EFFECT OF POTASSIUM LEVEL ON IN VITRO MAGNESIUM TRANSPORT ACROSS RUMEN AND OMASUM EPITHELIUM OF CATTLE

by

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INTRODUCTION

Severe economic losses from acute hypomagnesemia in ruminants has spurred interest in studying Mg metabolism over the years. Acute hypomagnesemia, also known as grass tetany or grass staggers, occurs most commonly in older, lactating beef cows grazing lush spring pasture, but also occurs in lactating dairy cows. Although rare, hypomagnesemic tetany has also been observed in sheep, cattle on low Mg winter rations and calves fed exclusively on milk.

Cows most often afflicted have calved between 2 wk and 4 mo prior to the disturbance. Hypomagnesemia is exacerbated by the stress of lactation and inclement weather. The primary cause of grass tetany is a metabolic deficiency of Mg due to low Mg in the forage or inability of the animal to absorb Mg from the digestive tract. Lowered absorption of Mg occurs when components of the forage depress the absorbance capabilities of the ruminant gut.

Potassium can be high in tetany prone grasses. In metabolism trials where cattle and sheep were fed high K diets, K depressed Mg absorption when fed at levels equivalent to those necessary for plant growth.

The study to be discussed in this thesis relates in vitro Mg transport across the epithelium of two parts of the bovine stomach; the rumen and the omasum. A technique was developed to measure the transport of Mg and the effects of one of the inhibitors of its absorption, K.

REVIEW OF LITERATURE

Physiology of Magnesium

Magnesium is the fourth most abundant cation in the vertebrate body and is involved either directly or indirectly in all phases of homeostasis (Wacker and Parisi, 1968). Approximately 60% of the total body Mg is in the skeletal system, two-thirds to three-quarters of which is adsorbed to the apatite, the rest replacing Ca in phosphate complexes. The remaining 40% of body Mg is in the soft tissue and participates in over 300 enzymatic reactions (Ebel and Guther, 1980). One-half of the Mg in the soft tissue is intracellular within the muscle where it functions in the cessation of contraction. Magnesium is part of a Ca-Mg-ATPase complex bound to the membrane of the sarcoplasmic reticulum. This complex acts as a Ca pump, exchanging Ca for Mg against a gradient, making Ca unavailable for the contractile process.

Normal plasma Mg concentrations in cattle range from 1.7 to 3.3 mg/dl (Littledike and Cox, 1979). Magnesium is transported in the blood either as a free ion or complexed with plasma proteins such as albumin (Ebel and Gunther, 1980). The level of Mg in plasma or serum is not a good in-

dication of the onset of clinical Mg deficiency. A high degree of animal variation exists and some animals with serum Mg levels as low as .28 mg/dl may never develop clinical tetany (Sims et al., 1980). Because plasma concentrations of Mg are considered a poor indication of Mg status, many researchers report that cerebro-spinal fluid (CSF) may be a better indicator, but the difficulty in obtaining samples make its use impractical (Littledike and Cox, 1979; Martens and Rayssiguier, 1980).

Magnesium is transferred from the plasma to the CSF via the choroid plexus (Ames et al., 1964). The concentration of Mg in CSF in humans ranges between 1.2 to 5.0 mEq/l, with the value for the individual animal remaining fairly constant (Barrio, 1923). The concentration of CSF Mg is generally 125% of the level found in the blood. Contrary to other species, the total CSF Mg concentration of cattle, sheep and goats is lower than their respective plasma levels, but the ionized Mg level is higher in CSF than plasma (Allsop and Pauli, 1975). The concentration gradient between the CSF and plasma is maintained by the blood-brain barrier which also protects the central nervous system from fluctuations in the level of Mg (Pallis et al., 1968). Even in severely deficient animals the CSF Mg concentrations often remain within the normal range (Pauli and Allsop, 1974). A

higher correlation has been observed between grass tetany and CSF Mg concentrations than for plasma content (Allsop and Pauli, 1975).

When the level of Mg available for metabolic function becomes critically low, clinical tetany is observed. The signs of hypomagnesemic tetany are undue excitement, uncoordination, muscular twitching, profuse salivation, grinding of teeth, general tetanic contractions, labored breathing, pounding heart, convulsions and death (Fontenot, 1979). The convulsions and tetanic contractions observed in cows afflicted with grass tetany have been attributed to low Mg concentrations at the nerve end plate failing to inhibit Ca stimulated acetylcholine release (Hubbard et al., 1968). Tetany may also be related to the failure of the Mg dependent Ca pump on the sarcoplasmic reticulum (Martens and Rayssiguier, 1980).

Biochemical Function of Magnesium

Magnesium is involved in a multitude of biochemical processes due to its ability to form chelates (Ingraham and Green, 1958). This charateristic makes Mg invaluable in the metabolism of all the components of biological life, both in plants and animals. Magnesium is essential for synthesis of proteins, the integrity of nucleotidal structures, and the

production of energy. All reactions involving utilization of ATP or the transfer of a phosphate group requires Mg.

Since ATP is required in the energy processes of many biological systems, including membrane transport, amino acid activation, succinate activation, acetate activation, protein synthesis, nucleic acid synthesis, fat synthesis, coenzyme synthesis, nerve impulse generation and transmission, muscle contraction, and oxidative phosphorylation, the function of Mg may extend to all such processes (Wacker et al., 1977).

The primary mechanism of Mg action in energy production is through the activation of enzymes. Magnesium acts either as a part of a substrate or as a simple cofactor in enzyme activation (Ebel and Gunther, 1980).

Magnesium is involved in many energy production related reactions; hexokinase and pyruvate kinase will serve as examples of how Mg helps make these reactions more favorable. By chelating with ATP, Mg becomes part of the substrate in the reaction catalyzed by hexokinase (Cohn, 1963). This chelate facilitates the phosphate transfer by lowering the free energy of activation. In the pyruvate kinase reaction Mg acts as a cofactor, stimulating conformation change which results in enzyme activation (Seubert and Schoner 1980). Both of these enzymes have an absolute requirement for Mg

but the mechanism by which Mg facilitates their reactions differs.

Protein metabolism is affected by Mg, both directly and indirectly. Magnesium acts as a cofactor in the degradation of lysine and the conversion of aspartate to argininosuccinate (White et al., 1978). Magnesium also functions in ribosome stabilization (Wacker and Parisi, 1968). When Mg is deficient, ribosomes dissociate into their subunits and protein synthesis cannot occur. This reaction is reversible with the addition of Mg until a concentration of 10 uM is reached. Furthermore, Mg forms salt bridges between the ribosome and RNA phosphates, bringing them in close association so translation can be accomplished (Morgan et al., 1966).

Recent research has increased the interest in the role of Mg in lipid metabolism. Rayssigiuer et.al. (1981) fed weanling rats a Mg-deficient, high-carbohydrate diet. Mg-deficient rats had increased plasma triglycerides and free cholesterol levels, and depressed esterified cholesterol, when compared to controls. Hypertriglyceridemia may be caused by excessive synthesis by the liver, a decreased uptake and utilization by other tissues or a combination of both. An increased incorporation of ^{14}C-acetate into hepatic tissue in Mg deficient animals strongly supports the

former. Whether these changes in lipid metabolism are due to a direct effect of Mg shifting the energy metabolism is unknown.

Magnesium is essential in the biosynthesis of cholesterol as a cofactor of three enzymes involved in this process; mevalonic kinase, phosphomevalonic kinase and pyrophosphate mevalonic dicarboxylase (White et al, 1978). Magnesium is also a component of phospholipids present in cell membranes. The presence of divalent cations decreases the mobility of the phospholipid molecule and reduces membrane fluidity. When Mg is deficient in E. coli media, membrane permeability increases and the composition of the phospholipids is changed (Gunther et al., 1975). Membrane integrity is not restored by the addition of Mg to the media, protein synthesis must first take place.

Magnesium is essential for the formation of both the primary and secondary structure of nucleic acids. Aminoimidazol synthetase, which catalyzes an irreversible reaction in the synthesis of purines, has an absolute requirement for Mg (White et al, 1978). The intramolecular helices making up the secondary structure of the RNA molecule are stabilized when the negative charges of the phosphate backbone are neutralized through binding with Mg. Magnesium also binds to the phosphate groups of DNA that are not already

occupied by histone. One theory is that phosphate groups bound to Mg are active, whereas those bound to histone are not. Magnesium may also help stabilize DNA during transcription (Zubay, 1959).

Homeostasis of Magnesium

There is no evidence of a regulatory system for the control of Mg homeostasis. Although much research has been conducted on the effects of hormones, especially parathyroid hormone (PTH), there is no proof that their influence is of practical significance under physiological conditions (Heaton, 1981; Martens and Rayssiguier, 1980). Skeletal Mg stores are not readily released in times of Mg deprivation, especially in the adult animal where only 17% of total bone Mg is labile. Approximately 30% of bone Mg is present in the surface limited pool, a more readily available source than the other 70% which is incorporated in the hydroxy apatite crystals. Magnesium in the hydroxy apatite is only accessible through resorption of the bone (Alfrey and Miller, 1973). Since mobilization and deposition from the bone cannot contribute to Mg homeostasis the factors controlling plasma Mg concentrations are the absorption, secretion and excretion of the mineral (Todd, 1976).

Mayer and Hust (1978), compared the effects of Ca and Mg on the secretion rate of PTH in calves. Increased plasma Mg concentrations reversed high PTH levels caused by hypocalcemia. The repressive effects of Ca on PTH is 2.5 times greater than those of Mg. During hypomagnesemia the ability of PTH to respond to hypocalcemic stimuli is diminished (Targovnik et al., 1971; Martens and Rayssiguier, 1980). It may be theorized that Mg is important in the synthesis or release mechanism of PTH from the parathyroid gland.

The correlation of serum Mg with calcitonin (CT) release has not been established. Gitelman et al. (1971) observed CT release from isolated rat thyroid in the presence of Mg. Neither Rasmueson (1978) nor Roos et al. (1975) could find any effect of Mg on CT release. Rayssiguier et al. (1977) found no change in plasma CT in hypomagnesemic calves.

Hypoglycemia and ketosis, sometimes associated with the etiology of grass tetany, have stimulated research in the relationship between Mg and the carbohydrate regulatory hormones, insulin and glucagon. Magnesium and Ca must both be present and within a limited ratio before insulin is released from isolated rat pancreas (Bennet, 1979). In a similar experiment glucagon secretion was inhibited in the presence of either Mg or Ca, although their effects were

synergistic (Leclercq-Meyer et al., 1973). However, intravenous injections of insulin in human subjects results in a temporary increase in serum Mg followed by hypomagnesemia as the metabolic demands for Mg increase (Aikawa, 1963). Glucose infusion also results in a depression of serum Mg, but when insulin and glucose are infused together their effects are synergistic. These results suggest that insulin, either directly or indirectly, stimulates the release of Mg from pooled sources while increasing carbohydrate metabolism, which results in increased uptake of Mg by the tissues. The effects of glucose may be more direct. Lentz et al. (1978), report that serum insulin levels increased when KCl was infused IV into normal and Mg-deficient calves and intraruminally into nonpregnant cows. Serum glucose was also depressed in normal calves and cows but elevated in Mg-deficient calves. Low serum Mg in conjunction with high serum insulin may have stimulated the release of glucagon, which results in increased serum glucose. The effect of K on pancreatic hormones is especially important because it is considered an important inhibitor of Mg absorption in ruminants.

The IV infusion of adrenaline (epinephrine) into ewes has been reported to produce moderate to severe hypomagnesemia (Rayssiguier, 1977; Yano et al. 1979). Yano et al

(1979) further report that infusion of adrenaline results in elevated glucose and insulin levels and depressed serum K. These data are significant not only in the effects of environmental stress on producing beef cows, but also on the validity of some of the sampling techniques used in Mg research. Stress related drops in serum Mg and K could be an artifact of animal handling and blood sampling techniques.

Site of Magnesium Absorption

Many research groups have reported that Mg is absorbed pre-intestinally in sheep (Grace and MacRae, 1972; Strachan and Rook, 1975; Tomas and Potter, 1976b; Greene et al., 1983a; Giduck et al., 1981). Less research has been conducted using cattle. Kemp et al. (1973); Horn and Smith (1978) and Greene et al. (1983b) all report the stomach as the primary site of Mg absorption. Tomas and Potter (1976b) reported the Mg infused into the omasum or abomasum of sheep was recovered via a duodenal cannula, concluding the reticulo-rumen is the site of Mg absorption. These results were supported by Field and Munro (1970) in a similar experiment, but they added that a small amount of Mg was also absorbed in the omasum. Conversely, Fitt et al. (1979) reported that the omasum was as likely a site of absorption as the rumen in sheep. Horn and Smith (1978) used young steers and

found the omasum was the primary site of Mg absorption in cattle. The authors suggested a species difference in the site of Mg absorption to account for differences between studies. Martens et al. (1978) concluded the major site of Mg transport is the rumen in sheep. Further experiments were done in which the rumen of heifers was temporarily-isolated, emptied and washed, then exposed to buffer solutions containing varying levels of Mg. A saliva collector and a plug in the reticulo-omasal oriface prevented salivary and omasal influences. The authors conclude that the reticulorumen is the primary site of Mg absorption in cattle. Furthermore, kinetic studies support previous in vitro results that Mg is absorbed by a saturable process (Martens, 1983). The precise site of Mg absorption within the stomach region has not been conclusively established.

During in vitro experiments Martens et al (1978), exposed the serosal surface of isolated rumen tissue to ouabain, a Na-K-ATPase inhibitor. In the presence of ouabain Mg transport was eliminated. These results suggest that Mg is transported by a Na-K-ATPase dependent system. Temperature dependency and saturability of the system were also tested and support results that Mg is transported by an active transport system.

Excretion of Magnesium

The major portion of Mg consumed by ruminants is excreted in the feces. Most fecal Mg is either a component of, or adhered to, indigestible material. The remainder is either unabsorbed Mg or contributed by bile excretions and gut epithelial cells.

Martens (1983) used a washed rumen technique to measure the kinetics of Mg absorption in heifers. Maximum Mg uptake was achieved at 12.5 mmol Mg/liters, a concentration higher than expected in natural situations. However, the small amount of buffer used to replace rumen contents allowed contraction of the rumen wall and more than normal exposure of rumen cells to a given volume, which resulted in a greater Mg uptake per unit volume.

Most endogenous Mg is excreted via the kidney. When ^{28}Mg was injected IV into human subjects only 1 to 2% was recovered in the feces, the rest being excreted in the urine (Wacker and Parisi, 1968). Urine Mg is an indicator of Mg balance. Rook and Storry (1962) reported that because Mg absorbed in excess of body requirements is excreted by the kidney, urine Mg reflects nutritional adequacy of the diet. Chicco et al. (1972) reported that the amount of Mg absorbed and urinary Mg were highly correlated (r=.95). Furthermore, when the availibility of Mg is depressed urinary Mg also de-

creases (Newton et al., 1972). According to Todd (1976), when plasma Mg falls below 2.0 mg/dl urinary Mg output approaches zero.

Hypomagnesemic cows lose little Mg through urine, but lactation creates a high demand for Mg. Cow milk contains .75% ash, of which 1.7% is Mg or 12 mg Mg/dl milk (White, et al. 1979; Todd, 1976). A high yielding cow can secrete the equivalent of the total Mg in her extracellular fluid into the milk produced in a single day (Todd, 1976). Mammary tissue, regardless of stage of lactation, appears to have a greater affinity for Mg than other tissues (Littledike and Cox, 1979). They injected ^{28}Mg IV into milking and dry cows and observed that a very large percentage was taken up by the mammary gland. It was noted that the mammary tissue retained much of the ^{28}Mg; even after six milkings over a 3-d period. The authors suggest that mammary tissue could be considered a large exchangeable pool, which could yield Mg during deprivation. Conversely, if the affinity of the tissue is great enough it could be a Mg sink which would amass Mg to the detriment of other body processes.

The Effect of Potassium on Magnesium Utilization

The relationship between K fertilization and increased incidence of grass tetany has long been recognized (Dryerre, 1932; Nicholson and Shearer, 1938). Heavy use of K fertilization has been reported to lower serum Mg and increase the incidence of hypomagnesemic tetany (Kemp, 1958; Hvidsten et al. 1959) but Hemingway et al. (1963) concluded there was no effect of K fertilization on serum Mg levels of sheep.

Kemp et al. (1961) estimate apparent digestibility of Mg in fresh forage to range between 7 and 33%, with an average of 17%. In the early spring when hypomagnesemic tetany is most common, forage Mg content is at its lowest, averaging .15% Mg/g DM (Stewart and Holmes, 1953).

Forages considered to be "tetany-prone" are evaluated in terms of K content. If the ratio of K/(Ca+Mg) is greater than 2.2, the forage is considered to be tetany prone (Woodruff, 1972). This ratio is used to estimate risk of livestock losses so precautions such as spraying forage with MgO or supplementing MgO in a palatable mineral mix, may be utilized. Another method for evaluating tetany prone forages has been reported by the Committee on Mineral Nutrition, The Hague (1973) where the content of K x % crude protein is compared to forage Mg content, in a graphic form. Forages containing .2% or more Mg on a dry basis are considered ade-

quate to fulfill the animal needs (Mayland and Grimes, 1979).

Conventional metabolism trials, which used semi-purified diets helped to pinpoint K as the primary inhibitor of Mg absorption. Daniel et al. (1952) fed high K diets to sheep in the form of KCl. They reported no effect of K on weight of ewes, weight of lambs at birth, number of lambs weaned or plasma mineral levels. Kunkel et al. (1953) fed 5% K as $KHCO_3$ to ewes which resulted in subclinical hypomagnesemia. Fontenot et al. (1960) reported that lambs fed a high-protein, high K diet absorbed less Mg. Plasma Mg was also depressed. Kemp (1961) supplemented 400g KCl in diets of fresh cut grass fed to dairy cattle. Magnesium availibility was reduced from 18.5 to 16%.

When 4.2% KCl was added to feed, or introduced via rumen fistula of sheep with 7.5 liters of water, fecal Mg excretion was increased and urinary Mg decreased (Suttle and Field, 1976). When water alone was infused into the rumen only urine Mg increased while fecal Mg output was unaltered. It was concluded the transport of Mg across the rumen was inhibited by an increased potential difference created in the presence of K.

Newton and coworkers (1972) reported that feeding lambs a high K diet (4.9 vs .6%) resulted in a 46 % decrease in Mg

availibility over eight 3-d trials. Differences between treatments were established soon after the experiment began and continued throughout all trials. Fecal Mg was greater and urinary Mg less in animals fed the high K diet. In a second experiment, IV injections of ^{28}Mg were given to lambs fed a high K diet. Average fecal recovery was higher and urinary recovery lower than those animals fed low K diets.

Tomas and Potter (1976) found that a continuous infusion of KCl at a level equivalent to 3.9% of diet depressed Mg absorption when administered via the rumen, but not when duodenally infused. When Greene et al. (1983a,b) fed high K diets to sheep and cattle, Mg absorption was depressed preintestinally. There was no effect of the level of potassium on Mg absorption in the small and large intestine.

MacGregor and Armstrong (1979) fed four levels of K to sheep: .62, 1.36, 2.12, and 3.87%. Magnesium absorption was depressed only at the highest K level. Greene et al. (1983) studied the effects of K on Mg absorption in sheep. Magnesium was fed at .1% of diet in conjunction with four K levels, .6, 1.2, 2.4, and 4.8%. When expressed as a percent of intake, Mg absorption was depressed linearly. Serum Mg levels were depressed in animals fed the higher potassium diets.

Environmental Effects on Forage Magnesium Concentration

Magnesium is absorbed by the plant most optimally at pH 5.0. When the pH of the soil solution is raised from 3.5 to 5.0 Mg uptake increased three-fold, but a further increase to 6.5 had no effect (Hannaway et al., 1980).

The concentration of Mg in the plant is affected by many environmental and climatic effects. Crested wheatgrass grown at elevated temperatures (24 C) contain higher concentrations of Ca and Mg and a higher K/Ca+Mg ratio than plants grown at 18 C (Grimes et al., 1968). Magnesium concentrations in forages from well drained pastures are significantly higher than those grown on poorly drained soils. This could be a moisture or an oxygen effect or both (Elkin et al. 1977). When grasses were grown in 8 and 25% shade, levels of N, K, K/(Ca+Mg), Mg, organic acids, higher fatty acids, protein, and energy were all increased (Mayland and Grimes, 1974).

Legumes generally have higher Mg concentrations than grasses (NRC, 1980), but even within a plant type, there is considerable species variation. Tall fescue has greater levels of Mg than other grasses and smooth brome contains low levels of Mg at all stages of maturity (Powell et al., 1978). Tropical grasses are believed to be less tetany prone than C_3 plants because of their higher chlorophyll a/b

ratio (Black and Mayne, 1970). Chlorophyll b resists ruminal degradation longer than chlorophyll a (Dawson and Hemington, 1974). Recently the potential for genetic improvement of tall fescue to breed out potential tetany factors, was examined. Due to the high heritability of K/(Ca+Mg) considerable progress is believed possible in this area (Sleper et al. 1977).

Seasonal changes have been studied thoroughly in crested wheatgrass. The composition of the forage in early spring, when the cow's requirement for Mg is highest, is particularly tetany prone. The level of K is at its peak in early spring but Ca + Mg are at their lowest resulting in a very high K/(Ca+Mg) ratio. Organic acids are also at their highest in the earliest stages of maturity and particularly high in K fertilized grasses. These effects are exacerbated by ample moisture and high temperature (Stuart et al. 1973).

The Developement of In Vitro Transport Techniques

In vitro techniques can be less expensive and less time consuming than in vivo animal trials. Ferrira et al. (1964), developed a method for working with isolated sheets of rumen epithelium from which the muscle layer had been removed. The presence of oxygen and important metabolic sub-

strates, such as propionic and butyric acids, maintained the tissue for several hours as measured by its ability to maintain its electrical potential.

Martens and coworkers (1976) adapted this technique for use in the study of Mg transport through the rumen of sheep. Using this method the following conclusions were reached. First, the rumen is the primary site of Mg absorption in sheep. Second, Mg is absorbed by a Na-K-ATPase dependent active transport system. The authors used ^{28}Mg and cold Mg to measure Mg transport and found the results to be comparable.

JOURNAL ARTICLE

Summary

A procedure was developed to measure in vitro Mg absorption from the digestive tract. The procedure involved mounting epithelial tissue from different compartments of the bovine stomach in parabiotic chambers and incubating the chambers in a constant temperature water bath at 39C. It was found that glucose was transported, and temperature affected the system. Evidence was obtained that Mg was absorbed across the ruminal and omasal epithelia. Flow across the ruminal tissue appeared to be higher than across the omasal tissue. A high standard error between samples was observed using the procedure. Different K levels in the buffer were tested with both the ruminal and omasal tissues. The mean values indicate that K exhibited an effect on Mg flow, especially in the rumen tissue. The drawbacks of this in vitro technique limit its value as a tool for mineral metabolism. The composition of the medium is somewhat inflexible, so an effect must be great while using only a small amount of inhibitor. The advantages of isolating a particular element while excluding external factors could outweigh the disad-

vantages in a limited number of circumstances. The high degree of variation intrinsic in this technique makes the use of radioisotopes advantageous to maximize precision.

Key words: Magnesium, Absorption, Rumen, Omasum, Cattle, In Vitro

Introduction

Poor absorption of Mg from the digestive tract of ruminants is one of the primary causes of grass tetany in cattle (Fontenot, 1979). Although it is known that Mg is absorbed prior to the duodenum the exact site of Mg absorption is still uncertain. Potassium, which is abundant in young actively growing, tetany prone grasses, has been found to decrease the absorption of Mg in sheep (Newton et al., 1972) and cattle (Greene et al., 1983b).

Martens et al. (1976) used ^{28}Mg to measure the flow of Mg through isolated sheep rumen epithelium. A simple in vitro technique may have broad applications as a low cost, preliminary tool for measuring mineral transport through the ruminant gut and the effect of absorption antagonists. An in vitro technique was investigated to measure the flow of Mg across a concentration gradient, through isolated rumen and omasum epithelium from cattle. Also, the effect of different K levels on the flow of Mg across rumen and omasum epithelium was studied in vitro.

Materials and Methods

Prior to studying the effect of K on Mg absorption in vitro, a number of sequential experiments were conducted to develop a working model to study in vitro absorption. Tissues from the rumen wall and omasal leaf were used. For all experiments, the tissues were obtained from yearling crossbred heifers fed a diet consisting of 60% hay and 40% concentrate for a minimum of 2 wk prior to slaughter. The tissues were obtained by eviscerating the animal immediately after it was killed. The tissues were transported to the laboratory within 10 min after death in a buffer containing physiological levels of Mg and K (low Mg, low K, table 1). All buffer solutions used in the preparation, transport and incubation of the tissues were essentially as described by Martens et al. (1978). In the laboratory the tissues were washed in fresh low K-low Mg buffer. The muscle layer was separated from the ruminal epithelium and the omasal leaves were pulled apart to expose the serosal side of the tissue. This was done while the tissue was immersed in fresh buffer. The solutions were maintained at 39 C and aerated with 95% O_2 and 5% CO_2 for 2 h prior to the start of the experiment.

The apparatus consisted of a series of parabiotic chambers (figure 1) incubated in a constant temperature water bath at 39 C. Each chamber was individually aerated conti-

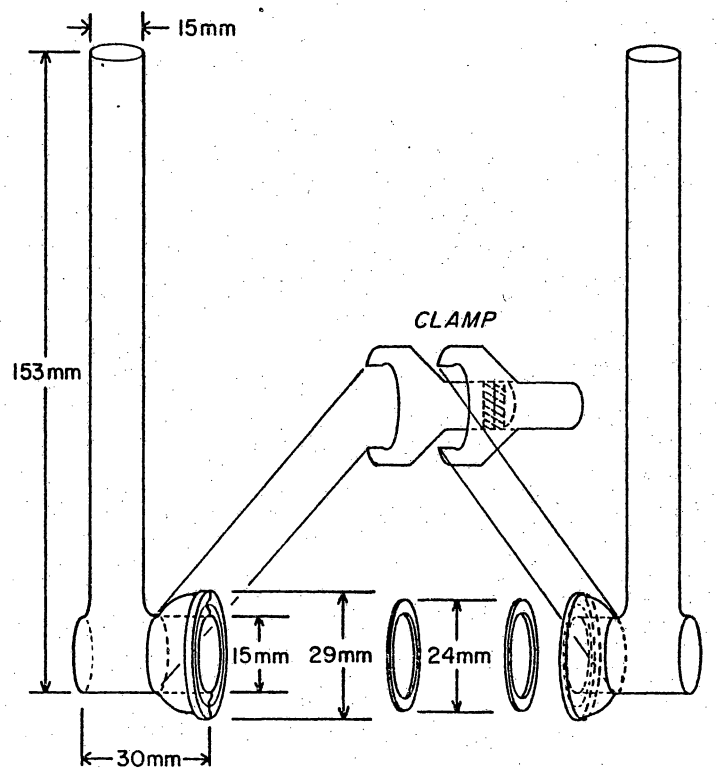

Figure 1: Parabiotic chamber used in the measurement of in vitro magnesium flow

TABLE 1

BUFFERS USED TO STUDY THE EFFECTS OF POTASSIUM ON MAGNESIUM TRANSPORT IN VITRO

Buffers	NaCl	$MgCl_2$	KCl
	mM	mM	mM
Low Mg-low K	99.7	.84	4.67
Plus Mg-low K	97.5	2.5	4.67
Plus Mg-medium K	78.01	2.5	18.66
Plus Mg-high K	59.36	2.5	37.31

All buffers also contained 25 mM $NaHCO_3$, 5 mM glucose, 15 mM Na-acetate, 15 mM propionic acid, 15 mM butyric acid and 2 mM $CaCl_2$.

nuously in both sides with 95% O_2 and 5% CO_2. The parabiotic chamber consists of two glass L-shaped tubes separated by two rubber O-rings and held together with a metal clamp[1]

The ruminal and omasal epithelia were mounted on metal-free parabiotic chambers and incubated in a 39 C water bath for 2 h. The time incubation began was noted for each chamber. All buffer solutions were prepared in metal-free glassware using deionized water. After complete solubilization the pH was adjusted with 10N NaOH to 7.3 for the buffers on the serosal side of the tissue and 6.75 for all others with the exception of the pH experiments. Buffers were prepared within 24 h of the start of the experiment and refrigerated until needed, then warmed slowly to 39 C.

Initially, the ability of the tissue to transport glucose was measured in isolated rumen epithelium. The low Mg-low K buffer was used on the serosal side of the tissue while a similar buffer, except higher in glucose (20 mM), was placed on the mucosal side. Osmolality was maintained at approximately 300 mosm. Blanks were included to adjust for glucose used by the tissue. The glucose content of the buffers was obtained using a glucose kit[2]. Flow of glucose

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[1] Parabiotic chambers were constructed in the VPI & SU glass shop.

[2] Glucose kit No. 510A was obtained from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO.

to the serosal side of the chamber was calculated by the following formula.

$$\text{Flow Mg} = \frac{C_1V_1 - C_0V_0}{A\Delta t}$$

where C_1 = the final concentration, C_0 = original concentration, V_1 = final volume, V_0 = original volume, Δt = incubation time and A = area of the tissue.

The effects of pH on in vitro transport of Mg was studied. Two animals were slaughtered and tissues were collected as previously described. The pH of the mucosal buffers were adjusted with 10 N NaOH to 6.5, 8.0 and 9.5 (table 2). The serosal buffer remained at pH 7.3 as in the other in vitro experiments. Equal volumes (15 ml) of the two buffers were used on both sides of the chamber. Chambers were incubated and final volumes were recorded. Buffers were analyzed and Mg flow calculated as described above.

The temperature dependency of Mg transport in rumen epithelium was measured. The epithelium was prepared as described above, then incubated simultaneously in a 39 C water bath, at room temperature (22 C) and 0 C ice bath. Blanks were included at all three temperatures. To maximize Mg transport the buffers used were the low Mg-low K and plus Mg-low K on the serosal and mucosal sides of the parabiotic chambers, respectively. The chambers were aerated from a

TABLE 2

BUFFERS USED TO STUDY THE EFFECT OF pH MAGNESIUM TRANSPORT IN VITRO

Buffers	NaCl	MgCl
	mM	mM
Low Mg-Low K	100	--
Plus Mg-Low K	97.5	2.5

All buffers also contained 25 mM $NaHCO_3$, 5 mM glucose, 15 mM Na-acetate, 15 mM propionic acid, 15 mM butyric acid, 2 mM $CaCl_2$ and 4.67 mM KCl. Adjustment of pH with 10 N NaOH.

single gas cylinder regardless of temperature treatment, and each chamber was individually regulated to insure consistent aeration. All chambers began incubation within 1 h after death of the animal.

After incubation the final volume was measured in individual, metal free, graduated cylinders. The buffers were then diluted with 1% $LaCl_2$ and analyzed with a Perkin-Elmer 403 atomic absorption spectrophotometer. The transport of Mg across the tissue was calculated by the equation given above.

Once the preliminary experiments designed to standardize the procedure were completed, a new set of experiments were conducted. The purpose of this second set was to study the relative Mg absorption in ruminal and omasal tissue and the effect of K levels on Mg transport. Five heifers were slaughtered on different days and rumen and omasum tissues were obtained. The treatment buffers were similar to those used in the tissue prepartion but were modified by varying levels of Mg and K (table 1). An attempt was made to keep osmolality constant by adjusting the NaCl concentration of the solution. Potassium was included in the plus Mg-low K buffer in concentrations relative to Mg, in order to approximate the K:Mg ratios in cattle diets containing .1% Mg, and .6% K. Potassium concentrations were then adjusted to

correspond to each other similarly to diets fed to cattle which were 2.4 and 4.8% K (4 times basal level and 8 times basal level, respectively). These values were obtained from work with cattle conducted by Greene et al. (1983b). Prior to incubation, low, medium and high K buffers were pipeted into the mucosal side of the parabiotic chamber and low Mg-low K buffer was placed on the serosal side (15 and 8 ml, respectively). A fourth treatment which contained low Mg-low K on both sides of the chamber was included as a blank. Blank, low, medium and high treatments were placed in the water bath sequentially and prepared from similar tissue. Blanks were used to adjust for the tissue effect within a sequence. For each tissue, rumen and omasum, 12 chambers were used, three for each treatment for a total of 24 chambers.

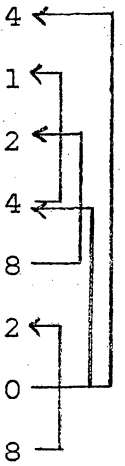
The tissues exposed to the treatment buffers during incubation were freeze-dried and acid digested with HNO_3 and $HClO_4$ (2:1) prior to analysis.

Data were statistically analyzed using the General Linear Models Procedure of SAS (1979) (table 3).

TABLE 3

ANALYSIS OF VARIANCE

Source	df
Animal	4
Tissue	1
Treatment	2
Animal x tissue	4
Animal x treatment	8
Tissue x treatment	2
Block (animal x tissue)	20
Animal x tissue x treatment	8
	49



*Arrows start at statement used to test the statement and end at the statement being tested.

Results and Discussion

Glucose Transport

Tissue viability was determined by the tissues ability to transport glucose to the serosal side of the parabiotic chamber. Glucose appeared to be transported across isolated rumen epithelium at a rate of 26.9 $ug.(cm^2)^{-1}.h^{-1}$, when measured down a concentration gradient. Glucose disappeared from the mucosal side of the chamber at a rate of 8.3 $ug.(cm^2)^{-1}.h^{-1}$. This transport capability indicates that the tissue in the in vitro system was alive and viable. The environment in the in vitro system is static; there is no blood flow bringing in fresh nutrients and carrying away metabolic wastes. The buffers bathing the tissues must contain metabolically essential compounds and oxygen. The conditions maintained by the buffers are then altered to fit an experimental aim. Caution must be taken not to deviate too far from the physiological norm. The CO_2 present in the gas bubbled through the medium may have altered the pH of the medium. This possibility should be considered in future experiments. The degree of deviation must be evaluated in terms of a similar in vivo experiment. Although final values will differ between the methods, trends should be similar.

Temperature Study

Magnesium transport across isolated rumen epithelium was temperature dependent. When all conditions were held constant except temperature, it was determined that Mg was not transported at 0 C or 22 C, but transport did occur at 39 C (table 4).

Effects of pH

There was no effect of pH on Mg transport through rumen or omasal epithelium (table 5). Storry (1961), reported that increased pH in the digesta of sheep resulted in a decreased availability of Mg. These results were not confirmed in this in vitro situation.

Potassium Levels

Potassium tended to decrease Mg transport in both tissues, but not linearly (table 6 and figure 2). The addition of K to the mucosal side of the chamber tended to reduce Mg transport through both tissues tested. Potassium reduced the flow of Mg in the rumen tissue by 27.1 and 21.9% at the medium and high levels, respectively. Decreases of 4.2 and 5.7 fold, respectively, were recorded for the omasal tissue This experiment was designed to correspond with an in vivo cattle trial conducted by Greene et al, (1983). If this in

TABLE 4

THE EFFECT OF TEMPERATURE OF THE TRANSPORT OF MAGNESIUM THROUGH ISOLATED RUMEN EPITHELIUM

Temperature (C)	Flow of Mg ug Mg/(cm^2).h^{-1}
39	1.26
22	-.50
0	-.03
SE	.084

TABLE 5

RATE OF FLOW OF MAGNESIUM THROUGH RUMEN AND OMASUM EPITHELIUM AT THREE pH LEVELS

Tissue	Treatment	Mucosal flow[a] ug Mg/(cm^2).h^{-1}	Serosal flow[b] ug Mg/(cm^2).h^{-1}
Rumen	Average of blanks	9.87	3.37
	6.5 minus blank	-19.46	5.17
	8.0 minus blank	-16.18	4.71
	9.5 minus blank	-9.63	4.79
	[c]SE	4.68	.54
Omasum	Average of blanks	2.72	1.24
	6.5 minus blank	-12.79	4.20
	8.0 minus blank	-6.64	4.27
	9.5 minus blank	-10.70	3.92
	[c]SE	3.29	.50

[a] $p<.68$

[b] $p<.25$

[c] Standard error does not include blanks.

TABLE 6

RATE OF FLOW OF MAGNESIUM THROUGH RUMEN AND OMASUM EPITHELIUM OF CATTLE IN THE PRESENCE OF THREE LEVELS OF POTASSIUM

Tissue	Treatment	Mucosal flow ug $Mg/(cm^2).h^{-1}$	Tissue Mg content ug $Mg/(cm^2).h^{-1}$	Serosal flow ug $Mg/(cm^2).h^{-1}$
Rumen	Average of blanks	7.10	.51	0.34
	Low minus blank	-13.76	.55	3.06
	Medium minus blank	-18.34	.57	2.23
	High minus blank	-11.94	.54	2.39
Omasum	Blank	-2.90	.16	-3.51
	Low minus blank	-8.45	.13	0.52
	Medium minus blank	-8.23	.20	0.12
	High minus blank	-14.51	.21	0.09
	[a]SE	2.62	.03	.43

[a]Standard error does not include blanks.

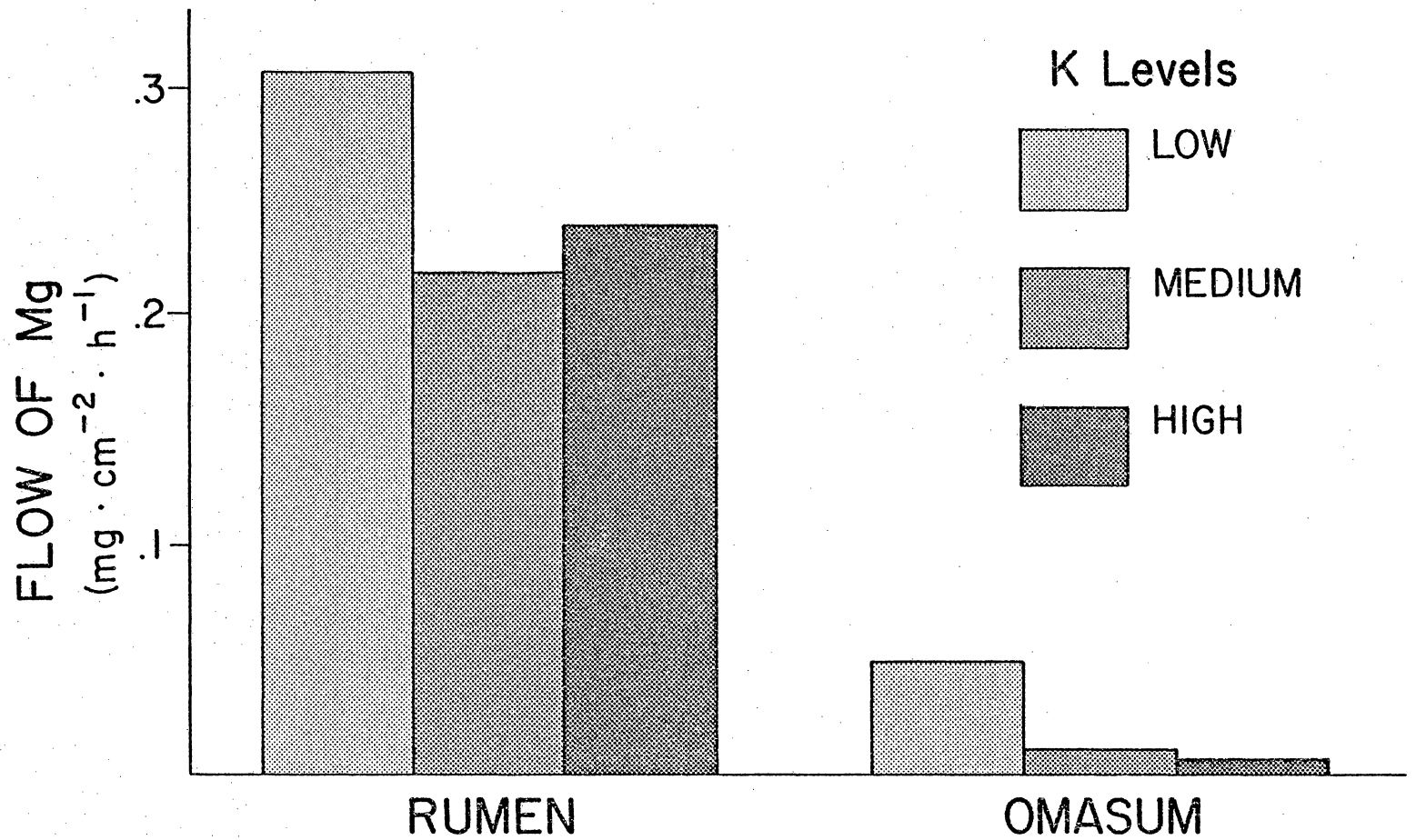


Figure 2: Flow of magnesium through epithelium from rumen and omasum as influenced by K level

vitro technique is a working model of an in vivo situation, the results from the two experiments should have the same trends. When Greene fed cattle diets containing .6, 2.4, and 4.8% K, K decreased Mg absorption linearly ($P<.05$). These differences could be due to several factors. The most obvious reason is that the in vitro system was not sufficiently similar to the physiological state, so the effects of K were not as marked. Previous researchers used a similar technique to measure the electrical potential of the tissue and found it to be similar to that measured in live, anesthetized animals (Keynes, 1969). This potential could not be maintained if the tissue was in a nonphysiological environment. Furthermore, glucose transport would not have occurred if the conditions were unfavorable.

Another possiblity for the differences between live and in vitro experiments, is that the Na:K ratios differ. The Na:K ratios in the three buffers were 30:1, 6:1, and 3:1 in the low, medium, and high treatments respectively. In the diet from the studies of Greene et al., (1983a,b), the Na:K ratios were 1:1, .2:1 and .1:1. The Na concentration in the rumen would likely be higher due to the contribution of Na from saliva (McDougall, 1948). Martens and Rayssiguier (1980) reported that the Na:K ratio must fall to 1:2 before a significant decrease in Mg absorption is observed. These

results could be challanged by Poe et al, (1983), who could not find any evidence that the Na:K ratio has an effect on Mg absorption. The differences in Na:K ratios could be compensated for by using a non-reactive, unabsorbed and metabolically inert compound such as polyethylene glycol to adjust the osmolality rather than NaCl.

The most significant difference between the two studies is the high degree of variation in the in vitro experiment. The animal trial showed minimal variablity between animals. The high degree of variability in the in vitro study, coupled with the small number of observations prevented statistical significance from being achieved.

A high standard error was inherent in this technique,but steps may be taken to minimize this problem. First, the data were analyzed by blocks. Blocks are defined as a sequence of chambers, containing blank, low, medium and high treatments of like tissue which start incubation within minutes of each other. There is a significant effect of the lenth of time between the death of the animal and the start of incubation so adjusting for the blank must be done within blocks. Secondly, radioisotopes would increase the precision of this technique. The isotope used by other researchers was ^{28}Mg, but its short half-life and great cost made it desirable to develop this technique using cold Mg. Martens

(personal communication) reported that experiments using ^{28}Mg and cold Mg yeilded similar results, but he did not report results of his statistical analysis. In future experiments the isotope should be used to improve sensitivity. Finally, a larger number of observations should be made to reduce the significance of the large standard error.

Magnesium Transport Across Ruminal and Omasal Tissue

Magnesium transport was measured across two tissue epithelium, rumen and omasum. It was observed in the K trial that Mg transport was 10 times greater through the rumen than the omasum ($P<.15$) (table 4). If flow of Mg was put on a per g of tissue basis there is a 5.3 fold difference between rumen and omasum, 44.71 and 8.48 ug $Mg.g^{-1}.h^{-1}$, respectively in the K experiments. While the degree of probability of the difference is not considered statistically significant, the degree of confidence placed on the "significance" of these data must be tempered by ability to measure differences. Because of the high standard error intrinsic in this method, these results alone may not be considered conclusive. These results were supported by the Mg transport recorded through these two tissues in the pH experiment. Magnesium flow towards the serosal side of the chamber was 18.4% greater through the rumen than the omasum

TABLE 7

RATE OF FLOW OF MAGNESIUM THROUGH RUMEN AND OMASUM EPITHELIUM OF CATTLE, IN VITRO

Tissue	Mucosal flow[a] ug Mg/(cm^2).h^{-1}	Tissue Mg content ug Mg/(cm^2).h^{-1}	Serosal[b] flow ug Mg/(cm^2).h^{-1}
Rumen minus blank	-14.52	.55	2.53
Omasum minus blank	-10.40	.20	.24

[a] (P<.20)

[b] (P<.15)

($p<.02$) (table 8). These results were supported in recent work conducted by Martens (1983), who measured Mg transport through the rumen of heifers using a washed rumen technique.

There was no difference in Mg content between rumen and omasum epithelium regardless of K treatment (table 6). Magnesium content of treated tissues was twice that of blanks. The differences may be explained by Mg loading in treated tissues and the loss of Mg from the blank tissues into the surrounding buffers. The tendency for higher transport of Mg through the rumen epithelium seemed to indicate that only the rumen tissue had the mechanism to pass Mg into the serosal side of the chamber.

These results support the theory of an active transport system. When Martens et al, (1978) exposed isolated rumen to ouabain, Mg transport ceased. These reseachers concluded that the mechanism of Mg transport was Na-K-ATPase dependent. Other indicators of active transport are saturability and temperature dependency. The latter was tested in this study. In future experiments transport inhibitors such as ouabain should be used in all phases of the study to insure that an active transport system exists.

Many research groups have reported that Mg is absorbed pre-intestinally in sheep (Grace and MacRae, 1972; Strachan and Rook, 1975; Tomas and Potter, 1976; Giduck et al., 1981;

TABLE 8

RATE OF MAGNESIUM FLOW THROUGH RUMEN AND OMASUM DURING pH EXPERIMENT

Tissue	Mucosal flow[a] ug $Mg/(cm^2).h^{-1}$	Serosal flow[b] ug $Mg/(cm^2).h^{-1}$
Rumen	-8.85	4.89
Omasum	-6.85	4.13

[a] $p<.06$
[b] $p<.02$

Greene et al., 1983a). Less research has been conducted using cattle, but Kemp et al. (1973), Horn and Smith (1978) and Greene et al. (1983b) all report the stomach as the primary site of Mg absorption. Tomas and Potter (1976) reported the Mg infused into the omasum or abomasum of sheep was recovered via a duodenal cannula, concluding the reticulorumen is the site of Mg absorption. These results were confirmed by Field and Munro (1970), but they added that small amounts of Mg were also absorbed in the omasum. Conversely, Fitt et al. (1979) reported that the omasum was as likely a site of absorption as the rumen in sheep. Horn and Smith (1978) used young steers and found the omasum was the primary site of Mg absorption in cattle. Martens et al. (1978) used an in vitro method similar to the technique used in this study and concluded that the major site of Mg transport in sheep, is the rumen.

The application of this technique in mineral research is limited, especially when studying inhibitors of mineral absorption. The environment bathing the tissue must not differ too far from the physiological norm, or the results will be over-shadowed by an abnormal tissue metabolism so pH, osmolarity, temperature and hydrostatic pressure should be carefully monitored. To minimize the effect of the latter, equal volumes on both sides of the chamber should be

used. The effect of inhibitors on the tissue being tested must be great so that only a small amount need be used to observe the effect. This method is best used to obtain preliminary data for a larger animal trial. But the advantage of isolating a particular element under study, and excluding external factor could outweigh these disadvantages in a limited number of circumstances.

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Appendix

APPENDIX TABLE 1

POST-INCUBATION VOLUMES AND MAGNESIUM CONCENTRATIONS, RUN 10

Block	Treatment	Tissue	Serosal		Mucosal	
			ml	mgMg/dl	ml	mgMg/dl
1	Blank	Rumen	7.6	1.97	13.8	2.34
2	Blank	Rumen	7.5	1.87	14.0	2.17
3	Blank	Rumen	7.4	2.07	14.4	2.35
1	Blank	Omasum	6.4	1.51	12.8	1.72
2	Blank	Omasum	7.5	1.74	13.3	1.72
3	Blank	Omasum	--	--	--	--
1	Low K	Rumen	7.6	2.13	12.4	6.97
2	Low K	Rumen	7.6	2.10	9.8	--
3	Low K	Rumen	--	--	--	--
1	Low K	Omasum	7.4	1.84	13.3	7.26
2	Low K	Omasum	7.6	1.87	14.0	7.15
3	Low K	Omasum	7.4	1.88	14.2	6.91
1	Med K	Rumen	7.2	2.17	13.8	6.77
2	Med K	Rumen	7.4	2.09	14.2	7.21
3	Med K	Rumen	7.4	2.18	--	--
1	Med K	Omasum	7.3	1.12	13.2	6.86
2	Med K	Omasum	7.6	1.88	14.3	6.65
3	Med K	Omasum	7.6	1.88	14.1	6.71
1	High K	Rumen	7.0	2.08	11.7	5.97
2	High K	Rumen	7.0	2.14	14.4	6.68
3	High K	Rumen	7.6	2.09	14.2	6.78
1	High K	Omasum	7.0	1.84	12.6	6.29
2	High K	Omasum	7.5	1.78	14.1	7.04
3	High K	Omasum	7.6	1.81	13.6	6.59

APPENDIX TABLE 2

POST-INCUBATION VOLUMES AND MAGNESIUM CONCENTRATIONS, RUN 12

Block	Treatment	Tissue	Serosal		Mucosal	
			ml	mgMg/dl	ml	mgMg/dl
1	Blank	Rumen	7.3	1.92	14.3	1.93
2	Blank	Rumen	7.4	1.95	14.2	1.96
3	Blank	Rumen	7.1	1.96	14.3	1.90
1	Blank	Omasum	7.4	1.87	14.1	1.91
2	Blank	Omasum	7.8	1.83	14.4	1.83
3	Blank	Omasum	--	--	--	--
1	Low K	Rumen	7.4	1.95	14.4	6.97
2	Low K	Rumen	7.6	1.83	14.4	7.04
3	Low K	Rumen	--	--	--	--
1	Low K	Omasum	7.4	1.89	14.1	7.43
2	Low K	Omasum	7.5	1.84	14.4	7.11
3	Low K	Omasum	7.8	1.88	14.4	7.09
1	Med K	Rumen	7.2	1.94	14.0	6.99
2	Med K	Rumen	7.5	2.08	14.3	6.94
3	Med K	Rumen	7.6	1.94	--	--
1	Med K	Omasum	7.0	1.94	14.2	7.11
2	Med K	Omasum	7.6	1.87	14.4	6.98
3	Med K	Omasum	7.5	1.90	14.5	7.04
1	High K	Rumen	7.6	1.92	13.9	7.19
2	High K	Rumen	7.6	1.86	14.4	7.00
3	High K	Rumen	7.4	2.04	14.4	6.89
1	High K	Omasum	7.0	1.98	14.2	7.02
2	High K	Omasum	7.7	1.81	14.2	6.94
3	High K	Omasum	7.4	1.87	14.6	6.91

APPENDIX TABLE 3

POST-INCUBATION VOLUMES AND MAGNESIUM CONCENTRATIONS, RUN 13

Block	Treatment	Tissue	Serosal		Mucosal	
			ml	mgMg/dl	ml	mgMg/dl
1	Blank	Rumen	7.3	1.30	14.0	2.09
2	Blank	Rumen	7.2	2.13	13.7	2.15
3	Blank	Rumen	6.4	2.38	13.2	2.17
1	Blank	Omasum	7.2	1.75	14.4	1.95
2	Blank	Omasum	7.0	2.01	13.4	1.99
3	Blank	Omasum	7.4	2.02	13.2	2.07
1	Low K	Rumen	7.1	2.03	13.8	7.72
2	Low K	Rumen	---	--	14.2	7.28
3	Low K	Rumen	7.6	2.33	13.9	7.32
1	Low K	Omasum	7.4	1.93	14.3	7.42
2	Low K	Omasum	7.4	1.89	14.0	7.21
1	Low K	Omasum	7.0	2.08	--	--
1	Med K	Rumen	6.6	2.25	13.0	8.02
2	Med K	Rumen	---	--	14.2	7.47
3	Med K	Rumen	6.8	2.18	14.3	7.38
1	Med K	Omasum	7.0	1.98	14.2	7.22
2	Med K	Omasum	7.0	1.99	13.6	7.71
3	Med K	Omasum	7.2	1.98	14.4	7.26
1	High K	Rumen	7.4	2.04	13.6	7.57
2	High K	Rumen	7.4	2.03	14.0	7.27
3	High K	Rumen	7.3	2.08	14.0	7.78
1	High K	Omasum	6.2	2.26	14.2	7.45
2	High K	Omasum	7.2	2.03	14.2	7.45
3	High K	Omasum	7.6	1.92	14.4	7.18

APPENDIX TABLE 4

POST-INCUBATION VOLUMES AND MAGNESIUM CONCENTRATIONS, RUN 14

Block	Treatment	Tissue	Serosal		Mucosal	
			ml	mgMg/dl	ml	mgMg/dl
1	Blank	Rumen	7.2	2.04	14.2	2.27
2	Blank	Rumen	7.6	1.96	14.2	2.24
3	Blank	Rumen	7.6	1.96	14.1	2.09
1	Blank	Omasum	7.6	1.86	14.2	--
2	Blank	Omasum	7.6	1.81	14.4	1.88
3	Blank	Omasum	7.0	1.93	14.0	1.98
1	Low K	Rumen	7.6	2.01	14.2	6.00
2	Low K	Rumen	7.6	2.02	14.2	6.00
3	Low K	Rumen	7.6	1.92	14.2	6.12
1	Low K	Omasum	7.9	1.88	--	--
2	Low K	Omasum	7.4	1.87	14.4	5.98
3	Low K	Omasum	7.5	1.85	14.0	5.86
1	Med K	Rumen	7.6	1.92	14.0	6.14
2	Med K	Rumen	7.6	1.99	14.1	6.00
3	Med K	Rumen	7.3	2.03	14.4	5.77
1	Med K	Omasum	7.6	1.83	14.4	5.85
2	Med K	Omasum	7.4	1.86	14.4	5.90
3	Med K	Omasum	--	--	14.4	5.87
1	High K	Rumen	7.6	1.99	14.2	6.06
2	High K	Rumen	7.6	1.94	14.4	6.01
3	High K	Rumen	7.7	1.94	14.0	6.03
1	High K	Omasum	7.6	1.80	14.4	5.86
2	High K	Omasum	7.8	1.77	14.2	5.95
3	High K	Omasum	7.6	1.77	14.2	5.84

APPENDIX TABLE 5

POST-INCUBATION VOLUMES AND MAGNESIUM CONCENTRATIONS, RUN 15

Block	Treatment	Tissue	Serosal		Mucosal	
			ml	mgMg/dl	ml	mgMg/dl
1	Blank	Rumen	7.5	2.01	14.0	2.19
2	Blank	Rumen	7.6	1.97	14.1	2.17
3	Blank	Rumen	7.4	2.01	14.2	2.02
1	Blank	Omasum	7.8	1.90	14.3	2.07
2	Blank	Omasum	7.4	1.90	14.2	2.05
3	Blank	Omasum	7.4	1.93	14.3	1.91
1	Low K	Rumen	7.5	2.06	14.2	6.38
2	Low K	Rumen	7.0	2.07	--	--
3	Low K	Rumen	7.0	1.99	14.2	6.25
1	Low K	Omasum	7.5	1.84	14.3	6.15
2	Low K	Omasum	7.6	1.90	14.4	6.14
3	Low K	Omasum	7.4	1.89	14.2	6.14
1	Med K	Rumen	7.6	2.00	13.2	6.19
2	Med K	Rumen	7.6	1.97	14.0	6.07
3	Med K	Rumen	7.5	2.02	14.0	6.26
1	Med K	Omasum	7.5	1.89	--	--
2	Med K	Omasum	7.0	2.03	13.7	6.43
3	Med K	Omasum	7.6	1.86	14.2	6.56
1	High K	Rumen	7.8	1.94	14.3	6.27
2	High K	Rumen	7.8	1.95	14.2	6.26
3	High K	Rumen	8.0	2.13	13.7	6.03
1	High K	Omasum	7.4	1.90	--	--
2	High K	Omasum	7.4	1.90	13.2	6.16
3	High K	Omasum	7.2	1.96	14.5	6.09

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EFFECT OF POTASSIUM LEVEL ON IN VITRO MAGNESIUM TRANSPORT ACROSS RUMEN AND OMASUM EPITHELIUM FROM CATTLE

by

Rebecca C. Gurley

(ABSTRACT)

Five crossbred heifers were slaughtered at different times and rumen and omasum tissues were removed. The epithelium was seperated from the muscle layer and mounted in parabiotic chambers. The tissues were incubated for 2 h in buffer at 39 C and aerated continuously in 95% O_2 5% CO_2. The buffers were similar to those which have been used previously in mineral transport studies, but were modified by varying the levels of Mg and K while keeping the osmolarity constant. Potassium was included in the buffers on the mucosal side in appropriate ratios to Mg to correspond to the K:Mg in diets fed to ruminants which would contain .1% Mg and .6, 2.4 and 4.8% K, (low, medium and high, respectively). A fourth buffer which contained physiological concentrations of K and Mg, was placed on the serosal side of the parabiotic chamber. Flow of Mg was calculated by:

$$F = \frac{C_1V_1 - C_0V_0}{A\Delta t}$$

where C_1 = final concentration, C_0 = original concentration, V_1 = final volume, V_0 = original volume, Δt = incubation

time, A = area of the tissue exposed to the buffer, and F = flow of Mg ($mg/cm^2/h$). Blanks were included which contained physiological levels of Mg on both side of the chamber to adjust for tissue effects. Magnesium transport tended to be 10 times greater through the rumen than the omasum. This indicates that the rumen is the primary site of Mg absorption in cattle. Potassium tended to depress Mg transport across both tissues. This technique has only limited application in mineral research.