or affect the utilization of Mg within the body. Mg metabolism and the way various factors affect Mg utilization varies greatly among animals. Many animals with severe hypomagnesemia may never develop tetany.

Meyer (1977) suggested that the occurrence of hypomagnesemic tetany was dependent on a drop in CSF Mg level. He suggested that when CSF Mg level dropped below 1.6 mg/dl the risk of hypomagnesemia was increased and that below 1.2 mg/dl Mg in the CSF, tetany occurred regularly. Work by Allsop and Pauli (1975a, 1975b) has added further evidence that supports this theory. If correct, this theory would help explain why

animals with extremely low plasma Mg may never develop tetany. Prolonged severe hypomagnesemia is usually required before CSF Mg level declines. High levels of Mg in the CSF have been associated with hypoexcitability and low levels of CSF Mg have been associated with hyperexcitability (Sims et al., 1980).

Meyer and Busse (1975b) and Bell et al. (1977) have reported on the use of $MgCl_2 \cdot 6H_2O$ enemas for elevating plasma Mg levels and have suggested its use as an emergency treatment for grass tetany. Meyer and Busse (1975a) have reported on the repletion of CSF Mg in sheep using intravenous injections of Mg gluconate but no reports were found on repletion of CSF Mg using Mg enemas.

While hypomagnesemic tetany occurs primarily in cows on pasture in early lactation, calves were used as models for hypomagnesemia in this study because of convenience in maintenance and handling. The objectives of this study were to study effects of Mg deficiency and excess dietary K on mineral metabolism in young male calves and to study the effects of $MgCl_2 \cdot 6H_2O$ enemas on CSF, plasma and RBC mineral levels in hypomagnesemic and normal calves.

CHAPTER II

LITERATURE REVIEW

Characterization of Hypomagnesemia

Hypomagnesemia is a metabolic disorder which has been researched extensively for many years. Low levels of serum Mg were first reported to be associated with the disease grass tetany by Sjollema in the Netherlands in 1928. In that region the disease had been a problem in lactating dairy cows for years before Sjollema's first paper (Grunes et al., 1970). Grass tetany has been reported in ruminants worldwide, primarily in cattle and sheep. It is a disease that has many names which vary regionally. These names include hypomagnesemic tetany, lactation tetany, grass staggers and wheat pasture poisoning.

The physical symptoms of hypomagnesemic tetany can include hyper-excitability, loss of coordination, anorexia, muscular twitching, profuse salivation, trismus, inability to stand, tetanic convulsions, labored breathing, increased pulse rate, opisthotonos, nystagmus and death (Aikawa, 1971; Fontenot, 1979; Rook and Storry, 1962; Stewart, 1954). Aikawa (1971) reported that 30% of the clinical cases of hypomagnesemic tetany are fatal and that death can occur within 30 minutes after onset of tetany. In some clinical cases lowered plasma Ca levels have been reported; however, the only consistent change in mineral composition found is lowered Mg concentration in the plasma. Normal plasma Mg levels in cows can vary from 1.5 to 3.1 mg/dl (Rook and Storry, 1962). Severe and prolonged hypomagnesemia is usually

present before clinical tetany occurs. Animal response to hypomagnesemia varies greatly and in many cases an animal with severe hypomagnesemia may never develop clinical tetany. If tetany does occur, anorexia, hyperexcitability and slight muscular twitching may be the only symptoms evident before the onset of tetany. The onset of an episode of tetany may be gradual or may occur when a sudden noise startles the animal and causes a reflex response which results in tetanic convulsions (Grunes et al., 1970). Other types of stress such as being handled or estrus can bring about the onset of clinical tetany in hypomagnesemic animals. Meyers and Scholz (1972) and Pauli and Allsop (1974) have reported that the onset of cases of grass clinical tetany is dependent on a drop in the level of CSF Mg. The danger of tetany is believed to be greater when Mg levels in the CSF drop below 1.6 mg/dl.

Practically all cases of hypomagnesemic tetany occur in adult females which are in the first 6 weeks of lactation or near parturition and on diets which are primarily forage. The disease has also been reported in steers and calves (Crookshank and Sims, 1955; Haggard et al., 1978).

In the United States, grass tetany is primarily a problem in beef cattle, although there are occasional cases in sheep and dairy cattle. Fontenot (1980) stated that the disease occurs in less than 1% of adult females, but the incidence can be much higher in individual herds. Fisher et al. (1978) reported that stockmen in the United States lose over $70,000,000 annually due to hypomagnesemic tetany. The incidence of tetany is greatest in early spring when lactating females are turned

out onto lush, rapidly growing pasture. Tetany can also occur during other periods of the year (Grunes, 1973; Kelley, 1979; McAleese et al., 1961). Tetany can also occur when cattle are fed wintering rations utilizing roughages (Henry et al., 1977).

The occurrence of tetany does not appear to be strictly a problem in herds with poor animal management. The incidence of tetany is high in well managed herds and often may be related to forage fertilization practices (Fontenot et al., 1973). Since the incidence of tetany is often high in individual herds it can be financially devastating for stockmen. Usually when 1 animal in a herd develops tetany, many other animals in the herd are also hypomagnesemic (Todd and Horvath, 1970). It would be difficult to estimate what percentage of the United States beef population is affected by non-clinical hypomagnesemia, although its occurrence is known to be much greater than hypomagnesemic tetany. Prolonged non-clinical hypomagnesemia can cause anorexia and irregular heart activity and therefore can limit animal production to a greater extent than hypomagnesemic tetany (Fisher et al., 1978). Bell (1980) reported that the financial losses from decreased productivity due to hypomagnesemia are greater than death losses due to hypomagnesemia. Non-clinical hypomagnesemia is only detectable by blood analysis.

The reason for the extremely varied animal response to hypomagnesemia is not certain. Research has shown that there are numerous agronomic and nutritional factors and interactions which can affect the incidence of the disorder. It appears that animals which are susceptible to hypomagnesemia are less able to compensate for changes in Mg excretion and/or absorption and therefore can not maintain a body pool of Mg

adequate for tissue requirements. Increased exertion of Mg in the milk during early lactation and grazing of lush spring pastures low in Mg content are certainly 2 major factors in the etiology of the disorder.

It seems that older cows are more susceptible to hypomagnesemia and this may be due to a decreased ability to mobilize body reserves of Mg (Jacobson et al., 1972; Fontenot et al., 1973; Meyer, 1976a; Fisher et al., 1978). Kemp (1960) reported that the mobilization of bone Mg in mature cows was insufficient to prevent hypomagnesemia. Rook and Storry (1962) reported that as an animal matures the blood supply to the skeleton decreases, reducing the mobility of skeletal Mg. In younger animals as much as 30% of skeletal Mg can be mobilized and translocated if needed by other tissues, while only 2% of bone Mg is available in mature animals. Smith (1959a) determined that the concentration of bone Mg decreased with increasing age in calves. Chicco et al. (1973b) stated that plasma Mg and voluntary feed intake dropped more rapidly in older sheep when various aged wethers were fed a Mg deficient diet. They attributed this effect to the older animals' decreased ability to mobilize bone Mg. It was suggested that the greater exchangeability of bone Mg in young animals might be due to smaller mineral crystal size and the presence of more water in the organic matrix surrounding the crystals.

Absorption of Magnesium

A great deal of Mg related research conducted over the last 30 years concerned the sites and mechanisms of Mg absorption from the gastrointestinal tract. The efficiency of absorption of Mg from various diets is extremely variable and is controlled and regulated in many ways. Many of the dietary factors which have been shown to play a role in

the development of hypomagnesemia do so by altering the absorption of Mg. More knowledge concerning the mechanisms of Mg absorption is needed in order to better understand the etiology of hypomagnesemia and how to prevent its occurrence.

Mg present in the gastrointestinal tract is from both dietary and endogenous sources. Many digestive secretions such as bile, pancreatic juice and saliva contain Mg (Care and Van't Klooster, 1965; Pfeffer et al., 1970) and some endogenous Mg is from cell sloughage in the digestive tract (Rook and Storry, 1962). Therefore any reduction in Mg absorption can result in a net loss of endogenous Mg (Wilson, 1964). Adequate absorption is essential for maintaining body levels of magnesium especially during periods of increased excretion, as with lactation.

Sites of absorption. Many early researchers concluded that the small intestine was the principal site of Mg absorption in ruminants. Field (1961) determined from studies using ^{28}Mg that absorption occurred primarily in the middle portion of the small intestine. Phillipson and Storry (1965) isolated portions of the digestive tract in sheep and added various concentrations of Mg to them, estimating absorption by measuring changes in the Mg concentration of the contents of the isolated portions. They found the rumen epithelium to be relatively impermeable to Mg. Secretion and absoprtion were noted in all loops of the small intestine; however, secretion was found most often in the duodenum and upper jejenum. Stewart and Moodie (1956) concluded that in sheep the major site of absorption was in the small intestine. They did find absorption occuring in other segments of the gastrointestinal tract, including the rumen.

Recent research has shown that the reticulo-rumen is a major site of Mg absorption. In a preliminary study, Grace (1970) placed cannulas in the rumen, the proximal duodenum and the terminal ileum of 4 sheep being fed forage. He found that 95% of the absorbed Mg was absorbed before the proximal duodenum. In a subsequent study Grace and Macrae (1972) obtained similar results, with net absorption occurring from the stomach and duodenum. Grace et al. (1974) found net absorption of Mg occurred in the stomach and net secretion of Mg occurred in the duodenum. Ben-Ghedalia et al. (1975) found the forestomach to be a major site of Mg absorption in sheep. They concluded that the omasum was probably the primary site of Mg absorption since other researchers found the reticulo-rumen to be impermeable to Mg ions.

Tomas and Potter (1976a) sought to determine in which portions of the ruminant stomach Mg absorption actually occurred. They placed cannulas in the rumen, omasum, abomasum and duodenum of sheep on a low Mg diet. They found that no Mg-acetate or Mg-gluconate was absorbed before it reached the duodenum when infused into the omasum or abomasum. However 36-61% of the Mg infused into the rumen was absorbed, indicating that absorption occurred in the reticulo-rumen. They concluded that Mg absorption postruminally was insufficient to maintain normal levels of Mg in the animal. Field and Munro (1977) obtained similar results working with sheep. They did find that there was some absorption occurring when Mg was infused into the omasum. Absorption was measured as the change in urinary excretion and apparent absorption observed when the infusion site was changed. Pfeffer et al. (1970),

Axford et al. (1975) and Stevenson and Unsworth (1978) have also found that net absorption does occur in the stomach of sheep.

Smith (1959b) found the large intestine to be a major site of absorption in the young milk fed calf. Between 40 and 70% of the Mg not absorbed in the small intestine was absorbed in the hindgut. He also found that the absorptive capability of the large intestine decreased with age. Aikawa (1959), using ^{28}Mg as the sulfate in rabbits concluded that there was no Mg absorption from the large intestine. Chutkow (1964a), using ^{28}Mg as the chloride in rats, determined that 70% of the total absorption of Mg occurred in the colon. Ben-Ghedalia et al. (1975) concluded that the colon and the forestomach were the major sites of Mg absorption in sheep. Other researches have also observed net absorption of Mg in the large intestine of sheep (Pfeffer et al., 1970; Grace et al., 1974; Tomas and Potter, 1976b; Stevenson and Unsworth, 1978; Green et al., 1981).

Apparently, Mg absorption occurs along almost the entire length of the gastrointestinal tract. The Mg absorption from the duodenum and jejunum is evidently masked by secretion of Mg (Ben-Ghedalia, et al., 1975). Perry et al. (1967) found that a net secretion of Mg occurred in the upper small intestine of calves.

Mechanisms of absorption. The actual mechanisms involved in Mg absorption are still uncertain. Rook and Storry (1962) theorized that Mg was absorbed from the duodenum by passive diffusion. They based this theory on the concentrations of ultrafilterable Mg in the lumen of the gastrointestinal tract and the electrical potentials present between the

lumen and the blood supply. Wilson (1964) reviewed the conflicting conclusions other researchers had made concerning the mechanism of Mg absorption and reported that absorption could occur by three methods: simple diffusion, facilitated diffusion and active transport. He also mentioned the possibility that Ca and Mg could be absorbed competitively by a common mechanism. Care and Van't Klooster (1965) also suggested that Mg and Ca were absorbed by the same mechanism. They suggested that absorption from the ileum was by a facilitated diffusion process involving a limiting carrier system.

Behar (1974) conducted studies using the ileum and colon of rats in vivo. His theory was that Mg is primarily transported by a solvent drag mechanism. He found that the amount and direction of water flow affected Mg transport and that sodium (Na), sugars, urea and hypertonic solutions could cause Mg transport by stimulating water flow. Behar found net absorption occurred against an electrochemical gradient and also suggested the possibility of another transport mechanism, the passive diffusion of ions into epithelial cells, which he believed was of minor consequence.

In subsequent studies with rat ileum in vivo, Behar (1975) found the absorption of Mg was saturable at higher Mg concentrations, a finding many researchers have interpreted as indicating that a facilitated or active transport mechanism was involved. He explained the observed saturability of the transport mechanism as being due to a reduction in membrane permeability caused by the higher concentration of Mg.

Tomas and Potter (1976a), working with sheep, interpreted their results as showing that Behar's solvent drag theory alone could not

account for the amount of Mg they observed being absorbed into the reticulo-rumen. The concentration of the rumen fluid (1.52 mg/dl Mg) was too low for Mg to have been absorbed totally as a solvent in water passing into the rumen epithelium. They also suggested that the reticulo-rumen may not be permeable to bulk flow transfer of Mg and believed that other transport mechanisms are involved.

Brown et al. (1978) measured the absorption of solutions of Mg varying in concentration. These solutions were infused into isolated dorsal rumen sacs in sheep. They also measured the potential difference between the rumen fluid in the pouch and venous blood. They found that transport of Mg occurred against an electrochemical gradient and that it was a saturable mechanism, indicating the involvement of an active transport mechanism.

In vitro evidence for an active transport mechanism of Mg absorption in the reticulo-rumen has been reported by Martens et al. (1978), who studied the movement of ^{28}Mg across isolated sheep rumen epithelium. They found that magnesium transport occurred without the presence of a concentration gradient and that Mg moved against a potential difference present in the rumen epithelium. They also found that the transport mechanism was saturable at higher concentrations of Mg and was sensitive to changes in the temperature of the bathing solutions. Ouabain, an inhibitor of Na-K-ATPases, inhibited Mg movement across the rumen epithelium by 90%. This may indicate the involvement of a Na-K transport system in Mg absorption. This study did not find evidence for the absorption of Mg via facilitated diffusion or bulk flow of water in the rumen.

It is quite possible that more than 1 mechanism is responsible for the absorption of Mg in the ruminant and that 1 mechanism may be more important in 1 region of the tract than the other. Significant Mg transport by passive diffusion is not likely in any region of the tract due to the potential difference between the blood and the lumen contents of the gastrointestinal tract. Whatever the mechanism, the efficiency of absorption of dietary Mg is low and is usually between 15 and 30% in mature animals (Field and Munro, 1977).

Excretion of Magnesium

Endogenous Mg can be excreted via secretions and cell sloughage into the digestive tract. Saliva and gastric juice are 2 of the major sources of endogenous fecal Mg, while cell sloughage accounts for only a minute amount. Saliva contains .6 to 1.58 mg/dl Mg in sheep and cows. Gastric juice, bile, pancreatic juice and Brunner's gland secretions contain from .84 to 3 mg/dl Mg (Rook and Storry, 1962). Daily endogenous excretion of Mg in calves has been reported to be 3.5 mg/kg body weight while it is estimated that cows excrete anywhere from .5 to 5 mg/kg body weight. The fecal Mg endogenously excreted in calves has been shown to increase per unit body weight as calves mature and this may be due to a decrease in absorption of Mg in the large intestine (Miller et al., 1972). Lomba et al. (1968) reports that fecal endogenous losses of Mg are highly variable. Simeson et al. (1962) reported that endogenous excretion of Mg into the gastrointestinal tract varies with type and amount of feed consumed; however, reports have differed (Miller et al., 1972).

House and Van Camapen (1971) reported that feeding wethers supplemental KCl increased endogenous fecal Mg losses. Losses of endogenous Mg in the feces have been shown to be similar in normal and hypomagnesemic calves (Rook and Storry, 1962). Net excretion of intravenous ^{28}Mg in the feces was 3.6 to 5% of the dose in control lambs and in Mg deficient lambs it was 1.7 to 2.1% of the dose (MCAleese et al., 1961).

Field et al. (1958) found that in wethers a change in dietary Mg was reflected in urinary Mg levels. Kemp et al. (1961) found that the amount of Mg an adult lactating cow excreted in the urine was positively correlated with the amount of "available" Mg which was not excreted in the milk. They concluded that the level of Mg in the urine was a better indicator of an animal's Mg status than plasma Mg since hypomagnesemia occurred only when the amount of "available" Mg in the urine was below 1 g/day. A fall in urinary Mg is closely followed by a drop in plasma Mg level. McAleese et al. (1961) found that urinary excretion of Mg was much greater in control lambs than in Mg deficient lambs. Storry and Rook (1963) studied Mg excretion using 2 cows given subcutaneous injections of $MgSo_4 \cdot 7H_2O$. They found that below plasma Mg concentrations of 1.7 mg/dl in 1 cow and 1.46 mg/dl in the other urinary excretion of Mg ceased. Apparently the threshold level for urinary Mg varies among animals. Virtually all plasma Mg above the threshold level is excreted in the urine and excretion stops when plasma levels drop below the threshold level. Excessive intake or kidney disfuction is required before plasma levels of Mg increase significantly (Miller et al., 1972). A correlation between absorption of Mg and

urinary excretion has been found and apparently the excretion of urinary Mg reflects the dietary Mg availability (Littledyke and Cox, 1979).

Meyer (1976a) describes Mg excretion in the kidney as being like a cuneiform slit which closes when plasma Mg levels drop below 1.8 mg/dl. Meyer believes that this level is when tubular reabsorption of almost all glomerular filtrated Mg is occurring. Reabsorption of glomerular filtrated Mg is lowered as plasma Mg rises until finally the "cuneiform slit" is wide open. Meyer estimates this point to be when plasma Mg level is at 3 to 3.2 mg/dl. Many factors have been shown to affect renal Mg excretion. Infusions of $NaCl_2$, $CaCl_2$ and acetylcholine have been shown to increase urinary Mg excretion (Ebel and Gunther, 1980), while parathyroid hormone is believed to decrease Mg excretion in the urine (Littledyke and Cox, 1979). The actual mechanism by which Mg is excreted in the kidney is uncertain. Excretion could be due to decreased tubular absorption or to secretion (Littledyke and Cox, 1979).

Factors Affecting Magnesium Utilization

Feeding schedule and age of animal. It has been shown that many factors affect Mg metabolism. Many of these factors have been shown to affect the site and extent of Mg absorption. Grace and Macrae (1972) found that the number of feedings per day affected the extent of absorption. With continuous feeding 90% of net Mg absorption occurred in the stomach, while with once a day feeding only 50% of net absorption occurred in the rumen. There was no effect on total absorption. Smith (1959b) found that age affected the extent of absorption in calves.

Organic constituents in ration. The apparent absorption of forage Mg is related to the crude protein content of the forage by Kemp (1960). He found that as crude protein increased there was a decrease in apparent Mg availability. Grace and Macrae (1972) found that there was no effect of protein supplementation with casein on absorption of Mg. In a study by Moore et al. (1972) wether lambs were fed 2 levels of non-protein nitrogen (N). There were no effects on apparent absorption of Mg observed with any treatment. Ruminal ammonia levels were increased with the high dietary level of crude protein and ruminal pH was increased by feeding the higher urea level.

It has been postulated that high levels of dietary protein reduce Mg absorption by causing high levels of ammonia in the rumen (Kelley, 1979). Henry et al. (1977) infused histamine, ammonia, histamine plus ammonia and deionized water into the rumen of fistulated lambs being fed a semi-purified diet low in Mg (.08%). One lamb in the group receiving the histamine plus ammonia infusions and 2 lambs receiving ammonia infusions developed hypomagnesemic tetany. The researchers concluded that histamine had no effect on serum Mg levels and that high levels of ammonia may lead to depression of plasma Mg levels. They suggested that excess ammonia may affect plasma Mg by altering ionic balance. Fontenot (1979) stated the the increased urinary Mg observed when feeding high levels of dietary N may indicate that there is interference with Mg utilization within the body.

Meyer (1976a) believes that the reduction in Mg absorption attributable to the intake of protein rich feeds is due to the increase in

ruminal pH it causes. As the pH of the rumen goes up, the solubility of Mg decreases. According to Rook and Storry (1962) the acidity of the digesta in the gastrointestinal tract is important for Mg absorption since a low pH is needed for Mg to be present in soluble or ultrafilterable form. The pH of the digesta increases as it passes along the small intestine from the abomasum and there is a decrease in Mg solubility (Ben-Ghedalia et al., 1975). Horn and Smith (1976) infused HCl (.2M) into the rumen of calves for 8 hours after they were fed. They observed a small decrease in rumen pH and a significant increase in Mg absorption. Their conclusion was that lactating cows grazing lush spring pasture may be more susceptible to hypomagnesemia due to a constant high ruminal pH.

The complexing of Mg by dietary compounds has been shown to interfere with Mg absorption in various species. Some of these Mg complexing agents include lignin, organic acids and phytate. Meintzer and Steenbock (1955) found that in rats fed a semi-synthetic diet, phytate reduced Mg absorption slightly. Dutton and Fontenot (1967) found there was no effect of form on Mg absorption in sheep fed purified rations containing either organic or inorganic phosphorus (P). It is unlikely that phytate has an effect on Mg absorption in ruminants since the rumen has been shown to contain phytase (Care, 1967; Kelley, 1979).

It has been shown that supplementation of ruminant diets with readily fermentable carbohydrates can increase the absorption of Mg. Madsen et al. (1976) found that supplementation with glucose increased the apparent absorption and urinary excretion of magnesium in sheep

fed vegetative grasss but did not affect apparent absorption. Energy supplementation resulted in higher serum Mg levels in lactating cows in a study by Boling et al. (1979) but the effect was dependent on time. The cows supplemented with MgO had the highest serum levels of Mg. House and Mayland (1976) found that in lambs fed supplemental sucrose, urinary excretion and apparent absorption of Mg was greater than in lambs fed the same diet without supplemental sucrose. There were no differences observed in plasma Mg levels. They also found that decreasing the level of starch in a semi-purified diet fed to a similar group of lambs had no effect on Mg utilization.

Lactose has been shown to increase Mg absorption and urinary excretion in rats (Forbes, 1961). Rayssiguier and Poncet (1980) found the addition of lactose to a hay diet increased the absorption of Mg in the stomach of sheep and also increased Mg secretion into the small intestine. They also found that the addition of lactose to the diet lowered ammonia levels and pH in the rumen and suggested that the increase in Mg absorption observed was due to changes in microbial fermentation.

Ration magnesium and other minerals. The quantity of magnesium in the diet can also affect its absorption. Field and Munro (1977) and Brown et al. (1978) have reported that Mg absorption is saturable when higher Mg concentrations are present. McAlesse et al. (1961) found that lambs on a Mg deficient diet absorbed more ^{28}Mg than control lambs.

Numerous interactions with other minerals have also been shown to affect Mg metabolism. The effects of Ca, P and K on Mg metabolism

have been researched extensively. Recent research has shown that high levels of aluminum (Al) can decrease the solubility of Mg in rumen fluid in vitro while high levels of manganese (Mn) had no effect on Mg solubility. High levels of Al (1,000 to 14,500 ppm) were found in forage samples from limited areas of pastures where tetany had occurred and in rumen samples from animals which had died from tetany (Allen and Robinson, 1980). The authors concluded that levels of Al above 500 ppm in forages can be involved in the etiology of grass tetany.

Feeding high levels of Ca and/or P can also interfere with the utilization of Mg. Feeding high levels of Ca and/or P to chicks had no effect on plasma Mg levels; however, the P did appear to reduce the absorption of Mg (Nugara and Edwards, 1961). Wise et al. (1963) fed varying levels of Ca and P to calves. The high P and low Ca ration depressed serum Mg. Feeding the high Ca level did not affect blood Mg level when fed with either high or low P levels. Toothill (1963) showed that in rats Mg absorption was decreased by increases in dietary Ca or P. The effect on Mg absorption was even greater when dietary Ca and P were both increased. In sheep, Ca appeared to interfere with Mg absorption in the middle portion of the ileum. This was believed to be due to competition for a common absorptive mechanism (Care and Van't Klooster, 1965). Garces and Evans (1971) found that feeding steers high levels of Ca had no effect on Mg absorption. Metabolism trials with labs using 4 combinations of high and low Ca were conducted by Pless et al. (1973). The high Ca and high P diets lowered the apparent absorption of Mg in these animals. Urinary excretion of Mg

was unaffected by Ca level, but was decreased when high P levels were fed. Blood serum Mg level was also unaffected by Ca level and lowered when the high P level was fed. Feeding high Ca levels to wethers decreased plasma and bone levels of Mg (Chicco et al., 1973a). Urinary excretion of Mg also tended to be greater with the high Ca diet when Mg was supplemented.

Behar (1975) studied the effect of Ca on Mg absorption from the ileum in rats. As Ca concentrations increased, Mg absorption decreased. Behar proposes that this decrease in Mg absorption can be explained by a reduction in membrane permeability to water which is caused by the increased Ca levels. If the solvent drag theory is correct then this would account for the reduction in Mg absorption.

The effect of K on Mg absorption and metabolism in ruminants has been investigated by numerous researchers and varing results have been reported. Pearson et al. (1949) fed ewes a diet supplemented with 5% K as $KHCO_3$ and observed no effect on plasma Mg levels. Daniel et al. (1952) also observed no effect of supplemental K as KCl on plasma Mg levels in sheep. A significant decrease in plasma Mg level as noted by Kunkel et al. (1953) who fed ewes 5% supplemental K as $KHCO_3$. Fontenot et al. (1960) observed an increase in fecal Mg excretion and a decrease in plasma Mg levels in lambs which were fed a high protein-high K diet. Blaxter et al. (1960) observed no effect of K on Mg metabolism in calves fed a diet supplemented with 4.6% K as KCl. The excess K did result in death due to K intoxication in some of the calves.

Kemp (1960) and Kemp et al. (1961) studied the effects of feeding forage which had been heavily fertilized with K and N on mineral metabolism in milking cows. It was found that feeding forage which had been fertilized with high N or high K (together or separately) caused a decrease in plasma Mg levels. Also, a decrease in urinary excretion of Mg was associated with increased intake of K. Hemingway et al. (1963) and Ritchie and Hemingway (1963) observed no effect of feeding forage that had received heavy applications of muriate of potash on plasma Mg levels in ewes. Butler (1963) examined the mineral content of spring pastures on which animals that had developed tetany were grazing. He found an increased level of K and a decreased level of Mg and Na.

Suttle and Field (1969) fed barren and lactating ewes a semi-purified diet with 3 levels of Mg (.05, .1, .15%) and either 0 or 4.6% supplemental K. The increased dietary K was associated with a decrease in plasma Mg in both the barren and lactating ewes. Increased dietary K had more of an effect on plasma Mg levels than decreased dietary Mg. Also, the effect of increased K intake on plasma Mg level was greater with the Mg deficient diets than with the control level of Mg. Clinical tetany was observed in 2 of the lactating ewes. Newton et al. (1972) fed wethers diets containing .6 and 4.9% K as $KHCO_3$. In 1 trial they conducted 8 3-day balance trials and in the second trial they measured excretion of orally administered ^{28}Mg in the urine and feces. They found that the wethers on the high K diet excreted more Mg in the feces and less in the urine. Serum Mg levels were not affected significantly, however, there were lower values observed at the midpoint of the trial. The reduction of apprent Mg absorption due to feeding increased levels of

various K compounds has been observed in sheep by many other researchers (Suttle and Field, 1967; House and Van Campen, 1971; Moore et al., 1972; Frye et al., 1975).

Powley et al. (1977) used ^{28}Mg to study endogenous fecal Mg excretion in sheep fed grass high in K or Na. They found that there was no effect of high K intake on endogenous fecal excretion of Mg. The decrease in apparent Mg absorption resulting from high levels of K is probably due to a decrease in absorption rather than an increase in endogenous fecal excretion of Mg (Suttle and Field, 1969; Fontenot et al., 1973). Studies in sheep using ruminal and intestinal cannulas have found that almost all of this effect of K on Mg absorption occurs in the stomach region (Macgregor and Armstrong, 1979; Greene et al., 1981). Tomas and Potter (1976c) observed no effect of K on Mg absorption when KCl was infused into the duodenum.

In addition to an effect on Mg absorption, it has been suggested that K affects Mg metabolism in other ways. Suttle and Field (1969) postulated that K could depress the level of circulating Mg. To determine if excess K affected the rate and extent of cellular uptake of Mg from the plasma, House and Bird (1975) conducted a Mg tolerance test in goats. They found that plasma Mg returned to near normal levels after an intravenous injection of Mg-acetate more rapidly in goats on a high K diet than controls and that urinary excretion accounted for only 80% of the drop in plasma Mg. Urinary Mg excretion accounted for 100% of the drop in plasma Mg after Mg infusion in control animals. It was suggested that this effect was due to increased cellular uptake and retention of Mg caused by high levels of cellular K.

It has been shown that hyperkalemia can increase plasma insulin levels in some species. Lentz et al. (1976) suggested that prolonged intake of high levels of dietary K could cause increased insulin levels in cows turned out on spring forage low in Mg and that this could increase glucagon secretion, eventually causing hypoglycemia and ketosis, which have been observed in some cases of clinical tetany. They observed higher levels of plasma K and immunoreactive insulin (IRI) in calves infused intravenously and cows infused intraruminally with KCl. The calves receiving the Mg deficient diet has lower plasma K and higher IRI than normal calves. The Mg deficient calves also had higher levels of plasma glucose than normal calves or cows. This elevation of plasma glucose was thought to be due to increased glucagon secretion which could eventually lead to ketosis, lowered blood pH and the coma associated with grass tetany. Miller et al. (1976) suggested that low readily available carbohydrates in high K-low Mg spring forage could also lead to altered carbohydrate metabolism and eventually grass tetany. They recommended feeding supplemental starchy carbohydrate and supplemental Mg during the spring tetany season. More recent work by Miller et al. (1980) found that beef cows supplemented with MgO or cornmeal when changed from a cornsilage and grassclover hay ration to wheat-rye pasture had lower IRI and higher plasma Mg levels than controls. Plasma Mg was higher in cows supplemented with MgO than in cows receiving cornmeal. It is not known whether the increased insulin levels associated with high K intake affects plasma Mg level.

Another suggested effect of K on Mg metabolism is that high K levels may increase urinary excretion of Mg. It has also been proposed

that high levels of dietary organic acids can increase urinary excretion of Mg. Bohman et al. (1969) produced experimental hypomagnesemic tetany in cattle given KCl and either citric or transaconitic acid orally. Neither of these acids or KCl produced hypomagnesemic tetany when given singly. Also, the effect of giving KCl with either organic acid on plasma Mg level was more rapid than the effect of feeding KCl alone. House and Van Campen (1971) observed no effect of dietary citric acid fed alone or with KCl on Mg metabolism in wethers. DeGregorio et al. (1981) infused ewes intravenously with KCl, citric acid, KCl plus citric acid or water and observed plasma and urinary Mg changes after an intravenous injection of $MgCl_2.6H_2O$ (given 7 minutes after the intraruminal infusion). Increased plasma Mg clearance was observed in ewes given either KCl plus citric acid or citric acid alone. Variable increases were observed in ewes given KCl. Increases in urinary Mg were observed when KCl and KCl plus citric acid were given to Mg deficient ewes in a similar study without intravenous injection of Mg. Though the effect of KCl or citric acid was greater with elevated plasma levels of Mg, these factors could play a role in the etiology of grass tetany.

Cerebrospinal Fluid Magnesium

The ionic concentration of CSF is important for proper function of the central nervous system (CNS). The secretion of CSF occurs almost entirely in the choroid plexus and these secretions are at a constant ionic concentration. Ions have been shown to be transported across the blood brain barrier as well (Littledyke and Cox, 1979). It is believed that an active transport mechanism is involved in the transfer of Mg

ions from the blood into the CSF (Oppelt et al., 1963). The concentration of ions in the CSF has been shown to remain constant in spite of short term increases and decreases in plasma ion levels. As early as 1927, researchers found that CSF Mg levels remained constant when plasma Mg levels were elevated by intramuscular injections of $MgSO_4$ (Pallis et al., 1965). Meyer and Busse (1975a) found that intravenous injections of Mg gluconate had little effect on the CSF Mg concentration in sheep with normal CSF Mg levels. Animals with extremely low plasma Mg levels often maintain normal Mg levels in the CSF for extended periods (Meyer and Scholz, 1972). Sims et al. (1980) observed normal CSF Mg levels in hypomagnesemic sheep having plasma Mg levels as low as .28 mg/dl. Prolonged and severe hypomagnesemia is often required before a significant drop in CSF Mg occurs. Pauli and Allsop (1974) found that in dairy cows the plasma level of Mg was not a reliable indicator of the level of Mg in the CSF.

Experimental alterations in the ionic concentration of CSF have been shown to produce a variety of results. Generally, excess Mg in the CNS causes hypoexcitability while lowered Mg concentrations cause hyperexcitability (Sims et al., 1980). Feldberg (1957) found that injecting $MgCl_2$ into the cerebral ventricles of cats produced a sleep-like condition. Injections of $CaCl_2$ into the cerebral ventricle have also been shown to have an anesthesia like effect on cats. These effects can be reversed by the injection of K salts. Seoane and Baile (1973) suggested that Ca and Na concentrations in the CSF may play a role in regulating body weight and internal temperature. They injected

Ca, Mg, Na and K into the ventricular region of sheep. Ca and Mg injections produced a feeding response 2 minutes after the injections. The duration of the feeding response was dependent on the magnitude of the dose. Ca injections produced a longer response than Mg injections. K injections decreased feed intake and Na injections had no effect on feed intake but did increase water consumption. Na and Mg injections also increased intraperitoneal temperature. They felt that these observed effects of ionic concentrations could be due to their action on the hypothalmus. Other researchers have found that varying CSF Ca concentration had a thermoregulatory effect while Mg concentration had no effect (Littledyke and Cox, 1979).

Chutkow (1964b) found that in rats the CSF Mg concentration remained normal despite severe hypomagnesemia and then began to decline gradually. The decreased Mg concentration in the CSF was associated with hyperexcitability. Allsop and Pauli (1975a), using sheep, perfused low Mg and low Ca synthetic CSF from the lateral ventricle to the lumbar region. Perfusion with synthetic CSF containing .6 mg/dl produced tetany. The sheep recovered when fluid with normal Mg concentration was perfused. Low Ca perfusions caused deeper and faster breathing and muscle tremors. Plasma levels of Mg and Ca remained unchanged throughout the perfusions and tetany episodes. When low Ca and Mg were perfused together the same symptoms occurred simultaneously.

Oppelt et al. (1963) infused $MgCl_2$ into dogs intravenously and maintained plasma Mg levels at 275 to 360% of control levels by the fourth hour. They also injected $^{28}MgCl_2$ intravenously into dogs.

Radioactivity appeared in the CSF quickly with peak levels occurring 150 minutes after the injection. The half times for ^{28}Mg in the plasma and CSF were both 2-3 hours. Radioactive Mg has also been injected intravenously into cows and radioactivity measured in the lateral ventricle, cisterna magna and lumbar region 10 minutes after the injections. The radioactivity increased most rapidly in the lateral ventricle. When ^{28}Mg was injected directly into the lateral ventricle it did not appear in the lumbar region for 10 hours (Littledyke and Cox, 1979). Chutkow and Meyers (1968) injected $MgCl_2$ intraperitoneally in rats with depleted CSF Mg levels. The CSF Mg concentration returned to normal within 1 hour. Meyer and Busse (1975a) injected Mg gluconate intravenously into sheep with normal and depleted CSF Mg levels. Samples were taken from the caudal, medial and cranial regions. They found that in normal sheep there was a slight, shortlasting increase in CSF Mg level. The greatest and most rapid increases in CSF Mg were observed in sheep with depleted CSF Mg concentrations. Magnesium level rose most rapidly in samples from the cranial region and increased more rapidly in samples from the medial region than the caudal region.

Meyer and Scholz (1972) observed that in prolonged Mg deficiency the Mg concentration in the CSF dropped more slowly in younger sheep than in older lactating ewes. They observed a strong relationship between CSF Mg level and the incidence of clinical tetany. It was suggested that tetany could occur at CSF Mg levels of less than 1.6 mg/dl and was almost certain to occur when levels dropped below 1.2 mg/dl. Meyer et al. (1974) produced tetany-like symptoms in sheep by infusing low Mg CSF into the ventricular system.

Meyer (1976b, 1977) seemed certain that hypomagnesemic tetany is of central origin and that its occurrence in ruminants is dependent on a decrease in CSF Mg concentration. This theory, if correct, would explain why plasma Mg levels are so variable in cases of clinical tetany and why individual animals with severely depleted plasma Mg may never develop clinical tetany. It was suggested that the changes in CNS function due to CSF Mg deficiency were the result of a drop in Mg concentration in the intracellular spaces adjacent to the ventricular system. This drop in intracellular Mg could lead to altered membrane permeability to glucose, the energy source for the neuron.

Pauli and Allsop (1974) compared CSF samples from 2 groups of dairy cows. Both groups had hypomagnesemia; however, 1 group's samples were taken from cows with clinical tetany. The CSF Mg concentration was found to be significantly lower in the group having clinical tetany. In cows fed a Mg deficient diet CSF Mg analyzed .92 and 1.26 mg/dl during tetany or after death due to tetany. The CSF Mg level was found to drop rapidly just before the onset of tetany. Similar observations were reported by Allsop and Pauli (1975b).

The theory that clinical hypomagnesemic tetany is dependent on a reduction of CSF Mg has not been disproven. The reasons low CSF Mg causes tetany have not yet been delineated. Littledyke and Cox (1979) suggest that low Mg levels in the fluid surrounding neural synapses would interfere with their normal function.

Magnesium Enemas

The first reported use of enemas for providing nutrients is probably Beaumont's (1883) use of nutrient enemas to sustain a soldier

having a gaping gunshot wound in the stomach. Magnesium sulfate enemas have been used as a treatment for various medical disorders in humans for years. Andrew et al. (1965) reported on the effects of hypertonic $MgSO_4$ enemas in lambs. They were concerned over the suggested use of hypertonic $MgSO_4$ enemas for the treatment of hyaline membrane disease in infants. Similar treatments had resulted in death when given to adults and children. Newborn lambs given 10 mg/lb body weight of a 50% $MgSO_4 \cdot 7H_2O$ solution rectally died in 23-46 minutes with death occurring at plasma levels of 18.2 to 28.2 mg/dl. One newborn lamb given 10 ml/lb body weight of 25% $MgSO_4 \cdot 7H_2O$ solution died and others exhibited signs of deep sleep and cyanosis. In young (60-90 days old) lambs given 10 ml/lb body weight of a 50% $MgSO_4 \cdot 7H_2O$ solution 3 of 5 developed deep sleep and one died. The authors voiced strong disapproval of the use of hypertonic$MgSO_4 \cdot 7H_2O$ enemas in newborn infants. Magnesium sulfate enemas have also been used as a treatment for constipation. Brown and Campbell (1978) reported a case where a 4 year old human infant was in a comatose state after receiving a hypertonic $MgSO_4 \cdot 7H_2O$ solution enema. His plasma Mg level was 14.3 mg/dl. Chutkow (1964a) cited 3 reports of hypermagnesemia in humans which had received hypertonic $MgSO_4 \cdot 7H_2O$ solutions rectally.

It has been reported that when plasma Mg levels reach 12 mg/dl in humans central depression begins and that anesthesia occurs when plasma concentration reaches 24 mg/dl. Very high plasma Mg can result in respiratory depression, coma and death in various experimental animals. Intravenous injections of $MgSO_4$ have been used as an euthanasia in animals (Shils, 1980).

Meyer and Busse (1975b) reported that rectal infusion of a 20-30% $MgCl_2 \cdot 6H_2O$ solution resulted in a rapid increase in plasma Mg level. Meyer (1976b) suggested that a 20% solution of $MgCl_2 \cdot 6H_2O$ solution could be used by the farmer as an emergency treatment for accute cases of grass tetany.

Bell et al. (1977, 1978) reported on the use of $MgCl_2 \cdot 6H_2O$ enemas for treating grass tetany in sheep and cattle. It was found that 30% $MgCl_2 \cdot 6H_2O$ solutions given rectally were effective in elevating plasma Mg level in beef cows, steers and wethers while $MgSO_4 \cdot 7H_2O$ enemas providing the same quantity of Mg did not significantly alter plasma Mg concentration in steers and wethers. Wethers given $MgSO_4 \cdot 7H_2O$ plus NaCl solutions rectally showed only slight increases in plasma Mg concentrations. Cows with clinical hypomagnesemic tetany were successfully treated with $MgCl_2 \cdot 6H_2O$ enemas. Fulgoni (1979, unpublished data) gave $MgCl_2 \cdot 6H_2O$ enemas to wethers fed Mg deficient diets supplemented with excess Ca and/or P. The only significant differences due to diet were with samples taken 10 minutes after the enemas were administered. Plasma Mg levels at this time were greater in sheep fed excess Ca or excess Ca and P. The excess Ca may have enhanced absorption from the rectum.

Flicoteaux et al. (1979) successfully treated 2 cows with hypomagnesemic tetany using an emema of a 30% $MgCl_2 \cdot 6H_2O$ solution. The serum Mg concentration reached peak levels 15 minutes after the enema was given.

Wilson (1980) compared milk production in 12 pairs of twin hypomagnesemic cows supplemented with $MgCl_2 \cdot 6H_2O$ solution providing 10 g of Mg daily. The $MgCl_2 \cdot 6H_2O$ solution was given either orally or rectally for the first week of the trial. Rectal administration

caused irritation and after 1 week this method was stopped and the cows were given $MgSO_4 \cdot 7H_2O$ subcutaneously instead. All 3 of these methods elevated plasma Mg similarly. Milk production and milk fat were elevated significantly by the oral route of administration but not by the others.

Red Blood Cell Magnesium

Magnesium contained in the erythrocyte is primarily found in hemoglobin where it is associated with the organic phosphates and enzymes. This Mg is released when the cells are lysed. About 2 to 6% of erythrocyte Mg is also bound to the membrane. The level of Mg in erythrocytes has often been used as an indicator of tissue Mg levels in humans; however, this has not been shown to be a reliable indicator of Mg status. Very broad normal ranges of erythrocyte Mg level have been reported (Seelig, 1980). Very few studies have reported the RBC status of domestic animals.

Various methods have been used to analyze or calculate RBC Mg levels. Initial studies found that the level of RBC Mg calculated by indirect measurement (subtracting plasma Mg from whole blood Mg and correcting for hematocrit) was comparable to the level obtained by directly measuring Mg in saline washed erythrocytes (Seelig, 1980). Blaxter et al. (1960) calculated the Mg concentration in RBC of calves using whole blood Mg level and hematocrits. They observed no significant lowering of RBC Mg after calves had been on a low Mg diet for 56 days, however, they did notice a slight drop. It was suggested that since the life of the erythrocyte is 15 weeks, a gross depletion of erythrocyte Mg would take much longer than 56 days if the Mg level in the erythrocyte reflects Mg status at the time of erythropoeisis.

The slight drop in total RBC Mg level was believed due to a dilution by RBC formed with lower Mg levels, rather than a loss of Mg from RBC already formed.

Work by McAleese et al. (1961) supported this theory that lowered total RBC Mg results from a dilution with RBC formed containing less Mg. They found very little ^{28}Mg uptake by the RBC and little or no activity above background in the bone marrow of Mg deficient and control lambs. Chutkow and Meyers (1968) found that in rats RBC Mg calculated using the difference method was not a reliable indicator of tissue Mg levels. Fertilization of pastures with $MgSO_4 \cdot H_2O$ caused a slight increase in RBC Mg in beef cows grazing the pastures for 2 weeks (Thompson and Reid, 1981).

It has been shown that older RBC contain less Mg than younger RBC. Prolonged slight hypomagnesemia has been shown to reduce RBC Mg values to half of control values. Most of the literature agrees that RBC Mg values indicate Mg status at the time of erythropoiesis and the age of the RBC (Seelig, 1980).

Magnesium Research with Calves

In studying hypomagnesemia, many researchers have used calves as a model for adult lactating cows. The rapid growth rate of the calf facilitates the production of hypomagnesemia. McClandish (1923) first suggested that the inability to raise calves to maturity on whole milk alone was somehow related to Mg metabolism. Duncan et al. (1935) observed low plasma Mg in calves fed whole milk and whole milk with

various supplements until tetany was produced. None of the supplements used prevented the development of tetany. Huffman and Duncan (1936) found that supplementing whole milk rations prevented the occurrence of tetany in calves. Moore et al. (1936, 1938) looked at cardiovascular and other lesions in Mg deficient calves and compared the effect of Mg deficiency in calves to arteriosclerosis in humans.

In studying various nutritional deficiencies, many researchers have produced those deficiencies in calves using semi-purified diets. Wiese et al. (1947) compared the palatability of various semi-purified liquid and dry calf diets. They found that calves did best on a liqiud diet containing casein, lard, cerelose, minerals and vitamins. Some of the vitamins were dissolved in wheat germ oil. The calves did much worse when switched to dry diets (fed as a slurry) after 6 weeks on the synthetic milk. Calves on the dry diet with starch did do better than calves receiving cerelose as the total carbohydrate source. Similar synthetic milks have been used in calves (Johnson et al., 1948; Sheppard and Johnson, 1957) and lambs (Draper and Johnson, 1951) to produce vitamin deficiencies. Flipse et al. (1950) compared the performance of calves fed synthetic milks containing various sources of carbohydrates. They found calves did much better when lactose was used than when corn syrup or glucose was used. Calves fed lactose had gained twice as much and had much better health compared to calves fed either of the other 2 carbohydrate sources.

Blaxter and Rook (1954a, 1954b) produced hypomagnesemia in calves using an artificial low Mg liquid diet. This diet consisted primarily

of casein, glucose, margarine, arachis oil, lecithin, minerals and vitamins. Clinical and pathological observations were made on calves which had tetany and balance trials were conducted to study the metabolism of Ca, Mg and N in deficient and normal calves. Varying levels of Mg were supplemented to determine the Mg requirement of the young calf. Parr (1957) produced tetany in calves by feeding whole milk with various supplements and artificial milks. The bone Mg in the Mg deficient calves was found to be much lower than controls. Smith (1957, 1958, 1959a, 1959b, 1959c, 1959d, 1961) has conducted extensive studies on the metabolism of Mg and Ca in young dairy calves. In some studies he used a synthetic milk similar to one used by Flipse et al. (1950). Hamada et al. (1969) produced hypomagnesemic tetany in calves using a dry purified diet containing casein, corn starch, lactose, minerals and vitamins which was offered to male dairy calves when they were 37 days old. Roughage was also fed with this diet.

Todd and Horvath (1970) fed 4 calves milk from which 1/3 to 1/2 the Mg had been removed by an ion exchange technique. Severe hypomagnesemia was produced and 2 calves developed tetany. Measurements were made of the electrical stimulus required to cause contraction of the triceps muscle using direct or indirect stimulation. The current required using direct stimulation was not affected by hypomagnesemia or tetany, while the current required using indirect stimulation fell to 1/3 during tetany.

Hypomagnesemic tetany normally does not occur in suckling calves in the United States. It has been reported to be a problem in some areas of Scotland. Haggard et al. (1978) reported on cases of tetany

in suckling calves which occurred in 2 beef herds. The calves which developed tetany were larger, faster growing calves about 4 months old. The cows in these herds were on a corn silage, grass haylage and non-protein nitrogen supplemented ration.

CHAPTER III

EXPERIMENTAL PROCEDURE

General Design of Experiment

This experiment was conducted to study effects of Mg deficiency and excess dietary K on mineral metabolism in young calves. The experiment also involved a study of the effects of $MgCl_2 \cdot 6H_2O$ enemas on CSF, plasma and RBC mineral levels in hypomagnesemic and normal calves. Preliminary studies involving 5 calves were used to establish standard diets and procedures. Sixteen additional calves were involved in 2 trials in the Mg depletion-repletion study during the fall of 1980 and the winter of 1981.

Preliminary Studies

During the fall of 1979, a preliminary study was initiated using 2 male Holstein calves to determine the palatability of a semi-purified low Mg dry diet. The calves were obtained from the University of Tennessee dairy when they were 3 to 4 days old and placed in wooden metabolism stalls for the duration of the study. The calves were fed a commercial milk replacer[1] and offered a dry semi-purified diet ad libitum. One calf was fed the basal low Mg diet and the other calf was fed the basal diet supplemented with .1% Mg as $MgCl_2 \cdot 6H_2O$. The composition of the basal diet is shown in table 1. The supplemental $Mg \cdot Cl_2 \cdot 6H_2O$ replaced an equivalent percentage of dextrose. When the calves began consuming the

[1]Tennessee Farmer's Cooperative, Lavergne, Tennessee.

Table 1. COMPOSITION OF PRELIMINARY CALF DIET

Ingredients	International Feed Number (IFN)	Composition (%)
Casein		25
Dextrose	4-02-125	40
Sucrose		9.2
Ground corn cobs[1]	1-02-783	10
Corn oil	4-02-882	10
Mineral mix[a]		4.8
Vitamin mix[b]		1

[a]Mineral mix	IFN	Grams per kg of Diet
$CaCO_3$	6-01-069	1.68
$CaHPO_4 \cdot 2H_2O$	6-01-080	23.32
KCl	6-03-755	10.36
$K_2S_2O_8$		8.84
NaCl	6-04-152	2.48
$FeSO_4 \cdot 7H_2O$		.48
$MnSO_4 \cdot H_2O$		.12
Se premix[2]		.2
$ZnSO_4 \cdot 7H_2O$		.16
$CuSO_4 \cdot 5H_2O$		.04
MoO_3		.00152
KI		.00032
$CoSO_4 \cdot 7H_2O$		.00048

Table 1. (Continued)

$MgCl_2 \cdot 6H_2O$ (substituted in control diet)	8.78
[b]Vitamin Diet Fortification Mixture[3]	Grams per kg of mix
Vitamin A (200,000 units per gram)	4.5
Vitamin D (4000,000 units per gram)	.25
Alpha tocopherol	5
Ascorbic acid	45
Inositol	5
Choline chloride	75
Menadione	2.25
p-Aminobenzoic acid	5
Niacin	4.5
Riboflavin	1
Pyrodoxine hydrochloride	1
Thiamine hydrochloride	1
Calcium pantothenate	1
Biotin	.02
Folic acid	.09
Vitamin B_{12}	.00125

[1]Grit-o'cobs #4, The Andersons, Maumee, Ohio.

[2]Calcium Carbonate Company, Quincy, Illinois.

[3]ICN Nutritional Biochemicals, Cleveland, Ohio.

dry diet the amount of milk replacer fed was reduced and the calves were soon weaned. Both calves did poorly after being weaned, especially the calf fed the diet supplemented with $MgCl_2 \cdot 6H_2O$. This calf consumed very little of the diet and lost weight throughout the trial. He was finally put back on milk replacer after a CSF sample was taken using a temporary catheter inserted into the cisterna magna. The calf fed the Mg deficient diet consumed more of the dry ration and gained some weight; however, his plasma Mg was never depleted lower than 1.44 mg/dl. This calf was given an enema of 50 ml of a 30% $MgCl_2 \cdot 6H_2O$ solution and subsequent samples of blood and CSF were taken to observe changes in mineral concentration following the administration of the enema. The CSF was sampled using a temporary catheter inserted into the cisterna magna 24 hours prior to the administration of the enema.

The diets used for these 2 calves were stored at room temperature in the metabolism room. After a few weeks the diets became rancid and it was thought that this might be 1 reason for the poor fed consumption. A second preliminary study was conducted during the winter of 1980 with the dry semi-purified diets (table 1); however, 200 ppm of an antioxidant (butylated hydroxy anisol) was added and the diets were kept under refrigeration to prevent spoilage. For this study 2 male Holstein calves were obtained from the University of Tennessee dairy when they were 5 to 7 days old and placed in wooden metabolism stalls. The amount of milk replacer fed was reduced immediately and they were weaned 1 week after procurement. After the calves had consumed the dry diet alone for 7 days, samples of feed, feces and urine were collected for a 7 day practice balance trial. Both calves did poorly on the dry diets and

lost weight. The calf consuming the basal diet supplemented with $MgCl_2 \cdot 6H_2O$ became severely emaciated and died 51 days after being weaned. The plasma Mg level of the calf consuming the basal Mg deficient diet was depleted as low as .47 mg/dl and his CSF Mg was depleted to 1.59 mg/dl. Both plasma and CSF Mg levels were successfully repleted using an enema of 50 ml of a 30% $MgCl_2 \cdot 6H_2O$ solution. CSF samples were taken using a temporary catheter inserted into the cisterma magna 24 hours prior to the administration of the enema.

A third preliminary study was conducted during the summer of 1980 using 1 male Holstein calf. This calf was 6 weeks old when obtained from the University of Tennessee dairy and was already consuming a concentrate ration. The calf was housed in a stall bedded with wood shavings for the duration of the trial. The composition of the dry diet was altered for this trial to include lactose instead of sucrose. Research has shown that calves have very little sucrase activity in the small intestine (Huber et al., 1961). Flipse et al. (1950) found that young calves did much better when fed a semi-purified diet containing lactose as the carbohydrate source compared to diets containing glucose or corn oil. One percent $NaHCO_3$ was also substituted for dextrose in the diet as a buffer and soybean protein was used instead of casein. The composition of the diet used in this trial and in subsequent trials is shown in table 2. Butylated hydroxy anisol (200 ppm) was also added to this diet which was stored under refrigeration. One month after this calf was weaned 5% starch was substituted for 5% of the dextrose. The calf did well on this diet and gained .45 kg/day after the addition of starch to the diet. After 7 weeks on the dry diet alone his plasma

Table 2. COMPOSITION OF BASAL DIET

Ingredients[a]	International Feed Number (IFN)	Composition %
Isolated soybean protein		25
Dextrose	4-02-125	33.2
D-Lactose		10
Corn starch	4-02-889	5
Corn oil	4-02-882	10
Ground corn cobs[1]	1-02-783	10
Mineral mix[b]		4.8
Vitamin mix[c]		1
$NaHCO_3$		1
[a]200 ppm butylated hydroxy anisol added as a preservative		100

[b]Mineral Mix	IFN	Grams per kg of diet
$CaCO_3$	6-01-069	1.68
$CaHPO_4 \cdot 2H_2O$	6-01-080	23.32
KCl	6-03-755	10.36
$K_2S_2O_8$		8.84
NaCl	6-04-152	2.54
$FeSO_4 \cdot 7H_2O$		.48
$MnSO_4 \cdot H_2O$		.12
Se premix[2]		.2
$ZnSO_4 \cdot 7H_2O$		.16
$CuSO_4 \cdot 5H_2O$		.04

Table 2. (Continued)

[b]Mineral Mix	IFN	Grams per kg of diet
MoO_3		.00152
KI		.00032
$CoSO_4 \cdot 7H_2O$		.00048
KCL (substituted in diets 2 and 4)	6-03-069	20
$KHCO_3$ (substituted in diets 2 and 4)		25.64
MgO (substituted in diets 3 and 4)	6-02-756	1.67

[b]Vitamin Diet Fortification Mixture	Grams per kg of mix
Vitamin A (200,000 units per gram)	4.5
Vitamin D (400,000 units per gram)	.25
Alpha tocopherol	5
Ascorbic acid	45
Inositol	5
Choline chloride	75
Menadione	2.25
p-Aminobenzoic acid	5
Niacin	4.5
Riboflavin	1
Pyridoxine hydrochloride	1
Thiamine hydrochloride	1

Table 2. (Continued)

[b]Vitamin Diet Fortification Mixture[3]	Grams per kg of mix
Calcium pantothenate	3
Biotin	.02
Folic acid	.09
Vitamin B_{12}	.00125

[1]Grit-o'cobs #4, The Andersons, Maumee, Ohio.

[2]Calcium Carbonate Company, Quincy, Illinois.

[3]ICN Nutritional Biochemicals, Cleveland, Ohio.

Mg level was .175 mg/dl and his CSF Mg level was 1.2 mg/dl. Plasma and CSF Mg were repleted to normal levels 23, 42 and 50 days after weaning using an enema of 50 ml of a 30% $MgCl_2 \cdot 6H_2O$ solution. For this trial, the enemas were given under anesthesia instead of with the calf awake as in the first trial. This was done so that the calf's head could be immobilized and a spinal needle inserted into the cisterna and magna and left for subsequent sampling. The procedure for CSF sampling was altered because the temporary catheters used in the first 2 trials did not work well in some animals.

Management and Procedures

Based on the experience of the preliminary tests, the following study was initiated. For this study, male Holstein calves were obtained from the Knoxville area and placed on a commercial milk replacer[2]. In each trial, calves were selected by weight and condition and assigned to 4 groups of 2 calves each. Calves were offered a dry semi-purified diet ad libitum until they were consuming enough dry diet to support growth without milk replacer. Group 1 received the basal Mg deficient diet, group 2 received the basal diet plus 2% K, group 3 received the basal diet plus .1% Mg and group 4 received the basal diet plus .1% Mg and 2% K (table 3). The composition of the diets is shown in table 2 and the mineral analyses of each diet is shown in table 4. Supplemental Mg was added as MgO and supplemental K was added as KCl and $KHCO_3$. These supplements replaced equal percentages of dextrose. Starch (5%) was

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[2]Land O'Lakes, Inc., Fort Dodge, Iowa.

Table 3. EXPERIMENTAL DESIGN

	Diets			
	1 Mg deficient	2 +K	3 +Mg	4 +Mg + K
Trial 1	2*	2	2	2
Trial 2	2	2	2	2

*Number of calves

Table 4. MINERAL ANALYSIS OF DIET (mg/g)

	Diet			
Mineral	1	2	3	4
Calcium	7.18	6.99	7.41	7.29
Magnesium	.20	.24	1.49	1.54
Potassium	9.00	26.49	8.86	27.23

added to the diet after the calves were over 4 weeks old. The starch was also substituted for dextrose.

The calves were housed in individual stalls bedded with wood shavings during the preliminary period. No attempts were made to prevent the consumption of wood shavings and a few calves were observed consuming some shavings. Wet shavings and fecal material were removed daily. Periodically the calves were removed from the stalls to be exercised and brushed. Rectal temperatures were taken every other day. Calves with diarrhea were treated with Sulkamycin-S Bolettes.[3] If the diarrhea persisted after 3 days, Biosol-M[4] was given orally. Calves with chronic viral dirrhea were given daily doses of Kaopectate.[5] Calves were weighed and blood samples taken for plasma mineral analysis weekly.

During the preliminary period the calves were fed twice a day and had continuous access to tap water. Bottles used to feed milk replacer were cleaned after each use with Novasan Solution (chlorhexidine)[6] during the first trial and bleach during the second trial. The amount of milk replacer fed was reduced gradually as the individual consumption of the dry diet increased. After all calves were consuming the dry diet alone they were transferred to dual type wooden metabolism stalls (Briggs and Gallup, 1949) which had been altered for use with calves.

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[3]Norden Laboratories, Inc., Lincoln, Nebraska.

[4]The Upjohn Company, Kalamazoo, Michigan.

[5]The Upjohn Company, Kalamazoo, Michigan.

[6]Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

While in metabolism stalls the calves were fed twice daily and offered distilled water 3 times a day. The distilled water analyzed less than .05 mg/dl Mg.

Data Collected

Balance Study. During the collection trial, the daily consumption feed was recorded for each animal. After a 5 day preliminary period in the metabolism stalls, samples were collected for a 5 day balance trial. Due to anorexia and diarrhea in some calves, samples were collected from 5 of the 8 calves during the first trial and 7 of the 8 calves during the second trial. Aliquots of urine (4%) were collected daily and stored in glass jars under refrigeration. Fecal samples were collected using canvas collection bags and harnesses in order to prevent urine contamination. The canvas collection bags were lined with plastic bags which worked well for calves with normal feces. Samples were stored under refrigeration in plastic bags. Daily aliquots (1%) of feed were also collected and combined for analysis.

Repletion Study. Immediately after the collection trial the calves were used for a repletion study. The procedures used were time consuming and in most cases only 1 calf was repleted per day. The first calves used were those which were anorexic or having problems with diarrhea. If healthy, the Mg deficient calves were usually repleted last in order to deplete their CSF Mg levels as low as possible. When feasible calves receiving similar K levels and dissimilar Mg levels were selected to be repleted on consecutive days.

Each calf was anesthetized using Bio-tal (sodium thiamyl)[7] and placed on a padded table in right lateral recumbency. An endotracheal tube[8] was inserted using a laryngoscope and anesthesia was maintained using Metofane (methoxy flurance)[9] and oxygen. An I-CATH intravenous cannula[10] was then placed in the jugular vein and taped in place for blood sampling. The calf's head was flexed ventrally and immobilized a nylon rope. A spinal needle fitted with a stylette was then inserted through the skin, subcutaneous structures and the nuchal ligament. The needle was then advanced through the atlanto occipital junction until the dura mater and arachnoid were penetrated, situating the needle in the cisterna magna. A reflex response was usually observed when these meninges were penetrated. After taking a baseline sample the needle was fitted with a stopcock and left in place for subsequent sampling.

At each sampling time 2 to 3 ml of CSF was taken and 20 ml of blood was drawn into heparinized syringes. The blood was then transferred to plastic centrifuge tubes. After taking baseline blood and CSF samples, an enema of 50 ml of a 30% $MgCl_2 \cdot 6H_2O$ solution was given and held in the animal for 5 minutes. The enema was given using a 200 ml wash bottle adapted for use with a metal esophageal calf probe.[11] Subsequent samples

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[7]Bio-Ceutic Laboratories, Inc., St. Joseph, Missouri.

[8]Ohio Medical Products, Madison, Wisconsin.

[9]Pitman-Moore, Incorporated, Washington Crossing, New Jersey.

[10]Bard Biomedical, Murray Hill, New Jersey.

[11]The Magrath Company, McCook, Nebraska.

of blood and CSF were taken at 5, 15, 30, 60, 120 and 180 minutes after the administration of the enema.

After determining hematocrits on the blood samples they were separated into equal volumes. One portion was centrifuged at 30,000 L.C. for 15 minutes and the plasma was removed using glass pipettes. The other portion was centrifuged at 9,000 for 30 minutes and the platelets and plasma were removed using plastic disposable pipettes. This procedure was then repeated and more platelets and plasma were removed. Next the sample was centrifuged at 30,000 for 30 minutes and the buffy coat and plasma were removed. A 1 ml sample of RBC was then taken from the bottom portion of the centrifuge tube and mixed with 1 ml of a sodium borate-Triton-X solution in order to lyse the cells. The plasma, CSF and lysed RBC were then stored in plastic tubes under refrigeration until analyzed for mineral content.

Chemical Analysis

Feed and Feces. Fecal samples were weighed, placed in a 60°C drying oven and dried for 7 days. The samples were air equilibrated for 24 hours and then weighed again for dry matter determination. After being ground in a Wiley Mill using a 2 mm screen, replicate samples of approximately 2 g of feed and feces were placed in crucibles and ashed at 300°C for 1 hour and at 600°C for at least 3 hours. These samples were then placed in solution using 3 ml of 6N HCL and diluted to a known volume for analysis of Ca, Mg and K using an Instrumental Laboratory Model 551 atomic absorption/atomic emmission spectrophotometer. Lanthanum trioxide (.1%) was added to all Ca and Mg dilutions to prevent phosphate interference

and cesium chloride (.1%) was added to K dilutions to prevent ionization.

Urine. Replicate samples (5 ml) of urine were allowed to dry overnight in a 100°C oven. They were then ashed at 200°C for 1 hour, 300°C for 1 hour and 600°C for at least 3 hours. After ashing the samples were put into solution using 3 ml of 6N HCl and taken to a known volume for the same analyses performed on the feed and fecal samples.

Plasma, Cerebrospinal Fluid and Red Blood Cells. These samples were diluted to a known volume and analyzed for Ca, Mg and K using the prescribed methods listed above.

Statistical Analysis

Data were analysed statistically using programs prepared by Barr et al. (1979). Analyses of variance were used to determine if any independent variables explained any variation in dependent variables. Analyses of variance were performed by trail first. A test for homogeneity of variance (Snedecor and Cochran, 1967) was used to determine if error mean squares were significantly different. The data were then combined and analyzed using the model:

$$Y_{ij} = u + d_i + e_{ij}$$

where: Y_{ij} = dependent variables

u = theoretical population mean

d_i = effect of diet, i = 1-4

e_{ij} = random error

If differences were determined by F test, least square means were separated using the Student Neuman-Keuls test (Sokal and Rohlf, 1969).

Analyses of variance for the repletion study were performed by trail first. The model used was:

$$Y_{ijk} = u + d_i + m_k + e_{ijk}$$

where:

Y_{ijk} = dependent variables

u = theoretical population mean

d_i = effect of diet, i = 1-4

m_k = effect of minute, i = 1-7

e_{ijk} = random error

If the F test determined the effect of minutes after enema was significant, orthogonal comparisons were used as a mean separation procedure. The comparisons used were baseline mineral level versus the mineral level of each subsequent sample. These same orthogonal comparisons were also made by diet to determine in which animals significant differences occurred. After comparison of error mean squares, data from both trials were combined and analyzed using the model:

$$Y_{ijkl} = u + t_i + d_j + m_k + t_{ij} + e_{ijkl}$$

where:

Y_{ijkl} = dependent variables

u = theoretical population mean

t_i = effect of trial, i = 1-2

d_j = effect of diet, j = 1-4

m_k = effect of minutes after enema, K = 1-7

td_{ij} = effect of trial x diet interaction

e_{ijkl} = random error

The same orthogonal comparisons used by trial were also used to separate means for the combined data. Mineral levels were also fitted to a trinomial regression on minute. The regression equation used was:

$$Y = a + b_1(min) + b_2(min^2) + b_3(min^3)$$

where:

Y = dependent variables

a = intercept

min = minute after enema

b_1, b_2, b_3 = the partial regression coefficients for each dependent variable on the independent variable = min, min^2, min^3

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary Results

During the first preliminary study, plasma and CSF Mg level for the calf consuming the basal diet supplemented with Mg remained normal. After administration of the $MgCl_2 \cdot 6H_2O$ enema, the plasma Mg level of the calf consuming the basal diet without supplementation went from 1.44 mg/dl to a peak level of 5.42 mg/dl in 30 minutes (table 5). The enema had no measurable effect on CSF Mg level and had no apparent effect on Ca or K level in the plasma or CSF.

The plasma and CSF mineral levels for the calf consuming the Mg supplemented basal diet during the second preliminary study also remained normal. The calf consuming the basal diet was given a $MgCl_2 \cdot 6H_2O$ enema when his plasma Mg level was .66 mg/dl and his CSF Mg level was 1.59 mg/dl (table 5). The peak plasma Mg level was 1.65 mg/dl for the 5 minute sample. CSF Mg peaked at 1.95 mg/dl for the 120 minute sample. Much of the enema was expelled within a minute after administration, which probably explains the small increases in plasma Mg compared with other trials.

The calf used during the summer of 1980 was given 3 $MgCl_2 \cdot 6H_2O$ enemas (table 5). The enema given 23 days after the calf was weaned (8-20-80) elevated plasma Mg levels from .53 mg/dl to a peak level of 2.65 mg/dl 15 minutes after administration. CSF Mg level was normal and appeared unaffected by the $MgCl_2 \cdot 6H_2O$ enema. The enema given 42 days after weaning (9-8-80) repleted plasma Mg from .24 mg/dl to 3.13

TABLE 5. EFFECT OF $MgCl_2.6H_2O$ ENEMA ON PLASMA AND CSF MINERAL LEVEL OF CALVES IN STUDIES 1, 2 AND 3

Minutes after enema	mg/dl					
	Plasma			CSF		
	Mg	Ca	K	Mg	Ca	K
	First Preliminary Study					
0	1.44	9.49	15.81	2.43	5.55	11.73
5	3.74	9.32	15.18	2.19	5.07	11.76
15	5.22	9.26	14.01	2.68	5.64	11.83
30	5.42	9.06	15.69	2.25	5.06	12.35
60	5.29	9.16	14.14	2.28	4.94	12.10
120	3.99	9.21	15.80	2.36	5.27	12.46
	Second Preliminary Study					
0	.66	11.05	18.74	1.59	----	----
5	1.65	11.84	20.13	1.75	5.22	13.24
15	1.36	10.34	18.79	1.84	5.10	12.46
30	1.18	10.70	20.46	1.79	4.80	10.70
60	1.12	10.96	19.64	1.91	5.74	10.89
120	.71	10.30	17.11	1.95	5.49	13.00
	Third Preliminary Study - Summer of 1980					
	8-20	9-8	9-16	8-20	9-8	9-16
	Plasma Mg			CSF Mg		
0	.53	.24	.17	2.31	1.32	1.20
55	2.20	1.72	1.19	2.22	1.34	1.21
15	2.65	2.53	1.80	2.25	1.50	1.28
30	2.42	3.00	1.71	2.33	1.70	1.38
45	2.22	----	----	2.28	----	----
60	----	3.10	1.87	----	1.86	1.60
120	----	3.13	2.22	----	2.03	1.86
180	----	2.94	2.25	----	2.32	2.03

mg/dl by 120 minutes. CSF Mg level increased from 1.32 mg/dl to 2.32 mg/dl by 180 minutes. A plot of the plasma and CSF Mg levels after administration of the $MgCl_2 \cdot 6H_2O$ enema given 50 days post weaning is illustrated in figure 1. Plasma Mg level went from .17 mg/dl to 2.25 mg/dl 180 minutes after administration of the enema. CSF Mg level increased from 1.2 mg/dl to 2.03 mg/dl by 180 minutes. The enemas had no apparent effect on Ca or K in the plasma or CSF.

Giving the enemas under anesthesia seemed to work better than the previous method. This approach allowed easier sampling of CSF and also allowed taking a larger volume sample more rapidly. Preventing the expulsion of the enema was also easier when anesthesia was used. This helped to assure that each calf retained an equivalent amount of $MgCl_2 \cdot 6H_2O$ solution. The use of a jugular cannula was preferred over repeated venous puncture since it caused less venous damage and assurred successful sampling at each time. This calf also seemed to respond favorably to being housed in a stall and being exercised and brushed regularly. No swelling was noticed in his front leg joints as has been observed in calves housed in metabolism stalls for extended periods. The calf also became very docile and easy to manage.

Depletion Phase

Research with the calves in the preliminary studies was very helpful in establishing procedures and protocol for the balance and repletion trials. During the first week of the first trial, when only

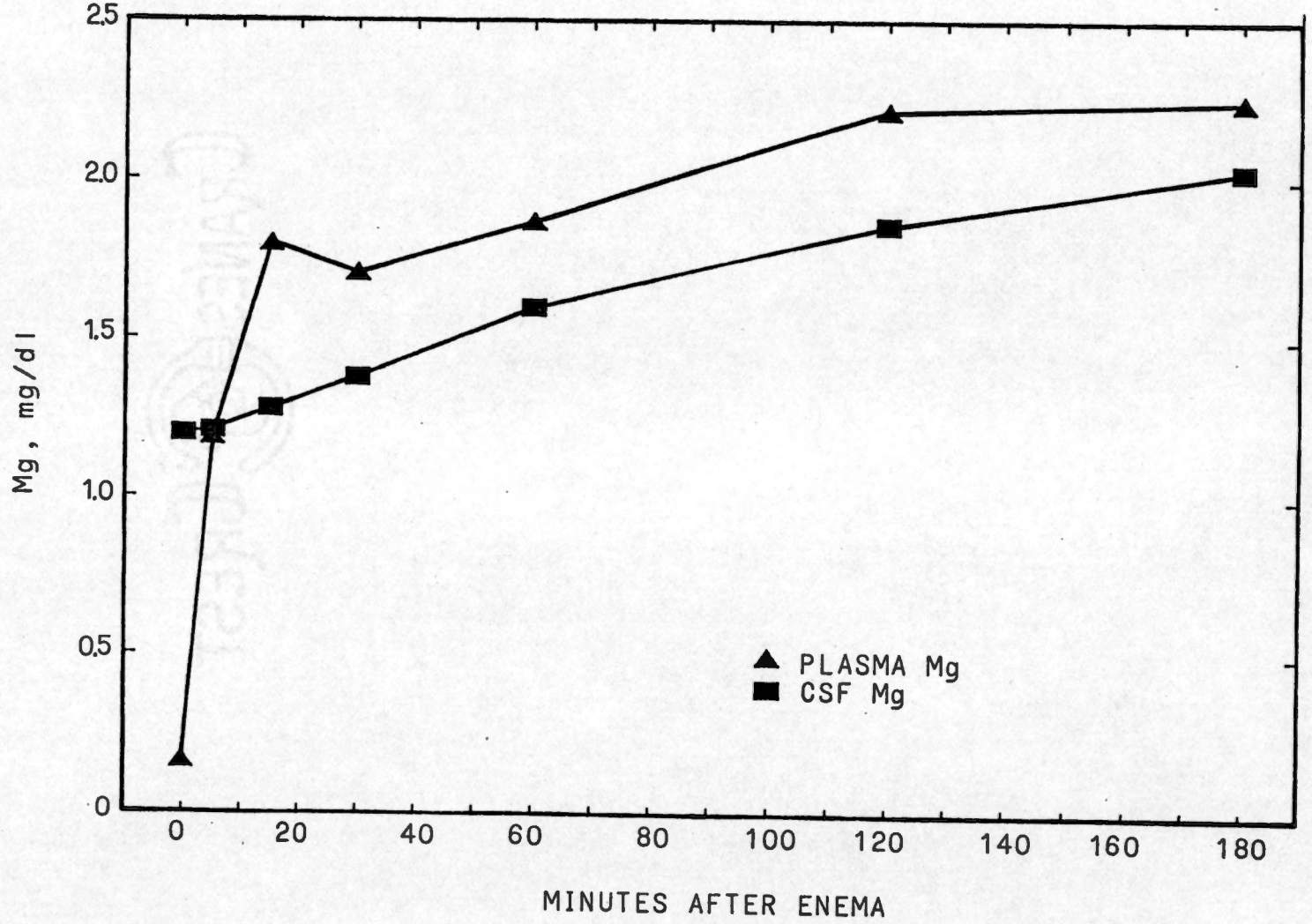


Figure 1. Effect of $MgCl_2 \cdot 6H_2O$ enema given 9-16-80 on plasma and CSF Mg level in calf during preliminary study.

a portion of the necessary calves had been obtained, the calves developed a severe projectile diarrhea. One calf became dehydrated and died despite efforts to rehydrate him with oral electrolytes. Tests conducted later determined that the calves had a corona virus infection. The other calves were kept from dehydrating using daily doses of Kao-pectate. All of the calves in the first trial and 4 of the calves in the second trial developed this highly infectious diarrhea. The 4 calves that did not develop the diarrhea had received an oral corona virus vaccination at or near birth. Calves were kept on milk replacer until they recovered from the infection, which took as long as 3 weeks in more severe cases. Once recovered, many calves appeared to be extremely prone to developing diarrhea and recurrent diarrhea was a problem in these animals.

The animals gained little weight while suffering from this diarrhea and continued to do poorly after they recovered. The initial mean weights and the mean weights after the balance trial are presented in table 6. Weekly plasma mineral levels were not statistically analyzed since many calves suffered from diarrhea throughout much of the preliminary period and were receiving medication. Blaxter and Rook (1954) observed that diarrhea decreased Mg retention in young calves fed a semi-purified diet. Generally, plasma Mg levels dropped below 1 mg/dl within 2 weeks after the calves fed the Mg deficient diet had been weaned. There were no observed effects of dietary K on plasma Mg levels in calves fed either the Mg deficient or Mg supplemented diets. Frye et al. (1975) observed an effect of supplemental K on

Table 6. MEAN CALF WEIGHTS (KG)

Trial	Diet 1	2	3	4
	Initial			
1	46	36	39	37
2	51	53	40	44
	After Balance Trial			
1	52	45	51	43
2	62	67	47	62

plasma Mg levels in wethers fed control levels of Mg but not in wethers fed Mg deficient diets. Perhaps similar results might be observed in these calves if a higher level of supplemental Mg had been fed. It is not known if these calves consumed substantial amounts of wood shavings. Smith (1961) estimated daily consumption of wood shavings by 13-14 week old calves to be 250 g/day. The consumption of wood shavings was found to decrease Mg absoprtion in those calves allowed free access to wood shavings when compared to control muzzled calves.

During the first trial, the 2 calves consuming the Mg deficient diet became severely hypomagnesemic before the collection trial began. One calf had an episode of tetany while still in a stall in the holding area. His plasma Mg level was .49 mg/dl and Ca was normal. He was treated intravenously with 50 ml of Cal-Dextro.[12] After the calves

[12]Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

were moved into the metabolism stalls the same calf again developed tetany. This episode began during the commotion and noise of morning feeding of animals in the metabolism room. The calf was removed from the metabolism stall, a catheter was placed in his juglar vein and a CSF sample was taken without anesthesia. The calf exhibited many of the classical symptoms of hypomagnesemic tetany. He stretched out on his side and intermittently paddled his feet. Opisthotonos and nystagmus were also observed. Occasionally his lips would pull from his teeth and he salivated excessively. He was given an enema of 50 ml of a 30% $MgCl_2 \cdot 6H_2O$ solution which was held in 5 minutes. He was sternal after 12 minutes and stood unassisted after 18 minutes. Plasma Mg before treatment analyzed .29 mg/dl Mg and CSF was 1.26 mg/dl Mg. The plasma Mg levels following enema treatment for this and 2 subsequent episodes of tetany are presented in table 7. The lowest observed CSF Mg level was 1.09 mg/dl during the third tetany episode. In treating this episode of tetany a second enema was given 180 minutes after the first. While the $MgCl_2 \cdot 6H_2O$ enemas successfully brought the calf out of tetany in all cases where it was used, this treatment did not elevate plasma Mg to normal levels after the last 2 episodes of tetany. It is not known at this time why this occurred, unless all body pools of Mg had been severely depleted. None of the enemas were expelled before 5 minutes after administration so this was not due to a loss of the repletion Mg. Calcium levels in the plasma remained normal during all episodes of tetany.

Table 7. EFFECT OF $MgCl_2 \cdot 6H_2O$ ENEMAS ON PLASMA MAGNESIUM LEVELS GIVEN DURING THREE EPISODES OF TETANY IN CALF #1.

	mg/dl		
Minutes after enema	12-10-80	12-15-80	12-19-80
0	.29	.29	.30
5	1.25	.78	1.57
15	2.00	.87	1.42
30	2.47	.90	1.28
60	2.22	.98	1.15
120	1.65	.70	1.03
180[a]	1.49	.53	.82
30	----	.80	----
180	----	1.14	----
	CSF		
Baseline sample	1.26	1.09	----

[a]Second enema given here on 12-15-80.

Tetany was observed in another calf consuming the Mg deficient plus K diet during the second trial. This calf recovered without treatment 5 minutes after the episode began. A plasma sample taken after the calf had recovered analyzed .4 mg/dl.

Balance Study

During the first trial, the 2 calves consuming the basal diet were severely hypomagnesemic. They became anorexic and their consumption of the dry feed dropped rapidly. Another calf consuming the diet supplemented with Mg and K also went off feed and so fecal and urine samples were collected from only 5 calves. One of those calves developed diarrhea during the collection period. During the second trial, the calf which had tetany also became anorexic and went off feed. Samples were collected from 7 calves, 2 of which were treated for diarrhea during the collection period. Those calves which developed diarrhea during the collection period were not included in the statistical analyses, leaving 9 calves. The distribution of those 9 calves across diets and trials is shown in table 8.

For comparison, 2 methods of urine and fecal analysis were used in this study. For 1 method, the average daily excretion of urinary and fecal minerals was used to calculate balance results for the collection period. The other method used a composite urine sample analysis and the average daily urine volume to calculate urinary mineral excretion. Fecal mineral excretion was calculated from average daily fecal volume and the average daily fecal mineral analysis. Intake of minerals was calculated using a composite sample for both methods.

Table 8. DISTRIBUTION OF CALVES USED FOR STATISTICAL ANALYSIS OF BALANCE STUDY

Trial	Diet			
	1	2	3	4
1	-	2	1	1
2	2	1	1	1

Analysis of variance of trial 1 indicated that for the average daily excretion analysis there were significant differences across diets for urinary Ca and Mg and for the intake of Ca, Mg and K. With the composite analysis, data significant differences occurred for urinary Mg and the intake of Ca, Mg and K. Analysis of variance of trial 2 revealed that for the average daily excretion analysis method urinary Mg and K and the intake of Mg were significantly different across diets. The same variables were significantly affected by diet when the composite analysis method was used. Intake of K approached significance with both methods of analysis.

Daily Mg balances for calves from both trials calculated from average daily excretion are presented in table 9. Mg intake was higher ($P < .05$) for calves on Mg supplemented diets. Fecal excretion of Mg was also higher ($P < .05$) for calves fed diets supplemented with Mg. Mg balance was greater ($P < .05$) for calves fed diets supplemented with Mg. Calves fed Mg deficient diets were in a negative balance for Mg. Similar results were obtained for Mg balance calculated using composite samples (table 9), the only difference being the level of

Table 9. LEAST SQUARE MEANS OF DAILY MAGNESIUM BALANCE IN GRAMS (± S.E.M.).

Item	Diet 1		Diet 2		Diet 3		Diet 4	
Average Daily Excretion								
No. of animals	2		3		2		2	
Intake	.197[a]	± .23	.196[a]	± .191	1.694[b]	± .232	1.813[b]	± .234
Urinary excretion	.007	± .045	.006	± .037	.148	± .045	.079	± .045
Fecal excretion	.364[a]	± .155	.367[a]	± .127	1.044[b]	± .155	1.141[b]	± .155
Balance	-.177[a]	± .091	-.178[a]	± .047	.502[b]	± .091	.593[b]	± .091
Composite Samples								
No. of animals	2		3		2		2	
Intake	.197[a]	± .234	.196[a]	± .191	1.694[b]	± .234	1.813[b]	± .234
Urinary excretion	.009	± .046	.008	± .037	.155	± .046	.075	± .046
Fecal excretion	.358[a]	± .150	.366[a]	± .122	1.048[b]	± .150	1.143[b]	± .150
Balance	-.170	± .090	-.178[a]	± .073	.492[a]	± .090	.595[b]	± .090

[ab]Least squares means within a row superscripted with different letters are significantly different ($P < .05$).

significance for Mg balance using average daily excretion was greater. The feces appeared to be the main route of excretion for calves fed all diets.

The daily Ca balances for both trials are presented in table 10. There were no significant differences across diets for Ca intake, excretion or balance. The feces was the major route of excretion of Ca, with very little Ca being excreted in the urine.

The daily K balances for both trials are presented in table 11. Similar results were obtained for K balance using both methods of calculation. Intake of K was greater ($P < .05$) for the calves fed diets supplemented with K. Urinary excretion was also greater for calves fed excess K. Analysis of variance revealed that fecal excretion of K was significantly affected by diet; however, mean separation using Student-Neuman-Keuls procedures did not indicate significant differences across least-squares means. Balance was significantly different for calves fed the diet supplemented with Mg and K. Student-Neuman-Keuls procedures did not find K balance to be significantly different for the calves fed the Mg deficient diet supplemented with K. This was probably because the intake of K was lower for these calves than for the calves fed the diet supplemented with Mg and K. The major route of excretion for K was the urine.

The excretory pattern of Mg observed in these calves agrees with the results of Blaxter and Rook (1954b) who found that the amount of Mg excreted in the urine was small compared to the amount excreted in the feces. Fecal excretion of Mg is also much greater than urinary excretion in sheep (Field, 1967) and lactating cows (Kemp et al., 1961)

Table 10. LEAST SQUARE MEANS OF DAILY CALCIUM BALANCE IN GRAMS (± S.E.M.)

Item	Diet 1	Diet 2	Diet 3	Diet 4
Average Daily Excretion				
No. of animals	2	3	2	2
Intake	6.169 ± 1.186	7.193 ± .968	7.825 ± 1.186	8.791 ± 1.186
Urinary excretion	.026 ± .005	.028 ± .004	.028 ± .005	.042 ± .005
Fecal excretion	2.720 ± 1.010	3.463 ± .894	4.173 ± 1.010	4.869 ± 1.010
Balance	3.424 ± 1.375	3.702 ± 1.122	3.625 ± 1.375	3.88 ± 1.375
Composite Samples				
No. of animals	2	3	2	2
Intake	6.169 ± 1.189	7.193 ± .968	7.825 ± 1.186	8.791 ± 1.186
Urinary excretion	.029 ± .008	.031 ± .007	.032 ± .008	.054 ± .008
Fecal excretion	2.703 ± 1.101	3.421 ± .899	4.203 ± 1.101	4.893 ± 1.101
Balance	3.438 ± 1.370	3.742 ± 1.118	3.590 ± 1.370	3.844 ± 1.370

[ab]Least square means within a row superscripted with different letters are significantly different (P .05).

TABLE 11. LEAST SQUARE MEANS OF DAILY POTASSIUM BALANCE IN GRAMS (± S.E.M.).

Item	Diet 1		Diet 2		Diet 3		Diet 4	
Average Daily Excretion								
No. of animals	2		3		2		2	
Intake	8.196[a]	± 2.558	26.721[b]	± 2.089	9.334[a]	± 2.558	32.402[b]	± 2.558
Urinary excretion	4.957[a]	± 3.022	18.599[b]	± 2.468	4.963[a]	± 3.022	20.356[b]	± 3.022
Fecal excretion	.335	± .058	.577	± .047	.550	± .058	.492	± .058
Balance	2.905[a]	± 1.075	7.544	± .878	3.822[a]	± 1.075	11.554[b]	± 1.075
Composite Sample								
No. of animals	2		3		2		2	
Intake	8.196[a]	± 2.558	26.721[b]	± 2.089	9.334[a]	± 2.558	32.402[b]	± 2.558
Urinary excretion	4.401	± 3.312	19.764	± 2.704	4.236	± 3.312	19.331	± 3.312
Fecal excretion	.334	± .057	.570	± .047	.559	± .057	.498	± .057
Balance	3.462[a]	± 1.454	6.387	± 1.187	4.540[a]	± 1.454	12.573[b]	± 1.454

[ab]Least square means within a row superscripted with different letters are significantly different ($P < .05$).

fed forage. Urinary Mg was higher for calves fed the Mg supplemented diets, which agrees with work in calves by Smith (1959c). Other researchers have shown in sheep and cows that the amount of Mg in the diet is reflected in urinary Mg levels (Miller et al., 1972). The Mg concentration of the urine in the calves fed the Mg deficient diets was extremely low. The mean plasma Mg level for the 2 calves on the Mg deficient diet at the start of collection was .7 mg/dl. Smith (1957) reported that as calves became hypomagnesemic, the urinary excretion of Mg dropped to a residual value and then was not affected by further drops in plasma Mg. The balance of Mg appeared to be unaffected by dietary K. Smith (1957) compared the percent of dietary Mg in the sum of retention and urinary excretion. In the calves fed diets supplemented with Mg the sum of Mg retained and excreted in the urine was a lower percentage of dietary Mg in calves fed supplemental K than in those fed supplemental Mg alone (37 versus 60%).

Smith (1957) reported results for Ca balance similar to the results in this study. Urinary excretion of Ca was never more than 1% of dietary intake which was also observed in this study. Smith (1957) did report greater values for the percent of dietary Ca retained, which may be because calves in the study reported by Smith received daily supplements of from 7000 to 140,000 I.U. of vitamin D in the diet. Calves on this study consumed about 18,000 I.U. vitamin D daily. The increased urinary excretion of K in calves fed supplemental K agrees with results in sheep reported by House and Van Campen (1971).

Repletion Data

Repletion data were obtained for all 16 calves. Analysis of variance of trial 1 showed diet and minute had a significant effect on plasma Mg level. Orthogonal comparisons of baseline versus subsequent sample plasma Mg levels were different at all sampling times ($P < .001$). When the same orthogonal comparisons were made by diet, all sampling times for each diet were significantly different from baseline values except for the 5 minute sample for diet 1. Means of plasma Mg levels for the 2 calves fed diet 4 in trial 1 showed the greatest increase in plasma Mg, peaking at 5.89 mg/dl at the 30 minute sampling time.

Analysis of variance of trial 2 showed diet and minute also had a significant effect on plasma Mg level. Orthogonal comparisons of baseline versus subsequent sampling times were different for each sampling time ($P < .001$). Orthogonal comparisons by diet were not significant for diet 3. Comparisons were significant at all sampling times for diets 1 and 2 ($P < .05$) and at 14, 30, 60 and 120 minutes for diet 4 ($P < .1$).

Analysis of variance for the combined data showed that diet, trial, minute and trial X diet interaction all had significant effects on plasma Mg levels. Orthogonal comparisons were different ($P < .001$) from baseline at each sampling time. Orthogonal comparisons by diet were significant at all sampling times for each diet. Differences were most significant for diet 1 and least significant for diet 3. Plots of the regression curves for each diet from the combined data are illustrated in figure 2. Plots of the regression curves versus

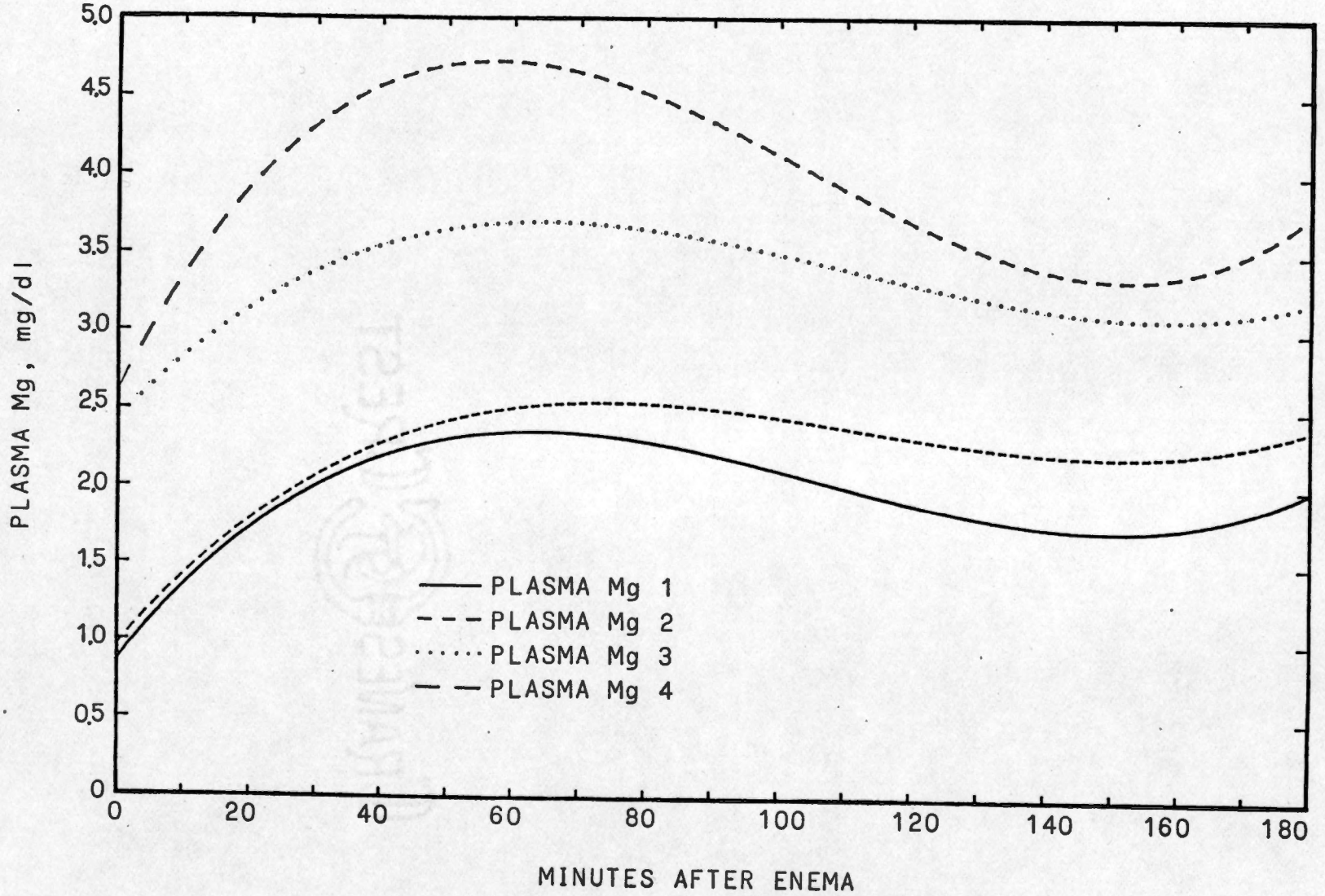


Figure 2. Plots of the regression curves for plasma Mg of calves on each diet.

the actual mean values for plasma Mg are shown in figure 3. Plasma Mg level responded similarly to the $MgCl_2 \cdot 6H_2O$ enema for diets 1 and 2 while calves fed diet 4 showed the greatest increase in plasma Mg level. With all diets, peak calf plasma Mg levels occurred near the 60 minute sampling time and then declined gradually.

Analysis of variance of trial 1 showed that minute had a significant effect on CSF Mg level. Differences across diets were not great enough to be significant. Orthogonal comparisons showed differences ($P < .05$) in CSF Mg level from baseline level at the 120 and 180 minute sampling times. Differences approached significance at the 30 and 60 minute sampling times. Orthogonal comparisons by diet showed no significant differences at any sampling times for diets 1, 3 and 4. With diet 1, CSF Mg level approached significant differences from baseline level at 120 and 180 minutes. With diet 2, CSF Mg level approached significant differences at 30 minutes and was different ($P < .01$) at 60 minutes and at 120 and 180 minutes ($P < .001$).

Analysis of variance of trial 2 showed diet and minute had an effect ($P < .001$) on CSF Mg level. Orthogonal comparisons indicated differences from baseline Mg level from 15 minutes through 180 minutes. The level of significance increased with subsequent samples. Orthogonal comparisons by diet showed that with calves fed diet 1, significance occurred at 120 and 180 minutes, while with calves fed diet 2 all CSF samples had significantly different Mg levels from baseline Mg level except the 15 minute sample. Significance level increased with subsequent samples. With calves fed diet 3, the 15 minute sample was different ($P < .1$) from baseline and with calves fed diet 4 the 180 minute sample was different ($P < .1$) from baseline.

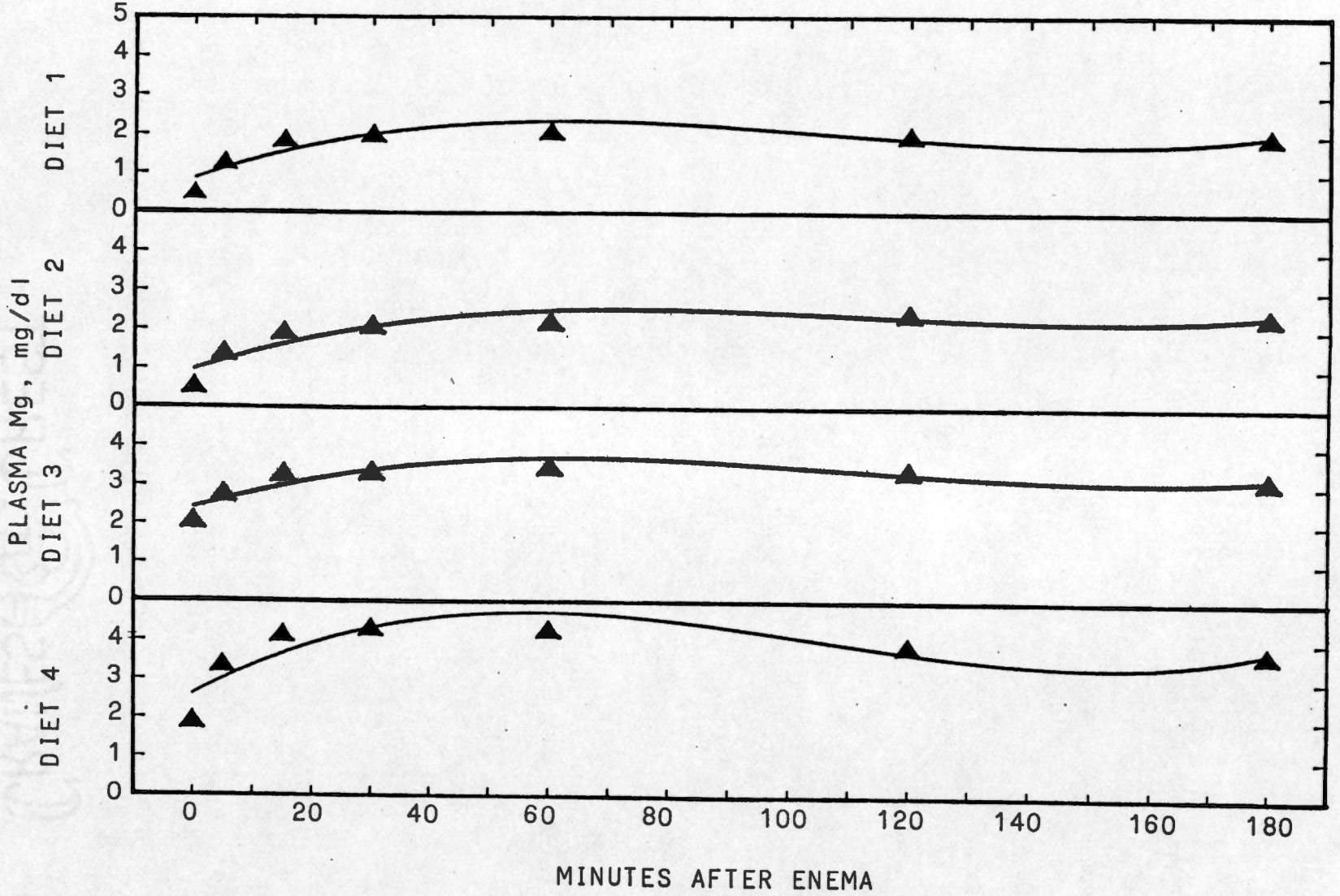


Figure 3. Regression curves for plasma Mg of calves on each diet and actual mean valves.

Analysis of variance of the combined data showed that diet, trial, minute and trial X diet interaction all had a significant effect on calf CSF Mg. Orthogonal comparisons showed significant differences from 30 minutes through 180 minutes. Orthogonal comparisons by diet showed significant differences from 30 to 180 minutes for calves fed diets 1 and 2. CSF Mg level was not significantly different from baseline level at any sampling time for calves fed diets 3 and 4. Plots of the regression curves for each diet was illustrated in figure 4. Plots of the regression curves versus the actual mean values for CSF Mg are shown in figure 5. CSF Mg levels responded similarly to the $MgCl_2 \cdot 6H_2O$ enema for calves fed diets 1 and 2, with gradual increases being seen until a plateau was reached at about the 120 minute sample. For calves fed diet 3 CSF Mg level increased slightly and then began to decline after 60 minutes. With calves fed diet 4 the CSF Mg level increased gradually until the 180 minute sample.

Analysis of variance of trial 1 showed that diet had a significant effect on RBC Mg. Analysis of variance of trial 2 also showed that diet had a significant effect of RBC Mg. Analysis of variance of the combined data showed that diet and diet X trial interaction had a significant effect on RBC Mg. Plots of regression curves for each diet are shown in figure 6. Calves fed diets 1 and 2 had the lowest levels of RBC Mg, while calves fed diet 3 had the highest Mg level. Calves on diet 2 showed a gradual increase in RBC Mg level after the $MgCl_2 \cdot 6H_2O$ enema, which increased until the last sample, while calves in diets 3 and 4 showed an increase and subsequent decline in RBC Mg level. None of these changes in RBC Mg levels were significant for the combined data.

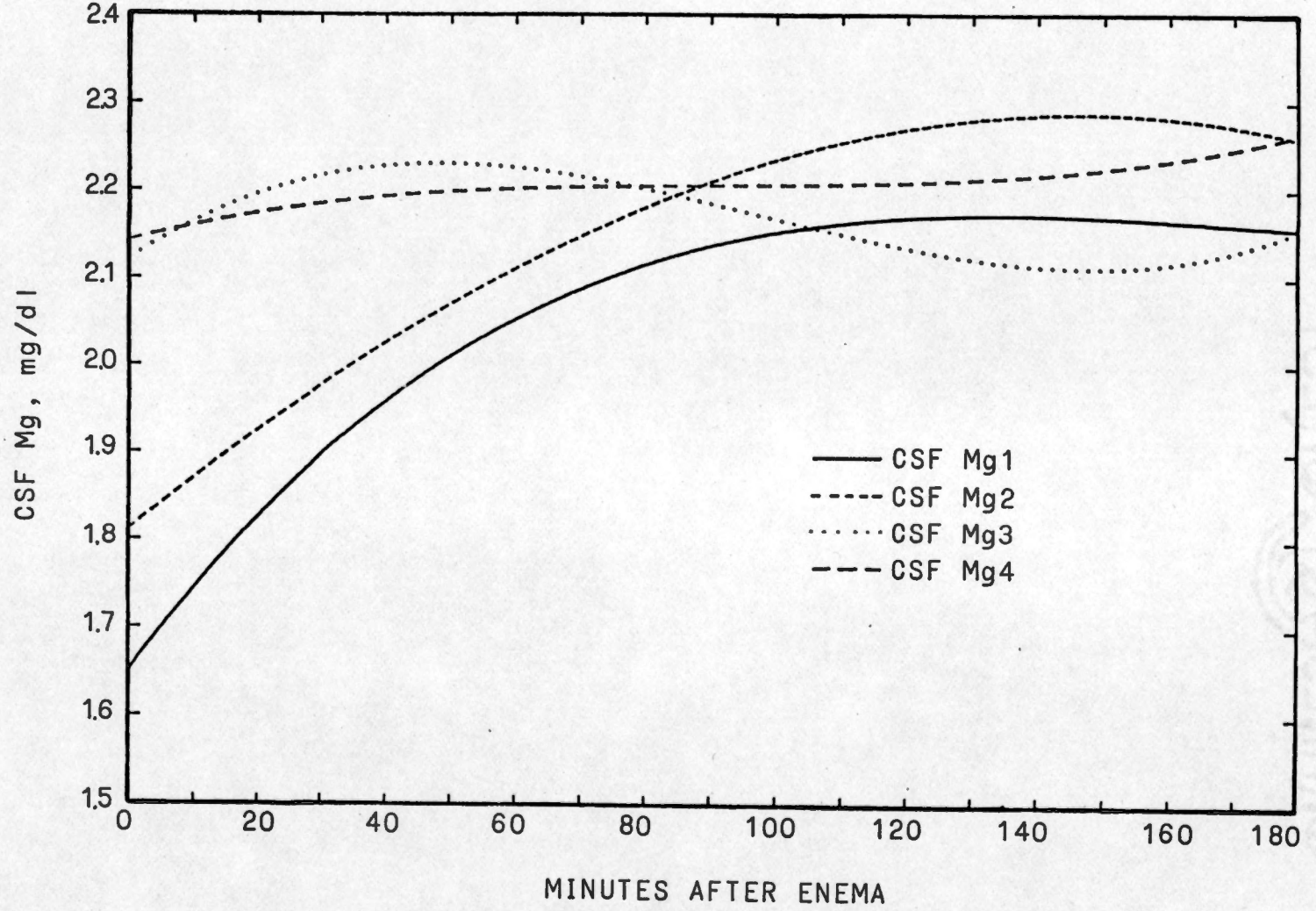


Figure 4. Plots of the regression curves for CSF Mg of calves on each diet.

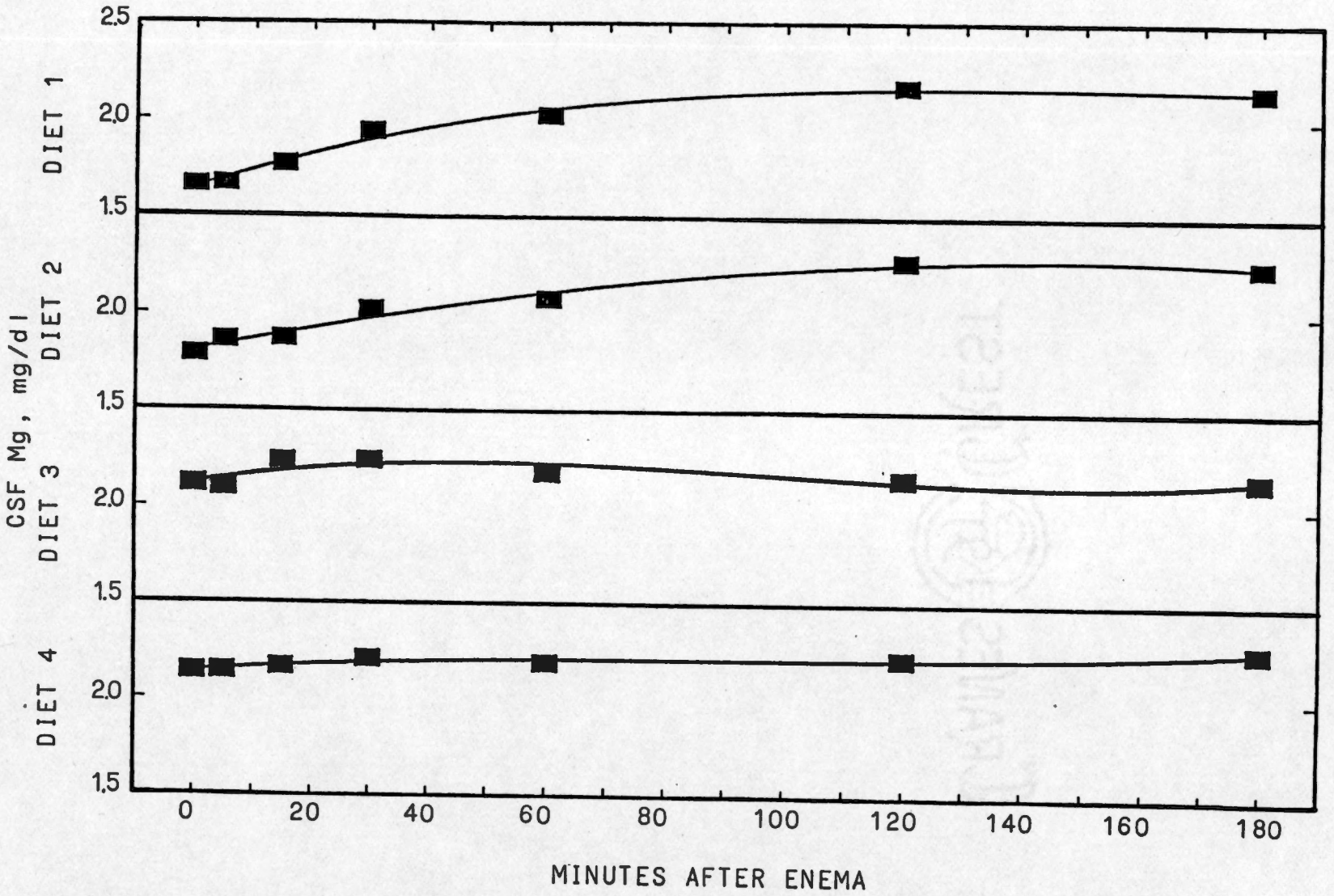


Figure 5. Regression curves for CSF Mg of calves on each diet and actual mean values.

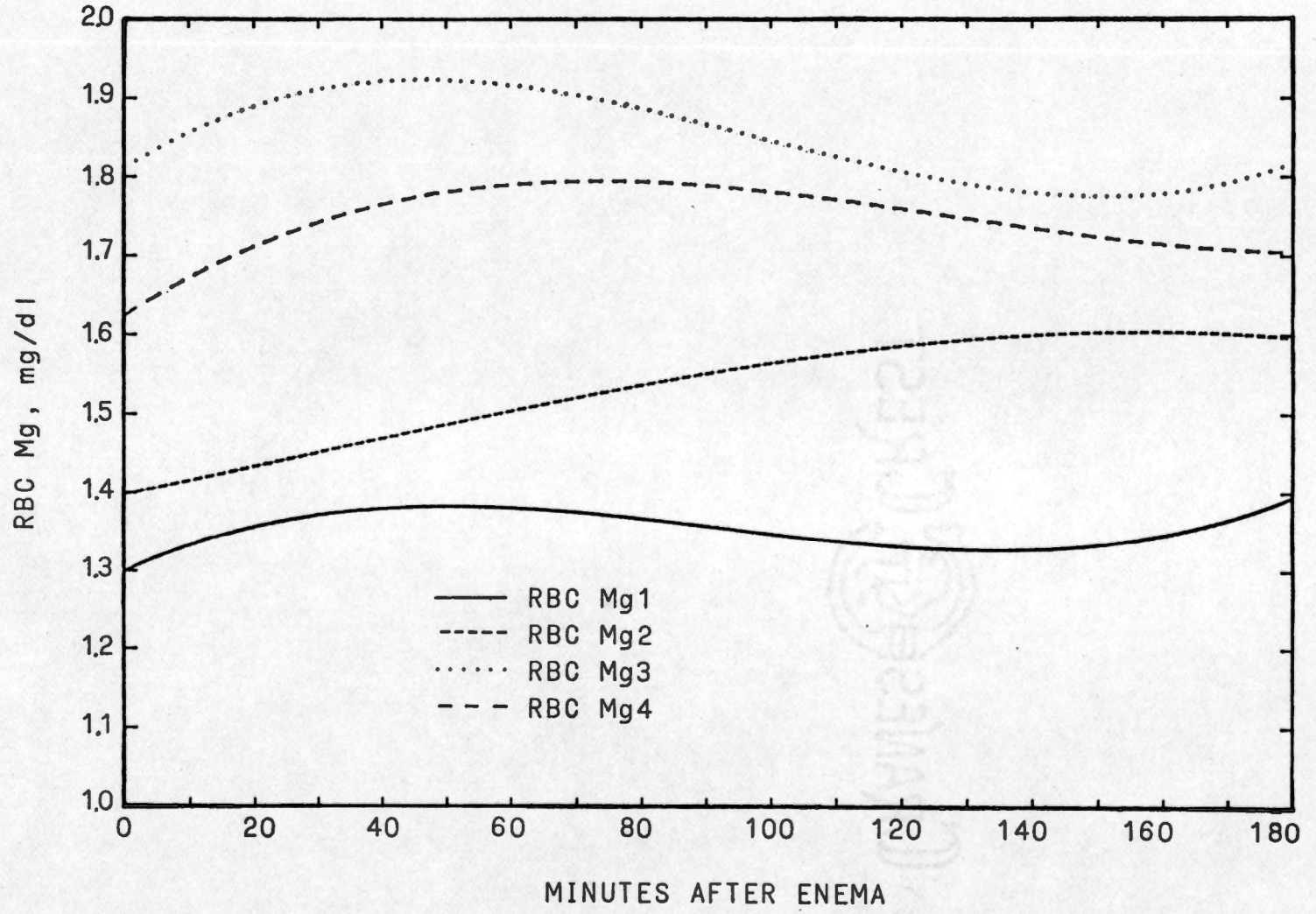


Figure 6. Plots of regression curves for RBC Mg of calves on each diet.

Analysis of variance of trial 1 showed diet had a significant effect on plasma Ca. Analysis of trial 2 showed that diet and minute had a significant effect on plasma Ca. Orthogonal comparisons showed that differences from baseline Ca levels occurred at 60, 120 and 180 minutes. Calf diet orthogonal comparisons showed differences (P .05) occurred at 180 minutes for diet 1 and 120 minutes for diet 2. Analysis of variance for the combined data showed diet, minute and diet X trial interaction had a significant effect on calf plasma Ca. Orthogonal comparisons showed significant differences from baseline levels at 60, 120 and 180 minutes. Calf diet orthogonal comparisons showed significant differences at 120 minutes for calves on diet 2 and 60 and 180 minutes for calves on diet 3. Plots of regression curves for each diet are illustrated in figure 7. Calves on diet 1 had the lowest plasma Ca levels and showed no changes in plasma Ca level after administration of the $MgCl_2 \cdot 6H_2O$ enema. Calves on diets 2, 3and 4 showed increases in plasma Ca after the enema. Calves fed diet 2 showed the greatest increases in plasma Ca which peaked at 120 minutes and then dropped sharply.

Analysis of variance of trial 1 showed diet had a significant effect on calf CSF Ca. Analysis of variance of trial 2 showed diet and minute had a significant effect on calf CSF Ca. Orthogonal comparisons were significant at 180 minutes. Orthogonal comparisons by diet showed that calf CSF Ca levels were significantly different from baseline levels at 60, 120 and 180 minutes for diet 3. Analysis of variance for both trials showed diet, trial and trial X diet interaction had a

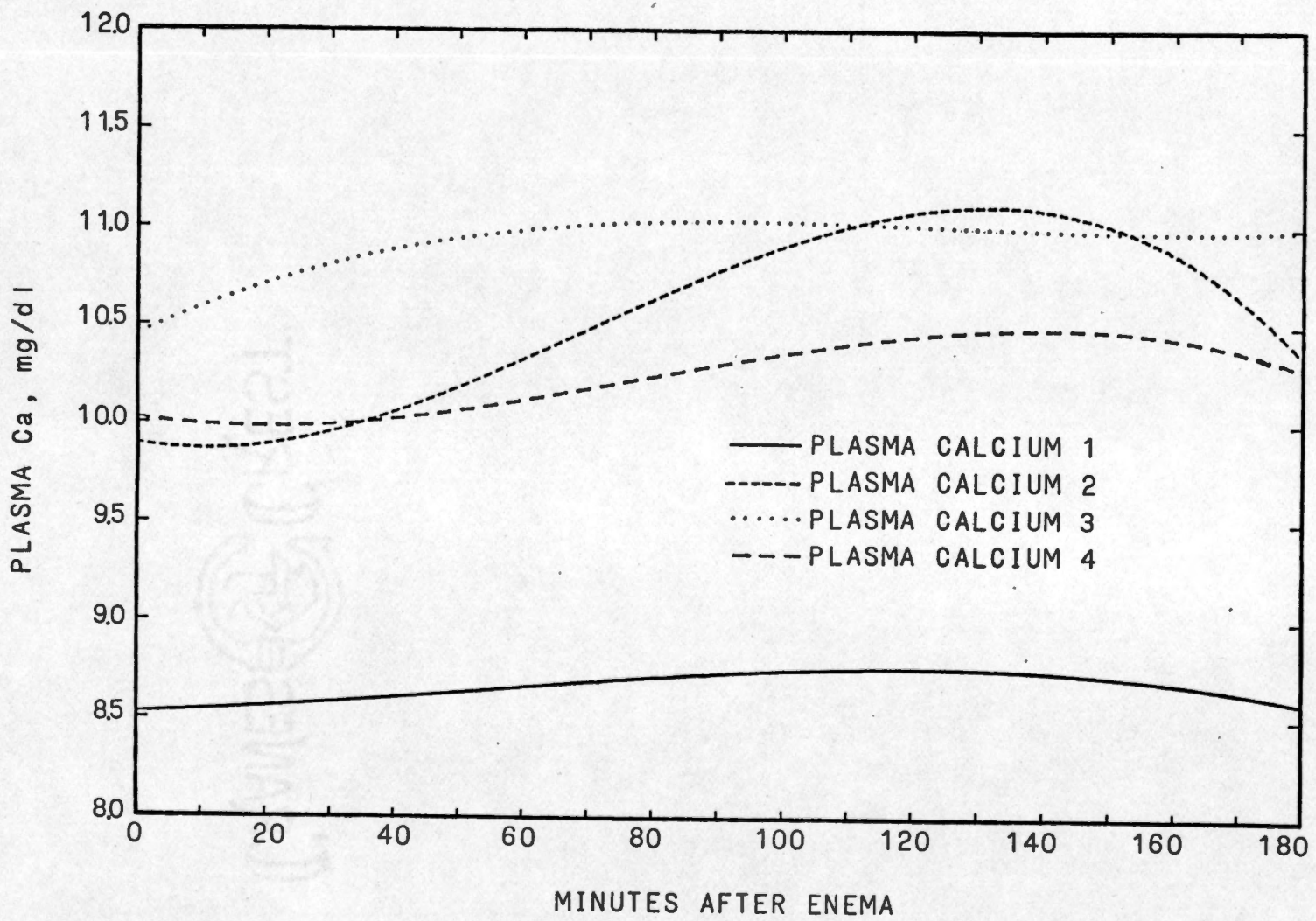


Figure 7. Plots of regression curves for plasma Ca of calves on each diet.

significant effect on CSF Ca. Minute did not have a significant effect on CSF Ca. Plots of regression curves for CSF Ca showed calves on diet 1 had the lowest CSF Ca levels. Cerebrosponal fluid Ca level remained fairly constant for diet 1 and decreased gradually for diet 2 after the $MgCl_2 \cdot 6H_2O$ enema was administered. For calves fed diets 3 and 4, CSF CA increased, peaking at about 60 minutes and then declined gradually.

Analysis of variance for trial 1 showed diet had a significant effect on calf RBC Ca, while analysis of variance for trial 2 showed that neither diet nor minute were significant. Analysis of variance of the combined data showed that only trial had a significant effect on calf RBC Ca. Plots of regression curves for RBC Ca showed varied changes after the $MgCl_2 \cdot 6H_2O$ enema was administered. For calves fed diet 2, RBC Ca increased slightly then began to decline after about 90 minutes, while for calves on diets 1, 2 and 4 RBC Ca decreased and then began to increase between 60 and 120 minutes.

Analysis of variance of trial 1 showed diet had a significant effect on plasma K, while analysis of variance of trial 2 showed diet and minute had a significant effect on plasma K. Orthogonal comparisons for trial 2 were significant for 60, 120 and 180 minutes. By diet orthogonal comparisons were significant for calves fed diet 2 at 120 and 180 minutes and for calves fed diet 3 at 60, 120 and 180 minutes. Analysis of variance of the combined data showed diet, trial and trial X interaction had a significant effect on plasma K. Plots of regression curves for plasma K showed that calves fed diet 1 had the lowest plasma K level while calves fed diets 2 and 3 had the highest

plasma K level. Plasma K levels for calves on all diets showed a slight increase in plasma K after the administration of the $MgCl_2 \cdot 6H_2O$ enemas.

Analysis of variance of trials 1 and 3 showed diet had a significant effect on CSF K level. Analysis of variance of the combined data showed diet, trial and diet X trial interaction had a significant effect on CSF K. Plots of regression curves for each diet showed calves fed diet 3 had the highest level of CSF K. No changes were observed in CSF K level for calves fed diet 3, while calves fed diet 2 had a slight increase in CSF K level which began at 90 minutes. With calves fed diet 1, CSF K decreased sharply, began increasing at 40 minutes and then decreased sharply at 130 minutes. For calves fed diet 4, CSF K responded in a near opposite symmetrical pattern compared to diet 1, increasing, decreasing and then increasing.

Analysis of variance of trials 1 and 2 showed diet had a significant effect on RBC K. Analysis of variance for the combined data showed trial X diet interaction had a significant effect on RBC K. Plots of regression curves for each diet showed calves on diet 1 and 4 had the highest level of RBC K. RBC K level remained fairly constant after the $MgCl_2 \cdot 6H_2O$ enema was administered to calves fed diet 1. RBC K level in calves fed diets 2, 3 and 4 decreased sharply and then began to increase after 60 minutes. Calves fed diet 4 showed the sharpest decrease and increase in RBC K level.

Calf packed cell volume (PCV) was significantly affected by diet in trials 1 and 2. Analysis of variance of the combined data showed diet, trial and trial X diet interaction had a significant effect on PCV. Plots of regression curves for each diet showed that

PCV remained fairly constant after the enema was administered to calves fed all 4 diets. There were slight decreases in PCV for calves fed diets 1, 2 and 3 but levels began to increase again after 30-60 minutes. Calves on diet 1 in trial 1 were slightly anemic when the $MgCl_2 \cdot 6H_2O$ enema was administered. Mg deficiency has been shown to cause anemia in rats (Elin, 1976).

Plots comaring regression curves for plasma, CSF and RBC Ca, Mg and K are presented by diet in figures 8 through 11.

The increases in plasma Mg level observed after administration of the $MgCl_2 \cdot 6H_2O$ enema to calves are similar to the results reported by other researchers (Meyer and Busse, 1975b; Bell et al., 1978). Plasma Mg increased the least in calves fed diet 1, which was probably due to their being the most hypomagnesemic when given the enema. The calves fed diet 4 showed the greatest increases in plasma Mg level. This was due to the large increases observed in the 2 calves fed diet 4 during trial 2 and was probably due to animal variation. This high level of plasma Mg was far from reported toxic levels (Shils, 1980) and was maintained for only a short time period. With calves fed all 4 diets peak levels of plasma Mg occurred at 60 minutes. The peak level of plasma Mg occurred more rapidly in the work by Meyer and Busse (1975b) in sheep (5 minutes) and in the work by Bell et al. (1977) in steers (20 minutes), cows (20 minutes) and sheep (10 minutes).

The increases in CSF Mg level after administration of the $MgCl_2 \cdot 6H_2O$ enema to calves are similar to results in sheep reported by Meyer and Busse (1975a). The largest and most rapid increases in CSF Mg level were observed in calves which had the lowest baseline

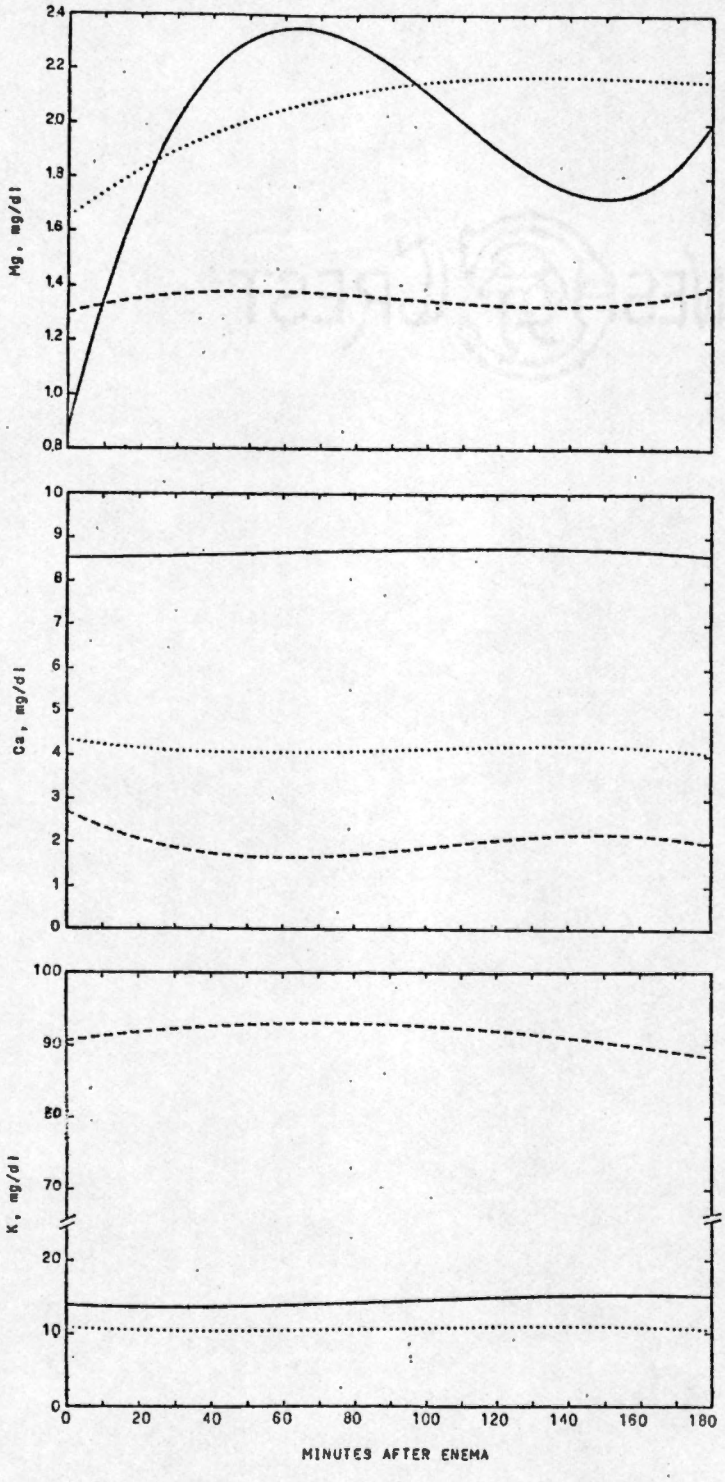


Figure 8. Plots of regression curves for plasma (——), CSF (···) and RBC (---) minerals for calves on diet 1.

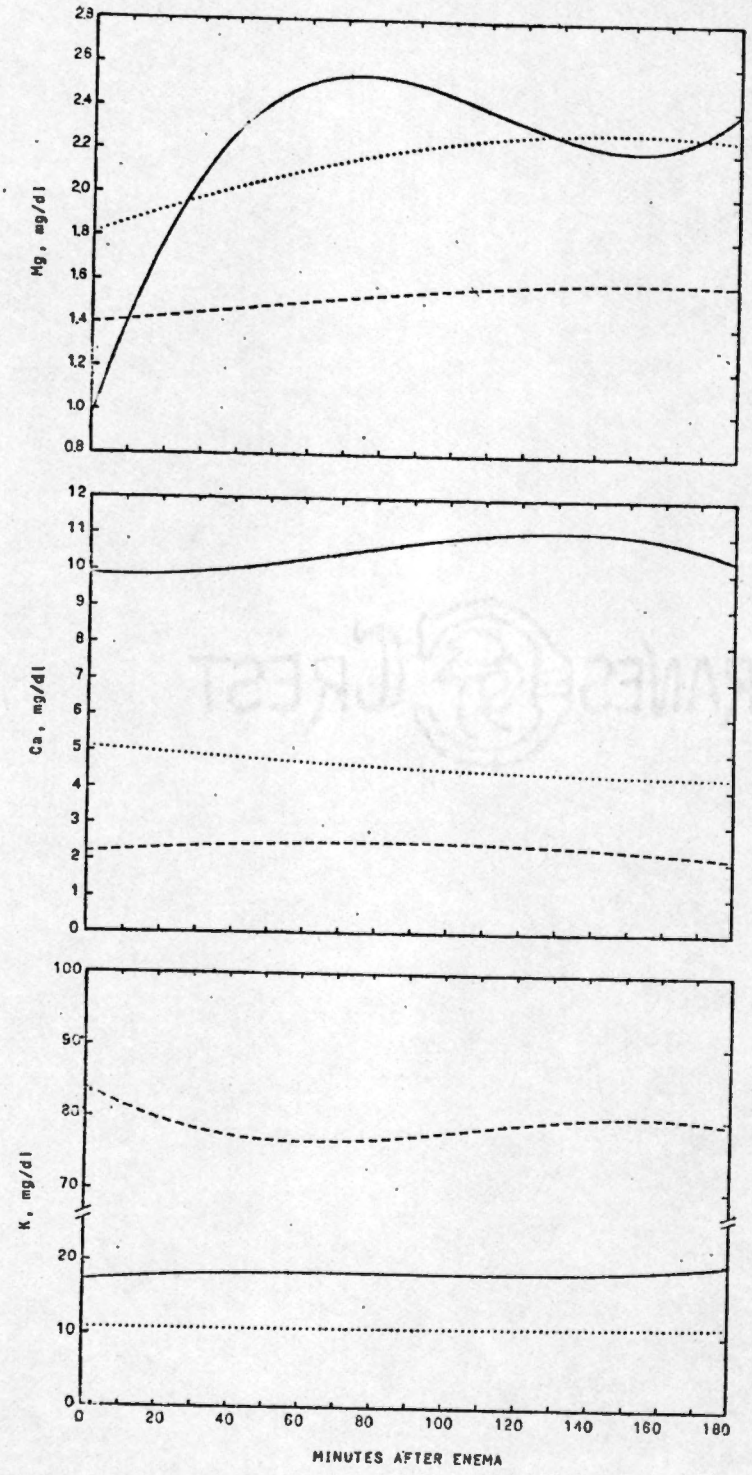


Figure 9. Plots of regression curves for plasma (——), CSF (···) and RBC (---) minerals for calves on diet 2.

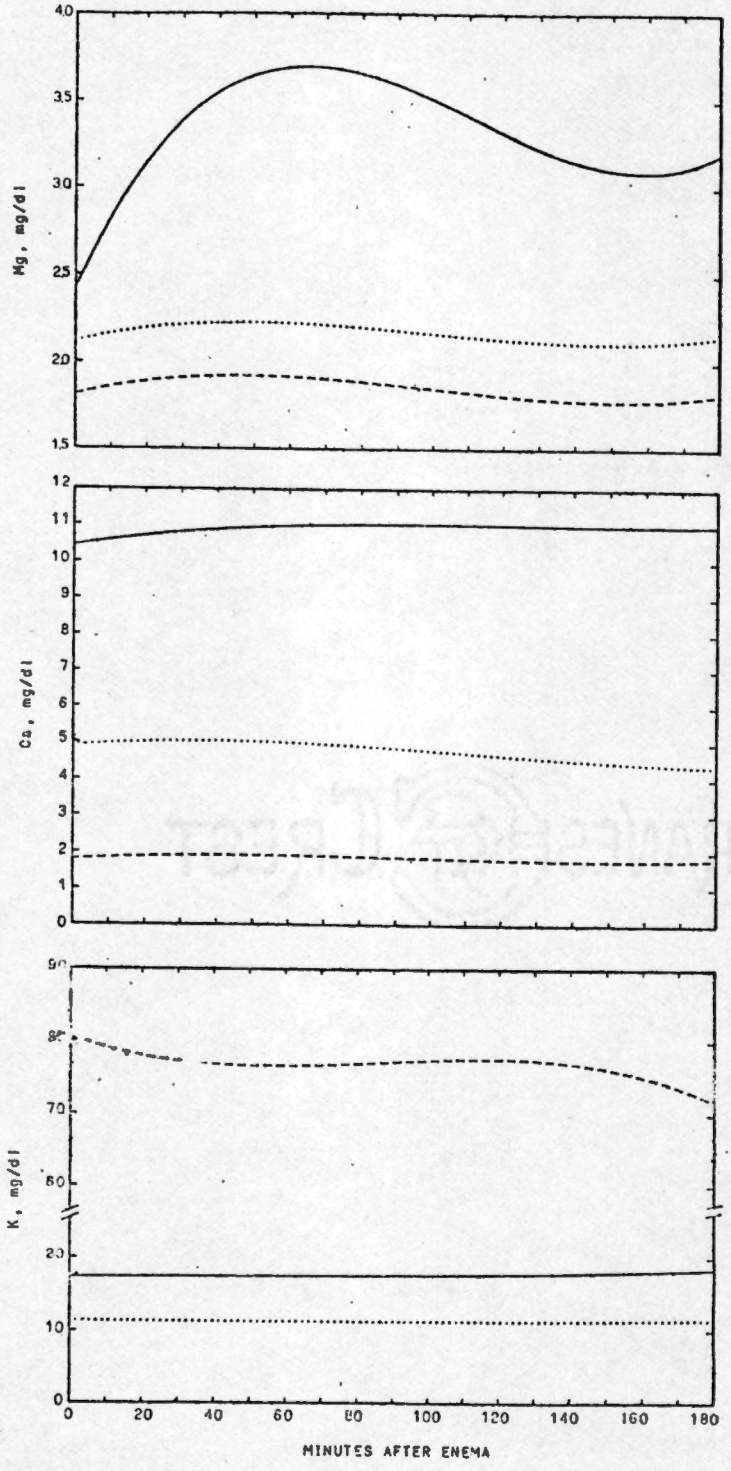


Figure 10. Plots of regression curves for plasma (——), CSF (···) and RBC (---) minerals for calves on diet 3.

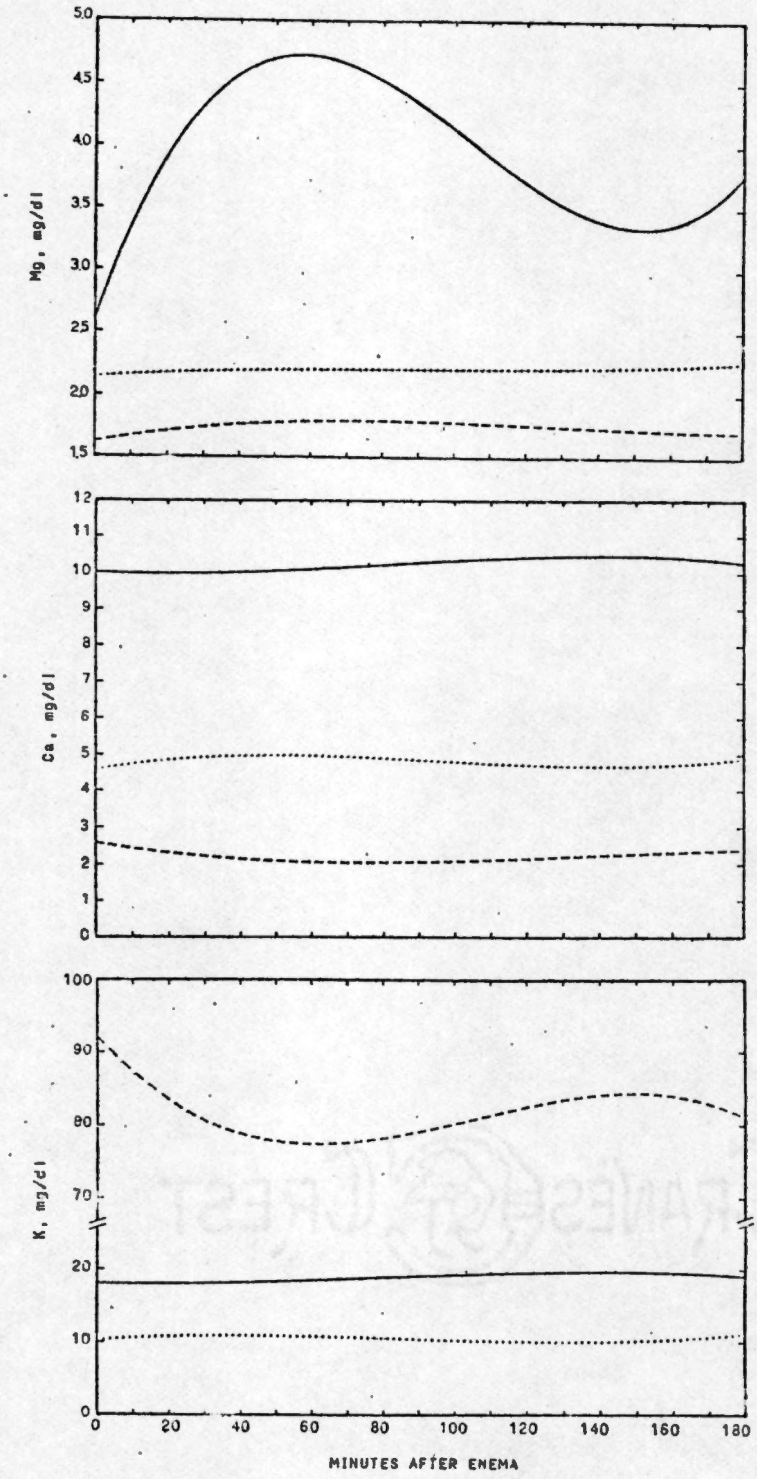


Figure 11. Plots of regression curves for plasma (___), CSF (···) and RBC (---) minerals for calves on diet 4.

CSF level. Meyer and Busse also observed the greatest increases in CSF Mg level in hypomagnesemic sheep with depleted CSF Mg given an intravenous injection of Mg gluconate. In this study, significant increases in CSF Mg level were observed after 30 minutes. In the study by Meyer and Busse, increases in CSF Mg were more rapid in samples taken from the cerebral ventricle than from the cisterna magna. Peak levels were observed after 15 minutes in samples taken from the cerebral ventricle while in samples taken from the cisterna magna the Mg level was approaching peak levels after 30 minutes. The slight, short lasting increase in CSF Mg level seen in calves fed diet 3 was also observed in sheep with normal CSF Mg levels given intravenous gluconate by Meyer and Busse. Meyer (1977) has reported that when CSF Mg level decreases below 1.6 mg/dl the risk of hypomagnesemic tetany is increased and that when CSF Mg drops below 1.2 mg/dl tetany occurs regularly. CSF samples taken from the same calf during separate episodes of tetany analyzed 1.26 mg/dl during 1 episode and 1.09 mg/dl during an episode occurring 5 days later. Lower levels of CSF Mg and more instances of tetany might have been observed in these trials if 2 calves which were severely hypomagnesemic had not developed anorexia and stopped eating the dry feed. The plasma Mg level did not decrease further in these calves after they went off feed.

The lower levels of RBC Mg observed in calves fed the Mg deficient diets agrees with earlier conclusions that lowered RBC Mg levels result from dilution with RBC formed with lower Mg levels (McAleese et al., 1961). Elin (1976) reported that studies of RBC membrane

permeability to Mg are unclear. In vivo studies have shown that ^{28}Mg is incorporated into RBC while in vitro studies using ^{28}Mg have not shown ^{28}Mg uptake by the RBC. In this study, no significant changes in RBC Mg level after the administration of $MgCl_2 \cdot 6H_2O$ enemas were observed.

No reason can be given for the increases in plasma Ca seen in calves fed diets 2 and 3. Calves fed diet 1 had the lowest plasma and CSF Ca levels. Hypocalcemia has been reported to accompany hypomagnesemia in many cases (Rook and Storry, 1962) and these calves were the most severely hypomagnesemic when the enemas were administered. The plasma and CSF Ca levels seen for calves on diet 1 were below normal according to Kaneko (1977). No conclusions can be made from the varied response seen in RBC Ca levels.

Plasma and CSF K values are all within normal ranges (Kaneko, 1977). There was a great deal of variation in the level of plasma, CSF and RBC K after administration of the $MgCl_2 \cdot 6H_2O$ enema, however, no significant effect was observed. Calves on diet 1 had high RBC K levels that were unaffected by the $MgCl_2 \cdot 6H_2O$ enema. No explanation can be given for the trends towards decreasing RBC K level seen for the calves on the other diets.

These results provide further evidence for the effectiveness of $MgCl_2 \cdot 6H_2O$ enemas in elevating plasma Mg levels in a short time period. The $MgCl_2 \cdot 6H_2O$ enema was also effective in repleting CSF Mg level in calves with subnormal CSF Mg level. A calf with tetany had subnormal

CSF Mg concentration and was treated successfully using a $MgCl_2 \cdot 6H_2O$ enema. The $MgCl_2 \cdot 6H_2O$ enema had no effect on plasma Ca and K, RBC minerals or PCV.

CHAPTER V

SUMMARY

Holstein bull calves were used as models to study metabolism of Mg. The calves were fed a dry semi-purified diet with either high or low Mg and high or low K content. During 2 5-day balance trials samples of feces, urine and feed were collected daily from calves not having diarrhea or anorexia. After the balance trial each calf was given an enema of 30% solution of $MgCl_2 \cdot 6H_2O$. Subsequent samples of blood and CSF were taken to determine the effect of the enema on plasma, CSF and RBC, Ca, Mg and K levels.

Calves on the diets supplemented with Mg had higher ($P < .05$) intake, fecal excretion and balance of Mg. The urinary excretion of Mg was not statistically different among calves on the 4 diets. Excess dietary K did not have a significant effect on Mg excretion or balance. This is in contrast to results reported in adult cows and sheep. The reason that no effect of dietary K on Mg metabolism was observed in these calves is not clear. The differences in results may be due to differences in Mg utilization and excretory patterns between the young growing animals and the older lactating animals or it may be due to differences in diet and stress levels.

There were no differences in intake, excretion or balance of Ca. The intake and urinary excretion of K were greater ($P < .05$) for calves consuming the diets supplemented with K. The balance of K was greater ($P < .05$) for calves consuming the diet supplemented with Mg and K.

There appeared to be no effect of K on plasma Mg level. Calves consuming the Mg deficient diets became hypomagnesemic within a few weeks. Severely hypomagnesemic calves on the basal diet also had subnormal plasma Ca levels.

The $MgCl_2 \cdot 6H_2O$ enemas elevated plasma Mg levels significantly within 5 minutes when given to calves on each diet. Calves on the Mg deficient diets which had subnormal levels of CSF Mg had significant increases in CSF Mg level within 30 minutes after administration of the enema. There appeared to be no effect of $MgCl_2 \cdot 6H_2O$ enemas on plasma Ca and K, RBC minerals or PCV. A calf on the basal diet had 4 episodes of tetany, 3 of which were treated successfully with a $MgCl_2 \cdot 6H_2O$ enema. CSF samples taken during 2 episodes of tetany had subnormal Mg levels. These results show that $MgCl_2 \cdot 6H_2O$ enemas can be used to elevate plasma and CSF Mg level and are a possible emergency treatment method for hypomagnesemic tetany.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aikawa, Jerry K. 1959. Gastrointestinal absorption of Mg-28 in rabbits. Proc. Soc. Exp. Biol. Med. 104:293.

Aikawa, Jerry K. 1971. The Relationship of Magnesium to Disease in Domestic Animals and in Humans. Charles C. Thomas, Springfield, Ill.

Allen, G., and D.L. Robinson. 1980. Occurrence of aluminum and magnesium in grass tetany in cases and their effects on the solubility of calcium and magnesium in vitro. Agron. J. 72:957.

Allsop, T.F. and J.V. Pauli. 1975a. Responses to the lowering of magnesium and calcium concentrations in the cerebrospinal fluid of unanesthetized sheep. Australian J. Bio. Sci. 28:475.

Allsop, T.F. and J.V. Pauli. 1975b. Cerebrospinal fluid magnesium concentrations in hypomagnesaemic tetany. Proc. New Zealand Soc. Anim. Prod. 35:170.

Andrews, B.F., D.R. Campbell, and P. Thomas. 1965. Effects of hypertonic magnesium-sulfate enemas on newborn and young lambs. The Lancet 1965:64.

Axford, R.F.E., M.F. Tas, R.A. Evans, and N.W. Offer. 1975. The absorption of magnesium from the forestomachs, stomach and small intestine of sheep. Res. Vet. Sci. 19:333.

Barr, A.J., J.H. Goodnight, J.P. Sall and J.T. Helwig. 1979. A User's Guide to SAS 79. SAS Institute Inc., Raleigh, N.C.

Beaumont, William. 1933. Experiments and observation on the gastric juices and the physiology of digestion. Reported as a Nutrition Classic in Nutr. Rev. 35:144. 1977.

Behar, J. 1974. Magnesium absorption by the rat ileum and colon. Amer. J. Physiol. 227:334.

Behar, Jose. 1975. Effect of calcium on magnesium absorption. Amer. J. Physiol. 229:1590.

Bell, M.C. 1980. Prevention of grass tetany. Proc. of the John Lee Pratt Internat'l Symp. on Role of Magnesium in Animal Nutrition, Blacksburg, VA.

Bell, M.C., J.A. Oluokun, N. Ramsey and G.E. Beckmann. 1977. Magnesium enemas for cows with grass tetany. Tenn. Farm and Home Sci. 104:22.

Bell, M.C., J.A. Oluokun, N. Ramsey and G.E. Beckman. 1978. Magnesium treatment of cows for grass tetany. Feedstuffs 50:24, February 26.

Ben-Ghedalia, D., H. Tagari, S. Zanwel and A. Bondi. 1975. Solubility and net exchange of calcium, magnesium and phosphorus in digesta flowing along the gut of sheep. Brit. J. Nutr. 33:87.

Blaxter, K.L., B. Cowlishaw and C.B. Boyles. 1960. Potassium and hypomagnesemic tetany in calves. Anim. Prod. 2:1.

Blaxter, K.L., J.A.F. Rook and A.M. MacDonald. 1954a. Experimental magnesium deficiency in calves. I. Clinical and pathological observations. J. Comp. Path. 64:157.

Blaxter, K.L. and J.A.F. Rook. 1954b. Experimental magnesium deficiency in calves. 2. The metabolism of calcium, magnesium and nitrogen and magnesium requirements. J. Comp. Path. 64:176.

Bohman, V.R., A.L. Lesperance, G.D. Harding and D.L. Grunes. 1969. Induction of experimental tetany in cattle. J. Anim. Sci. 29:99.

Boling, J.A., T.O. Okolo, N. Gay and N.W. Bradley. 1979. Effect of magnesium and energy supplementation on blood constituents of fall calving beef cows. J. Anim. Sci. 48:1209.

Briggs, H.M. and W.D. Gallop. 1949. Metabolism stalls for wethers and steers. J. Anim. Sci. 8:479.

Brown, A.T. and W.A.B. Campbell. 1978. Hazards of hypertonic magnesium enema therapy. Archives of Disease in Childhood. 53:920.

Brown, R.C., A.D. Care and D.W. Pickard. 1978. Magnesium absorption from the rumen of sheep. J. Physiol. 276:62P.

Butler, E.J. 1963. The mineral element content of spring pasture in relation to the occurence of grass tetany and hypomagnesemia in dairy cows. J. Agric. Sci. 60:329.

Care, A.D. 1967. Magnesium homeostasis in ruminants. World Rev. Nutr. Diet. 8:127.

Care, A.D. and Th. Van't Klooster. 1965. In vivo transport of magnesium and other cations across the wall of the gastrointestinal tract of sheep. J. Physiol. 177:174.

Chicco, C.F., C.B. Ammerman, J.P. Feaster and B.G. Dunavant. 1973a. Nutritional interrelationships of dietary calcium, phosphorus and magnesium in sheep. J. Anim. Sci. 36:986.

Chicco, C.F., C.B. Ammerman and P.E. Loggins. 1973b. Effect of age and dietary magnesium on voluntary feed intake and plasma magnesium in ruminants. J. Dairy Sci. 56:822.

Chutkow, J.G. 1964a. Sites of magnesium absorption and excretion in the intestinal tract of the rat. J. Lab. Clin. Med. 63:71.

Chutkow, J.G. 1964b. Metabolism of Mg in the central nervous system: Relationships between concentrations of Mg in cerebrospinal fluid and brain during Mg deficiency. Neurology 4:780.

Chutkow, J.G. and S. Meyers. 1968. Chemical changes in the cerebrospinal fluid and brain in magnesium deficiency. Neurology 18:963.

Crookshank, H.R. and F.H. Sims. Serum values in wheat pasture poisoning cases. J. Anim. Sci. 14:964.

Daniel, D., E.E. Hatfield, W.C. Shrewsberry, M.E. Gibson and R. MacVicar, 1952. Effect of high dietary potassium on sheep. J. Anim. Sci. 11:790.

DeGregorio, R.M., S. Kaentrakoon, R.E. Tucker and G.E. Mitchell, Jr. 1981. Renal clearance of magnesium in sheep given citric acid and (or) potassium chloride. J. Anim. Sci. 52:895.

Draper, H.H. and B. Johnson. 1951. Thiamine deficiency in the lamb. J. Nutri. 43:413.

Duncan, C.W., C.F. Huffman and C.S. Robinson. 1935. Magnesium studies in calves. I. Tetany produced by a ration of milk or milk with various supplements. J. Biol. Chem. 108:35.

Dutton, J.E. and J.P. Fontenot. 1967. Effect of dietary organic phosphorus on magnesium metabolism in sheep. J. Anim. Sci. 26:1409.

Ebel, J.E. and J.P. Fontenot. 1967. Effect of dietary organic phosphorus on magnesium metabolism in sheep. J. Anim. Sci. 26:1409.

Elin, R.J. 1976. Role of magnesium in membrane structure and function in erythrocytes and platelet membranes. Abstracted in Proc. 2nd International Symp. on Magnesium, Montreal.

Feldberg, W. 1957. Anesthesia and sleep-like conditions produced by injections into the cerebral ventricles of the cat. J. Physiol. 140:20P.

Field, A.C. 1961. Studies on magnesium in ruminant nutrition, III. Distribution of magnesium 28 in the gastrointestinal tract and tissues of sheep. Brit. J. Nutr. 15:349.

Field, A.C. 1967. Studies on magnesium in ruminant nutrition. 4. Balance trials on sheep given different amounts of grass nuts. Brit. J. Nutr. 16:99.

Field, A.C. and C.S. Munro. 1977. The effect of site and quantity on the extent of absorption of magnesium infused into the gastrointestinal track of sheep. J. Agric. Sci. 89:365.

Field, A.C., W.J. McCallum and E.J. Butler. 1958. Studies in magnesium in ruminant nutrition. Balance experiments on sheep with herbage from fields associated with lactation tetany and from control pastures. Brit. J. Nutr. 12:433.

Fisher, D.D., L.L. Wilson, P.C. Lootens and R.W. Scholz. 1978. Grass tetany in beef cows on low magnesium pastures. Sci. Agr. 25:10.

Flicoteaux, J.R., P. Levy and L. Ostre. 1979. Influence de chlorude de magnesium hydrate administre par voie sur la magnesemic. Bul. Soc. Vet. Prat., France.

Flipse, R.J., C.F. Huffman, H.D. Webster and C.W. Duncan. 1950. Carbohydrate utilization in the young calf. I. Nutritive value of glucose, corn syrup and lactose as carbohydrate sources in synthetic milk. J. Dairy Sci. 33:548.

Fontenot, J.P. 1979. Animal nutrition aspects of grass tetany. Amer. Soc. Agron. Spec. Publ. 35:51.

Fontenot, J.P. 1980. Magnesium. Anim. Nutr. and Health. 35:38.

Fontenot, J.P., M.B. Wise and K.E. Webb, Jr. 1973. Interrelationships of potassium, nitrogen and magnesium in ruminants. Fed. Proc. 32:1925.

Fontenot, J.P., R.W. Miller, C.K. Whitehair and R. MacVicar. 1960. The effect of a high protein high potassium ration on the mineral metabolism of lambs. J. Anim. Sci. 19:127.

Forbes, R.M. 1961. Excretory patterns and bone deposition of zinc, calcium and magnesium in the rat as influenced by zinc deficiency, EDTA and lactose. J. Nutr. 74:194.

Frye, T.M., J.P. Fontenot and K.E. Webb, Jr. 1975. Effects of dietary potassium level on utilization of magnesium by sheep fed different magnesium levels. V.P.I. & S.U. Res. Div. Rep. 163:80.

Garces, M.A. and J.L. Evans. 1971. Calcium and magnesium absorption in growing cattle as influenced by age of animal and source of dietary nitrogen. J. Anim. Sci. 32:789.

Grace, N.D. 1970. Magnesium absorption in the digestive track of sheep. Proc. New Zealand Soc. Anim. Prod. 30:21.

Grace, N.D. and J.C. Macrae. 1972. Influence of feeding regimes and protein supplementation on the site of net absorption of magnesium in sheep. Brit. J. Nutr. 27:51.

Grace, N.D., M.J. Ulyatt and J.C. Macrae. 1974. Quantitative movement of fresh herbage by sheep. 3. The movement of magnesium, calcium, phosphorus, potassium and sodium in the digestive tract. J. Agric. Sic. 82:321.

Greene, L.W., J.P. Fontenot and K.E. Webb, Jr. 1981. Effect of potassium level on site of magnesium absorption in ruminants. Abstr. Amer. Soc. Anim. Sci. Southern Sec. Meetings.

Grunes, D.L., P.R. Stout and J.R. Brownell. 1970. Grass tetany of ruminants. Advances in Agron. 22:331.

Grunes, D.L. 1973. Grass tetany of cattle and sheep. In A.G. Matches (Ed.) Antiquality Components of Forages. Spec. Publ. 4, Crop Sci. Soc. Am., Madison Wisc. p. 113.

Haggard, D.L., C.K. Whitehair and R.F. Langham. 1978. Tetany associated with magnesium deficiency in suckling beef calves. J. Amer. Vet. Med. Assoc. 172:495.

Hamada, T., S. Tanabe, S. Omori, K. Kameoka and H. Morimoto. 1969. Experimental production of hypomagnesemia of early-weaned dairy calves. Japanese J. Zootech. Sci. 40:74.

Hemingway, R.G., N.S. Ritchie, A.R. Rutherford and G.M. Jolly. 1963. Effects of potassium fertilizers, age of ewe and small magnesium supplementation on blood magnesium and calcium levels of lactating ewes. J. Agri. Sci. 60:307.

Henry, Pamela, R., W.H. Smith and M.D. Cunningham. 1977. Effect of histamine and ammonia on hypomagnesemia in ruminants. J. Anim. Sci. 44:276.

Horn, J.P. and R.H. Smith. 1976. Absorption of magnesium in the stomach of the ruminant. Proc. Nutr. Soc. 35:32A.

House, W.A. and D. VanCampen. 1971. Magnesium metabolism of sheep fed different levels of potassium and citric acid. J. Nutr. 101:1483.

House, W.A. and H.F. Mayland. 1976. Magnesium utilization in wethers fed diets with varying ratios of nitrogen to readily fermentable carbohydrate. J. Anim. Sci. 43:842.

House, W.A. and R.J. Bird. Magnesium tolerance in goats fed two levels of potassium. J. Anim. Sci. 41:1134.

Huber, J.T., N.L. Jacobson, R.S. Allen and P.A. Hartman. 1961. Digestive enzyme activities in the young calf. J. Dairy Sci. 44:1494.

Huffman, C.F. and C.W. Duncan. 1936. Magnesium carbonate and magnesium oxide supplements to a whole milk ration for dairy calves. J. Dairy Sci. 19:440.

Jacobson, D.R., R.W. Hemken, F.S. Button and R.H. Hatlon. 1972. Mineral nutrition, calcium, phosphorus, magnesium and potassium interrelationships. J. Dairy Sci. 55:935.

Johnson, B., T.S. Hamilton, W.B. Nevens and L.E. Boley. 1948. Thiamine deficiency in the calf. J. Nutr. 35:137.

Kaneko, J.J. 1977. In H.M. Stahr (Ed.) Analytical Toxicology Methods Manual, Iowa State University Press, Ames.

Kelley, P. 1979. Hypomagnesemia in sheep. A review. ADAS Quarterly Rev. 34:151.

Kemp, A. 1960. Hypomagnesemia in milking cows: The response of serum magnesium to alterations in herbage composition resulting from potash and nitrogen dressings on pasture. Netherlands J. Agri. Sci. 8:281.

Kemp, A., W.B. Deijs, O.J. Hemkes and A.J.H. Van Es. 1961. Hypomagnesaemia in milking cows: Intake and utilization of magnesium from herbage by lactating cows. Netherlands J. Agr. Sci. 9:134.

Kunkel, H.O., K.H. Burns and B.J. Camp. 1953. A study of sheep fed high levels of potassium bicarbonate with particular reference to induced hypomagnesemia. J. Anim. Sci. 12:451.

Lentz, D.E., F.C. Madsen, J.K. Miller and S.L. Hansard. 1976. Effect of potassium and hypomagnesemia on insulin in the bovine. J. Anim. Sci. 43:1082.

Littledike, E.T. and P.S. Cox. 1979. Clinical, mineral and endocrine interrelationships in hypomagnesemic tetany. Amer. Soc. Agron. Spec. Publ. 35:1.

Lombar, F., R. Paguay, V. Bienfet and A. Lousse. 1968. Statistical research on the fate of dietary mineral elements in dry and lactating cows. 2. Magnesium. J. Agric. Sci. 71:181.

McAleese, D.M., M.C. Bell and R.M. Forbes. 1961. Magnesium-28 studies in lambs. J. Nutr. 74:505.

McClandish, A.C. 1923. Studies in the growth and nutrition of dairy calves. 5. Milk as the sole ration for calves. J. Dairy Sci. 6:54.

Macgregor, R.C. and D.G. Armstrong. 1979. The effect of increasing potassium intake on absorption of magnesium by sheep. Proc. Nutr. Soc. 38:66A.

Madsen, F.D., D.E. Lentz, J.K. Miller, D. Lowrey-Harnden and S.L. Hansard 1976. Dietary carbohydrate effects upon magnesium metabolism in sheep. J. Anim. Sci. 42:1316.

Martens, H., J. Harmeyer and H. Michael. 1978. Magnesium transport in isolated rumen epithelium in sheep. Res. Vet. Sci. 24:161.

Meintzer, R.B. and H. Steenbock. 1955. Vitamin D and magnesium absorption. J. Nutr. 56:285.

Meyer, H. 1976a. The physiology of magnesium metabolism in ruminants. In V. Lavon (Ed.), Magnesium in Ruminant Nutrition. Israel Chemicals LTD, Tel-aviv, Israel: p. 35.

Meyer, J. 1976b. The pathogenesis and prophylaxis of hypomagnesaemic tetany in ruminants. In V. Lavon (Ed.) Magnesium in Ruminant Nutrition. Israel Chemicals LTD, Tel-aviv, Israel: p. 66.

Meyer, H. 1977. Pathogenisis of the clinical signs of hypomagnesemia in ruminants. Vet. Sci. Communications. 1:43.

Meyer, H. and F.W. Busse. 1975a. Effect of intravenous magnesium on the magnesium level in the cerebrospinal fluid of sheep. Zentralbl. Veterinaer med. Reihe A. 22:780.

Meyer, H. and F.W. Busse. 1975b. Zur rektalen absorption von magnesium beim wiederkauer. Dtsch. Tieraerztl. Wochen schr. 82:140 (123).

Meyer, H., F.W. Busse and H. Scholz. 1974. Veranderungen des Mg-Gehaltes im Liguor cerebrospinalis bei der Hypomagnesaemie und ihre Bedeutung fur die Klinishce Symptomatologie. 7. Internat. Kongrefs fur Rinderkrankheiten, Mailand.

Meyer, H. and H. Scholz. 1972. Investigations on the pathogenesis of hypomagnesaemic tetany. I. Relationships between magnesium-content in blood and cerebrospinal fluid. Dtsch. Tieraerztl. Wochenschr. 79:55.

Miller, J.K., D.E. Lentz, F.C. Madsen and S.L. Hansard. 1976. Relationship of dietary carbohydrate, magnesium and potassium to grass tetany. Tenn. Farm and Home Sci. 98:2.

Miller, J.K., F.C. Madsen, D.E. Lentz, Wei-o Wong, Nancy Ramsey, C.E. Tysinger and S.L. Hansard. 1980. Blood plasma magnesium, potassium, glucose and immunoreactive insulin changes in cows moved abruptly from barn feeding to early spring pasture. J. Dairy Sci. 63:1073.

Miller, W.J., W.M. Britton and M.S. Ansari. 1972. In J.C., Jr., M.C. Blount and S.R. Wilkinson (Ed.) Magnesium in the Environment Soils, Crops, Animals and Man. Fort Valley State College, Fort Valley.

Moore, L.A., E.T. Hallman and L.B. Sholl. 1938. Cardiovascular and other lesions in calves fed diets low in magnesium. Arch. Path. 26:820.

Moore, L.A., L.B. Sholl and E.T. Hallman. 1936. Gross and microscopic pathology associated with low blood magnesium in dairy calves. J. Dairy Sci. 19:441.

Moore, W.F., J.P. Fontenot and K.E. Webb, Jr. 1972. Effect of form and level of nitrogen on magnesium utilization. J. Anim. Sci. 35:1046.

Newton, G.L., J.P. Fontenot, R.E. Tucker and C.E. Polan. 1972. Effects of high dietary potassium intake on the metabolism of magnesium by sheep. J. Anim. Sci. 35:440.

Nugara, D. and J.M. Edwards, Jr. 1961. Effect of calcium and phosphorus on magnesium metabolism in chicks. Fed. Proc. 20:294.

Oppelt, W.W., I. MacIntyre and D.P. Rall. 1963. Mg exchange between blood and cerebrospinal fluid. Am. J. Physiol. 205:959.

Pallis, C., I. MacIntyre and H. Anstall. 1965. Some observations on magnesium in cerebrospinal fluid. J. Clin. Path. 18:762.

Parr, W.H. 1957. Hypomagnesemic tetany in calves fed on milk diets. Vet. Rec. 69:71.

Pauli, J.V. and T.F. Allsop. 1974. Plasma and cerebrospinal fluid magnesium, calcium and potassium concentrations in dairy cows with hypomagnesemic tetany. New Zealand Vet. J. 22:227.

Pearson, P.B., J.A. Gray and R. Reiser. 1949. The calcium, magnesium and potassium contents of the serum of ewes fed high levels of potassium. J. Anim. Sci. 8:52.

Perry, S.C., R.G. Cragle and J.K. Miller. 1967. Intestinal distribution of Ca, Mg, Na, K and N in calves fed three rations. J. Nutri. 93:283.

Pfeffer, E., A. Thompson and D.G. Armstrong. 1970. Studies on intestinal digestion in the sheep. 3. Net movement of certain inorganic elements in the digestive tract on rations containing different proportions of hay and rolled barley. Brit. J. Nutr. 24:197.

Phillipson, A.T. and J.E. Storry. 1965. The absorption of calcium and magnesium from the rumen and small intestine of the sheep. J. Physiol. 181:130.

Pless, C.D., J.P. Fontenot, and K.E. Webb, Jr. 1973. Effect of dietary calcium and phosphorus levels on magnesium utilization in sheep. VPI & S.U. Res. Rep. 153:104.

Powley, G. A.D. Care and C.L. Johnson. 1977. Comparison of the daily endogenous faecal magnesium excretion form sheep eating grass with high sodium or high potassium concentrations. Res. Vet. Sci. 23:43.

Rayssiguier, Y. and C. Poncet. 1980. Effect of lactose supplement on digestion of lucerne hay by sheep. 2. Absorption of magnesium and calcium in the stomach. J. Anim. Sci. 51:186.

Ritchie, N.S. and R.G. Hemingway. 1963. Effects of conventional daily magnesium supplementation, breed of ewe and continued potassium fertilizer applications on plasma magnesium and calcium levels of ewes. J. Agric. Sci. 61:411.

Rook, J.A.F. and J.E. Storry. 1962. Magnesium in the nutrition of farm animals. Nutr. Abst. and Rev. 32:1055.

Seelig, M.S. 1980. Magnesium deficiency in the pathogenesis of disease. Early roots of cardiovascular, skeletal, and renal abnormalities. Plenum Medical Book Co., New York. p. 361.

Seonae, J. and C. Baile. 1973. Ionic changes in cerebrospinal fluid and feeding, drinking and temperature of sheep. Physiology and Behavior. 10:915.

Sheppard, A.J. and B. Johnson. 1957. Pantothenic acid deficiency in the growing calf. J. Nutr. 61:195.

Shils, M.E. 1980. Magnesium. In R.S. Goodhart and M.E. Shils (Ed.) Modern Nutrition in Health and Disease. Lea and Febiger, Philadelphia.

Simeson, M.G., T. Lonaas, T.A. Rogers and J.R. Luick. 1962. The endogenous excretion of magnesium in cattle. Acta Vet. Scand. 3:175.

Sims, M.H., M.C. Bell and N. Ramsey. 1980. Electrodiagnostic evaluation of hypomagnesemia in sheep. J. Anim. Sci. 50:539.

Smith, R.H. 1957. Calcium and magnesium metabolism in calves. 1. Plasma levels and retention in milk fed calves. Biochem. J. 67:472.

Smith, R.H. 1958. Calcium and magnesium metabolism in calves. 2. Effect of dietary vitamin D and ultraviolet irradiation on milk fed calves. Biochem. J. 70:201.

Smith, R.H. 1959a. Calcium and magnesium metabolism in calves. 4. Bone composition in magnesium deficiency and the control of plasma magnesium. Biochem. J. 71:609.

Smith, R.H. 1959b. Absorption of magnesium in the large intestine of the calf. Nature. 184:821.

Smith, R.H. 1959c. Calcium and magnesium metabolism in calves. 3. Endogenous faecal excretion and absorption of magnesium. Biochem. J. 71:306.

Smith, R.H. 1959d. The development and function of the rumen in milk fed calves. J. Agric. Sci. 52:72.

Smith, R.H. 1961. Effect of the ingestion of wood shavings on magnesium and calcium utilization by milk fed calves. J. Agric. Sci. 56:343.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. Wlt. Freeman and Co., San Francisco.

Stevenson, M.H. and E.F. Unsworth. 1978. Studies on the absorption of calcium, phosphorus, magnesium, copper and zinc by sheep fed on roughage cereal diets. Brit. J. Nutr. 40:491.

Stewart, J. 1954. Hypomagnesemia and tetany of cattle and sheep. Scot. Agr. 34:68.

Stewart, J. and E.W. Moodie. 1956. The absorption of magnesium from the alimentary tract of sheep. J. Comp. Pathol. 66:10.

Storry, J.E. and J.A.F. Rook. 1963. Magnesium metabolism in the dairy cow. 5. Experimental observations with a purified diet low in magnesium. J. Agri. Sci. 61:167.

Suttle, N.F. and A.C. Field. 1967. Studies on magnesium in ruminant nutrition. 8. Effect of increased intakes of potassium and water on the metabolism of magnesium, phosphorus, sodium, potassium and calcium. Brit. J. Nutr. 21:819.

Suttle, N.F. and A.C. Field. 1969. Studies on magnesium in ruminant nutrition. 9. Effect of potassium and magnesium intakes on development of hypomagnesemia in sheep. Brit. J. Nutr. 23:81.

Thompson, J.K. and R.L. Reid. 1981. Mineral status of beef cows and sheep on spring pasture fertilized with kieserite. J. Anim. Sci. 52:969.

Todd, J.R. and D.J. Horvath. 1970. Magnesium and neuromuscular irritability in calves, with particular reference to hypomagnesemic tetany. Brit. Vet. J. 126:333.

Tomas, F.M. and B.J. Potter. 1976a. The site of magnesium absorption from the ruminant stomach. Brit. J. Nutr. 36:37.

Tomas, F.M. and B.J. Potter. 1976b. Interaction between sites of magnesium absorption in the digestive tract of sheep. Australian J. Agric. Res. 27:437.

Tomas, F.M. and B.J. Potter. 1976c. The effect and site of action of potassium upon magnesium absorption in sheep. Australian J. Agric. Res. 27:873.

Toothill, J. 1963. The effect of certain dietary factors on the apparent absorption of magnesium by the rat. Brit. J. Nutr. 17:125.

Wiese, A.C., B. Conner Johnson, H.H. Mitchell and W.B. Nevens. 1947. Synthetic rations for the dairy calf. J. Dairy Sci. 30:87.

Wilson, A.A. 1964. Hypomagnesaemia and magnesium metabolism. Vet. Rec. 76:1382.

Wilson, G.F. 1980. Effects of magnesium supplements on the digestion of forages and milk production of cows with hypomagnesaemia. Anim. Prod. 31:153.

Wise, M.B., A.L. Ordoveza and E.R. Barrick. 1963. Influences of variations in dietary calcium-phosphorus ratio on performance and blood constituents of calves. J. Nutr. 79:79.

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