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Ruminal Magnesium Absorption: Mechanisms, Modulation and Meaning for Assessment of Mg Intake

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Dedicated to
my georgeous husband
and my parents
for their continues support
love and understanding

CONTENT

Abbreviations

CFS	Cerebrospinal fluid
CNS	Central nervous system
DCAD	Dietary Cation/Anion Difference
DCT	Distal collecting tubule
DM	Dry matter
DNP	Dinitrophenol
ECS	Extracellular space
EGF	Epidermic growth factor
FC	Fermentable carbohydrates
GIT	Gastro-intestinal-tract
GRF	Glomerular filtration rate
ICS	Intracellular space
$\mathbf{J_{ms}}$	Unidirectional ion transport in mucosal to serosal direction ($\mu eq \cdot cm^{-2} \cdot h^{-1}$)
$\mathbf{J_{net}}$	Net-transport of Ions ($\mu eq \cdot cm^{-2} \cdot h^{-1}$)
$\mathbf{J_{sm}}$	Unidirectional ion transport in serosal to mucosal direction ($\mu eq \cdot cm^{-2} \cdot h^{-1}$)
KCNA1	Potassium channel
MagT1	Magnesium channel
MDCT	Mouse distal convoluted tubule
NHE	Na/H exchanger
NEFA	Non-esterified fatty acids
25(OH) D_3	25 Hydoxycholecalciferol
1,25(OH)2D_3	Dihydroxycholecalciferol or calcitriol
$\mathbf{PD_a}$	Apicale potential difference (mV)
$\mathbf{PD_b}$	Basolaterale potential difference (mV)
$\mathbf{PD_e}$	Epithelial potential difference (mV)
$\mathbf{P_{Mg}}$	Passive Mg transport
$\mathbf{PD_t}$	Transepithelial potential difference (mV)
$\mathbf{pH_i}$	Intraepithelial pH
REC	Rumen epithelial cells
SCFA	Short chain fatty acids

SLC41A1	Solute carrier family 41 member 1
TAL	Ascending limb of Henle loop
TRP	Transient receptor potential
TRPM7	Transient receptor potential melastatin subtype 7
TRPV5	Transient receptor potential channel subfamily V member 5

Aspects of Magnesium Metabolism in Ruminants: Mg Absorption, its Modulation, and Meaning for Assessment of Mg Intake

1. INTRODUCTION

Magnesium (Mg) is an essential mineral (Leroy, 1926). It is the fourth-most abundant cation in the body, and within the cells, the second-most abundant after potassium. Many physiological and biochemical functions depend on Mg (Romani and Scarpa, 2000), with one of the best studied being the activation of enzymes. Mg is a required activator of more than 300 enzymes (Günther, 1981) and is either directly bound to the enzyme or to the substrate, as for example ATP^{4-} (Lüthi et al., 1999; Cowan, 2002). The catalytic effect of Mg is concentration-dependent and exhibits a bell-shaped curve with a maximum of activation at approximately 1 mmol/l (Günther, 1981), which is within the range of the ionized intracellular Mg concentration (McGuigen et al., 2007). The total intracellular concentration is much higher (Lüthi et al., 1999), because the divalent cation Mg neutralizes negative charges in phospholipids and particularly the charged phosphate groups in nucleic acids (Günther, 1981). Furthermore, Mg acts as modulator of synaptic transmission during excitation-contraction coupling in skeletal muscle (Lamb and Stephanson, 1994) and of ion channels (Vemana et al., 2008), and the unspecific cation conductance of rumen epithelial cells is regulated by Mg in a voltage-dependent manner (Stumpff and Martens, 2007; Leonhard-Marek et al., 2005). Mg modifies the release of parathyroid hormone (PTH) and hypomagnesemia causes secondary hypocalcemia (Anast et al., 1972; Rayssiguier et al., 1977), because Mg deficiency inhibits the action of PTH on Ca mobilization from bone (MacManus et al., 1971; Samson et al., 1983; van de Braak et al., 1987). Recently, Mg has been shown to serve as a second messenger with a crucial role in the immune system (Li et al., 2011).

Hence, corresponding to the variety of Mg effects, hypomagnesemia causes complex symptoms such as the impairment of embryonic growth (Zhou and Clapham, 2009) and of bone formation (Günther et al., 1981). Further, interactions of Mg with insulin release and insulin resistance (Günther, 2010) and a role of Mg in inflammation and metabolic syndrome have been suggested (Rayssiguier et al., 2010). Furthermore, recent data indicate that cellular Mg extrusion is regulating energy metabolism and tumor progression (Funato et al., 2014).

The modulation of channel functions in excitable tissues by Mg is probably the reason for neurological symptoms such as uncertain gait, grinding of the teeth, salivation, ataxia, recumbency, convulsions, and finally tetanic muscle spasms in

hypomagnesemia. In cattle, this complex of symptoms has been well known for some 80 years (Sjollema, 1930) and is called tetany or grass staggers.

Mg metabolism is influenced by hormones such as insulin (Miller et al., 1980), catecholamine (Pearson and Luthmann, 1974; Rayssiguier, 1977a; Romani, 2012), and PTH (Goff et al., 1986; Rayssiguier, 1977b), but a hormonal system for the controlled regulation of blood or body Mg as is found for Ca and Na, including a feedback mechanism, is missing, although a magnesiotropic hormone, epidermic growth factor (EGF), has recently been demonstrated. EGF modulates Mg transport in the kidney in an autocrine/paracrine manner (Groenestege et al., 2007).

Despite the absence of such a regulation system, Mg in blood is kept within the range of 0.8 – 1.2 $mmol \cdot l^{-1}$ when Mg influx (absorption) into the extracellular space (ECS) is larger than the efflux (requirement) from ECS. Hence, the normal blood Mg concentration and metabolism primary depends on influx being > efflux which is also influenced in man by six genomic regions (Meyer et al., 2010).

This review summarizes current knowledge about aspects of Mg metabolism in ruminants and pays attention to Mg absorption (influx) from the gastrointestinal tract and efflux via the kidneys. Our improved understanding of the site and mechanism of Mg absorption is combined with a meta-analysis of Mg digestibility in dairy cows. It has been shown that results from *in vitro* studies with isolated ruminal epithelia help explain data from traditional balance studies in dairy cows. Mg intake according the actual requirement for maintenance and milk production can quantitatively be estimated and qualitatively explained. The inclusion of a safety margin allows an assessment of Mg intake for actual requirements of maintenance and milk production. Mg homeostasis is further determined by the adjustment of renal Mg excretion. This function of Mg efflux is the second important part of Mg metabolism and is closely regulated according to requirement.

2. MAGNESIUM HOMEOSTASIS

2.1. Distribution of Magnesium

Mg is a constituent of bones, and the majority of Mg is found in the skeleton (60 – 70 %), with some 30 % in the intracellular space (ICS) and only some 1 % in the extracellular space (ECS). The total content of Mg within the body has been analyzed for calves by (Blaxter and McGill, 1956):

$$Mg\ (g) = 0.655x - 3.5g \quad (1)$$
$$x = \text{body weight in kg}$$

Hence, if the relationship is also true for cows, the total amount of Mg increases with body weight (BW). A cow with a BW of 700 kg has approximately 455 g Mg: ≈ 320 g in bones, ≈ 130 g intracellular, and only ≈ 4 - 5 g in the ECS including blood Mg.

2.2. Regulation of Magnesium Homeostasis

Despite its multiple and essential functions, magnesium homeostasis is not regulated by a hormonal controlled feedback system. Without hormonal regulation, blood Mg (0.8 – 1.20 $mmol \cdot l^{-1}$) depends, under steady-state conditions, on the influx of Mg from the gastrointestinal tract (GIT) into the ECS including blood (a) and on the efflux from ECS into the milk (most important) b), into the ICS (≈ growth including fetus during pregnancy and bones) (c), and into the intestine (endogenous losses) (d). Mg not required for b – c is excreted, via the kidneys, into urine (e). Hence, the blood Mg concentration varies within the physiological range, if

$$a = b + c + d + e \quad (2)$$

a = Mg absorption (g/d) (influx)
b = Mg efflux in milk (g/d)
c = Mg uptake (efflux) into the ICS (tissue, bone, fetus) (g/d)
d = Endogenous Mg secretion (efflux) (g/d)
e = (Surplus) Mg excretion (efflux) in urine (g/d)

A scheme of Mg metabolism is given in figure 1 for a cow with a BW of 700 kg and giving 40 kg/d milk.

Scheme of Mg Metabolism in a Dairy Cow (BW 700 kg)

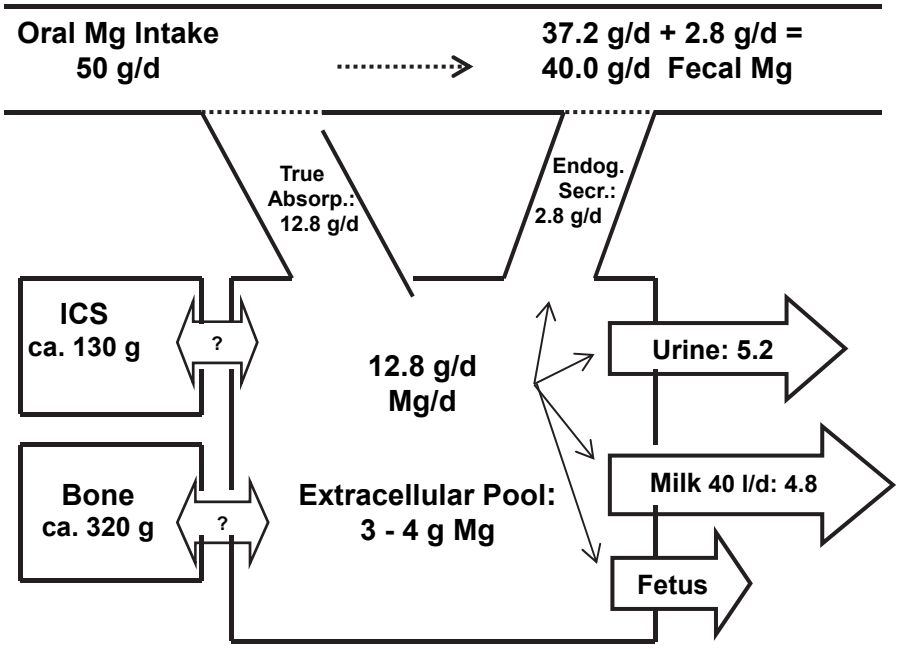


Figure 1: Scheme of Mg metabolism in a non-pregnant dairy cow of 700 kg BW. The daily Mg intake is 50 g, with true Mg absorption being 12.8 g/d (25.6 %). The true absorption is reduced by endogenous secretion of 2.8 g/d (4 mg/kg), which accounts for an apparent absorption or Mg digestibility of 10 g/d (20 %). 4.8 g/d Mg is used for milk secretion (12 mg/l) and the surplus (5.2 g/d) is excreted via kidneys in urine. The pool in the extracellular space has been calculated by assuming that the plasma volume and interstitial space represent 5 % and 15 % of BW, respectively (Storry, 1961a).The unidirectional flow of Mg into and out of the ICS and bone is not known (?). It is assumed that the net flow of Mg into ICS and bone is zero at constant body weight. The possible fetal requirement is not included. It accounts for 0.2 Mg/d in late gestation (House and Bell, 1993).

In an adult and non-pregnant cow, net uptake of Mg into the ICS, bone, and fetus is zero, and equation (2) can be simplified to

$$a = b + d + e \quad (3)$$

a = Mg absorption (g/d) (influx)
b = Mg efflux in milk (g/d)
d = Endogenous Mg secretion (efflux) (g/d)
e = (Surplus) Mg excretion (efflux) in urine (g/d)

Because Mg influx (a) rarely equals Mg efflux (b + d), additional mechanisms are necessary for the adjustment of possible differences. This fine tuning is controlled by the kidneys. A potential surplus (Mg influx > Mg efflux) is rapidly compensated by excretion via the kidneys (e). However, the functions of the kidneys are limited, and

they can handle only a Mg surplus. If Mg influx is lower than Mg efflux, particularly on secretion into milk, urinary Mg excretion becomes extremely small (< 1.0 $mmol \cdot l^{-1}$) and finally causes hypomagnesemia; because of the lack of hormonal regulation, the relatively large Mg pools in the ICS (130 g) or bones (ca. 320 g) cannot acutely be mobilized to maintain physiological blood Mg concentrations (Blaxter and McGill, 1956) or to give the small amount of some 0.5 g/d (Storry and Rook, 1963). Significant mobilization of Mg from bone appears to be unlikely because the ratio between Ca and Mg in bone is 42 to 1, and substantial withdrawn from bone would disrupt Ca homeostasis (Fontenot et al., 1989). Furthermore, Mg absorption from the rumen is neither increased at hypomagnesemia (Martens and Stössel, 1988), nor stimulated by intravenous injection of PTH or PTHrP (Dua et al., 1994).

This simple regulation of Mg homeostasis suggests that Mg influx was very rarely limited during evolution. Obviously, Mg intake and absorption (a) were generally above requirement (b + c + d), and the variable surplus was effectively excreted in urine (e). Moreover, Mg is not very toxic, and hence transient hypermagnesemia (rapid influx > efflux) is well tolerated (Martens and Stössel, 1988). Nevertheless, this type of regulation of Mg homeostasis primarily relies on a Mg surplus, clearly showing that Mg influx is of predominant importance, and means that Mg absorption from the GIT determines blood Mg (Mg_{Bl}):

$$\text{Normal } Mg_{Bl}\text{: Influx (a = absorption)} - [\text{efflux (b + d)} + \text{urinary Mg (e)}] \quad (4)$$

Therefore, the blood Mg concentration can only be kept constant when the daily Mg requirement, especially for milk production, is replaced by an adequate absorption of Mg. Vice versa, a high Mg requirement for milk production must lead to hypomagnesemia at reduced Mg intake or absorption. Hence, better comprehension of the absorption of Mg (influx) and its large variation (Schonewille et al., 2008) appears to be key to understanding hypomagnesemia. This knowledge will include improved information about a) the site of Mg absorption, b) mechanisms of Mg absorption, and c) factors modulating Mg absorption.

3. Mg ABSORPTION FROM THE GASTROINTESTINAL TRACT

3.1. Site of Magnesium Absorption

3.1.1. Early studies: The first study of the site of Mg absorption in the gastrointestinal tract (GIT) of ruminants was performed by Stewart and Moodie (1956). Sheep were anesthetized, and Mg salts were infused at various locations along the GIT. An increase of the venous blood Mg concentration of the corresponding part of the GIT was considered as absorption. An increase was observed in the venous blood of the rumen, abomasum, small intestine, and caecum. The absorption of Mg from the small intestine was considered as physiologically important. These findings concerning the net exchange of Mg in sheep GIT were confirmed by Field (1961), Care and van't Kloster (1964), Phillipson and Storry (1965), and Perry et al. (1967) and by Smith (1959, 1963) in calves. Hence, the results of the early studies suggested the distal part of the small intestine as the site of Mg absorption, whereas Mg secretion was observed in the proximal part of the intestine (Field, 1961; Care and van't Klooster, 1964; Phillipson and Storry, 1965; Smith, 1963; Perry et al., 1967), and "there is no evidence to assume that such a mechanism exists for the (active – the author) transport of calcium and magnesium across the rumen epithelium" (Storry, 1961a). In agreement with these studies was the conclusion of Poutainen (1971) that "no net absorption of Mg occurred from the reticulo-rumen". Hence, the common opinion at that time about the site of Mg absorption was the small intestine; the forestomachs were not mentioned or were even rejected (Storry, 1961a; Poutainen 1971), and possible epithelial transport mechanisms of Mg were not discussed. The transport of Mg was restricted to its appearance or disappearance.

In contrast to the studies mentioned above was the observation of Harrison et al. (1963). These authors applied the method of cannulae in the duodenum of sheep and observed, by measuring the flow rates of ingesta, that Mg disappeared proximal to the duodenum. This observation agrees with the data of Marongiu (1971) who suggested a disappearance of Mg from the rumen. The discrepancy of these data (intestine or forestomachs) was not discussed at that time but hinted at some uncertainties about the site of Mg absorption in the digestive tract of ruminants.

3.1.2. Flow rates: This insecurity was reinforced by the findings of Rogers and van't Klooster (1969). These authors, applying the method of cannulated cows, measured

the intake of minerals, flow rates at the duodenum and ileum, and fecal excretion. This technique determined the net movement of compounds along the alimentary tract. The obtained data demonstrated the absorption of Mg before the duodenum, a small amount of secretion into the small intestine, and the absorption of almost the same amount from the large intestine. Hence, the net movement of Mg in whole intestine was close to zero, and the total absorption equaled the disappearance before the duodenum. These early findings have now been confirmed in all studies with corresponding cannulated sheep and cows. Martens (1978) summarized these data from sheep and cows and concluded that Mg was absorbed before the duodenum. The absorption increased linearly with Mg intake (up to 35 g/d Mg in cows and ≈ 2.8 in sheep) (Martens 1981). Again, a net secretion was observed along the total length of the small intestine, and this small secretion was absorbed in the large intestine. Hence, the total absorption form the whole intestine was negligible, despite the absorptive capacity of the large intestine (see below).

Some uncertainty still existed regarding the exact location before the duodenum, because Edrise and Smith (1979) demonstrated Mg absorption also from the omasum. A comparison of Mg transport across isolated epithelia of the reticulum, rumen, and omasum from sheep showed that the absorptive capacity of the rumen was large and predominant (Martens and Rayssiguier, 1980). These *in vitro* findings agreed with *in vivo* observation of Tomas and Potter (1976a) and Field and Munro (1977). The main site of Mg absorption within the stomach area was the rumen. Only a small amount of Mg was absorbed from the omasum (Tomas and Potter, 1976a; Field and Munro, 1977) and not from the abomasum (Field and Munro, 1977).

A further and important finding underlined the physiological significance of Mg absorption before the duodenum. It turned out that Mg absorption from the forestomachs was essential for maintaining normal Mg_{Bl} (Pfeffer and Rahman, 1974; Baker et al., 1979), and that reduced Mg absorption from the forestomachs was not compensated by the intestine (Tomas and Potter, 1976b). Hence, the absorption of Mg from the forestomachs was a precondition of Mg homeostasis and represented Mg influx.

The estimated absorptive Mg capacity of the rumen exceeded the daily Mg requirement 4 – 5 times in sheep and was saturated at 5 $mmol \cdot l^{-1}$ in this species (Martens, 1979) and at some 12 $mmol \cdot l^{-1}$ in calves (Martens, 1983).

An important point to mention is that the Mg absorption switched from the hind gut in milk-fed calves (Smith, 1959) and lambs (Dillon and Scott, 1979) to the forestomachs in adult ruminants (Smith, 1959). The capability of the large intestine for Mg absorption is maintained in adult animals and can be used for first aid treatment of hypomagnesemia (Meyer and Busse, 1975; Bell et al., 1978). Furthermore, evidence has been presented of compensatory Mg absorption from the hind gut at high Mg and K intake which reduced ruminal Mg absorption (Dalley et al., 1997).

3.2. Mechanism of ruminal Mg transport: passive or active?

***3.2.1. Passive driving forces**:* Scott (1965) analyzed the passive driving forces across the rumen epithelium and concluded that the chemical gradient of Mg (rumen concentration > blood) for passive movement was opposed by the stronger electrical gradient (blood side positive 30 – 60 mV) and did not facilitate passive movement from the rumen to blood. A calculation of the equilibrium potential (PD_e) with the Nernst equation across the rumen epithelium at which no net movement of Mg occurred confirmed this conclusion. Figure 2 shows the PD_e at increasing rumen Mg concentrations assuming an ionized Mg concentration of 0.6 $mmol \cdot l^{-1}$ in the subepithelial layer of the epithelium. A potential difference across the rumen epithelium (transmural potential difference: PD_t) below the PD_e would allow passive absorption (chemical gradient from rumen to blood > electrical gradient in the opposite direction), and vice versa, a PD_t above PD_e (chemical gradient < electrical) would cause Mg secretion into the rumen. The calculation clearly shows that passive Mg absorption, even at high concentrations of 10 $mmol \cdot l^{-1}$, is only possible at a transmural potential difference of < 38 mV (fig. 2) and confirms the early conclusions of Storry (1961a) who excluded passive Mg and Ca transport from the rumen.

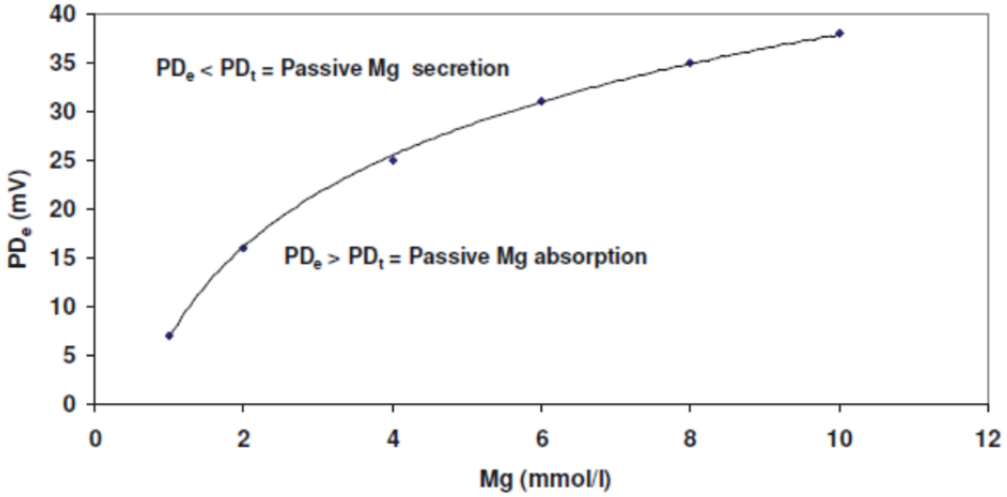


Figure 2: Calculated equilibrium potential (PD_e; blood side positive) across the rumen epithelium at increasing rumen (ionized) Mg concentrations and at an assumed constant ionized Mg concentration of 0.6 mmol·l^{-1} in the subepithelial layer. At a given Mg concentration and the corresponding PD_e, the chemical driving force (Mg gradient from rumen to blood) equals the opposite electrical gradient, and no passive net transport will occur. A potential difference, PD_t, across the rumen epithelium below PD_e at a given Mg concentration would allow a passive Mg absorption from and above the PD_e Mg secretion into the rumen. Under physiological conditions PD_e is in most cases < PD_t and would permit passive Mg secretion. However, the possible paracellular and passive movement of Mg is small and negligible (see text below).

***3.2.2. Evidence for active Mg transport**:* Hence, passive Mg transport appeared to be unlikely, and the suggested active transport across the rumen epithelium was deduced from two sets of *in vitro* experiments. First, the direction of Mg transport was tested, with the clear demonstration that J_{ms} Mg >> J_{sm} Mg with the same passive gradients. Further, J_{ms} exhibited saturation at some 5 mmol·l^{-1} (Martens et al., 1976). Because these flux rates were determined with concentration gradients (J_{ms} = mucosal 2.6 mmol·l^{-1} – serosal 0 mmol·l^{-1} Mg and vice versa for J_{sm}), Mg was transported “downhill” in both directions, and a firm conclusion about the possible driving forces was not possible, because asymmetric and passive cation flow cannot been excluded as it has been observed in MDCT cells (Ikari et al., 2004). However, the saturation hints for the involvement of a carrier and the J_{net} Mg in the mucosal-serosal direction (= absorption) suggested more than a simple assumption of diffusion.

Passive movement of Mg appeared therefore to be unlikely. For this reason, Martens and Harmeyer (1978) designed *in vitro* experiments to demonstrate the characteristics of active transport mechanisms. They observed, across the isolated rumen epithelium of sheep under open circuit conditions (blood side positive potential)

and with identical Mg concentration on both sides of the tissues, the transport of ^{28}Mg from the mucosal to the serosal side (J_{ms}) of 75 – 107 nmol·cm^{-2}·h^{-1}. This transport direction was some ten times larger than the flux rate in the opposite direction (J_{sm}) leading to a significant net Mg transport in the absorptive direction, J_{net}, against the slight positive transepithelial potential difference, PD_t, and in the absence of a chemical gradient. The transport of Mg, J_{ms}, was significantly reduced by 40 % by a decrease of the incubation temperature from 39° to 32° C (Martens and Harmeyer, 1978).

These observations supported the assumption of active transport, a finding that was further reinforced by the observation of the saturation of J_{ms} at 5 mmol·l^{-1} (Martens et al., 1976) and the almost total inhibition of J_{net} by ouabain, an inhibitor of the Na/K-ATPase in the basolateral membrane (Martens and Harmeyer, 1978). The function of the Na/K-ATPase for Mg transport was not clear at that time, because ouabain is an inhibitor of the Na/K-ATPase and an unspecific inhibitor of transport mechanisms (see below) that are indirectly linked to the Na/K-ATPase. Observation suggests the need of ATP for Mg transport. Inhibition of the synthesis of ATP by 0.1 mmol·l^{-1} dinitrophenol (DNP) by uncoupling the respiratory chain abolished Mg transport (Martens, 1985a).

All these data were in agreement with a putative model of active Mg transport, which was further corroborated by application of the Ussing equation (Martens, 1985a):

$$J_{ms}/J_{sm} = C_m/C_s \cdot e^{-zFE/RT} \quad (5)$$

J_{ms}, J_{sm}: flux rates in the mucosal-serosal (J_{ms}) or in the opposite direction (J_{sm})
C_m, C_s: concentration of the mucosal or serosal site
E = potential difference across the rumen epithelium
z = charge of the ion
F, R, T have the usual meanings

The equation calculates *passive* ion movement depending solely on chemical and electrical gradients as driving forces across an epithelium. The determined ratio of Mg flux rates (J_{ms}/J_{sm}) *in vitro* was larger than that computed (only passive movement) (Martens, 1985a) (table 1). Hence, the exclusion of passive movement of Mg across the ruminal epithelium validated an active transport system (table 1). Conversely, the application of DNP (inhibition of ATP synthesis by 0.1 mmol·l^{-1}) abolished J_{net}, and the remaining ratio of J_{ms}/J_{sm} agreed with that predicted by the Ussing equation and showed that Mg flux was passive if ATP synthesis had been inhibited.

The *in vitro* data were confirmed by corresponding *in vivo* results. The determination of both flux rates with the aid of ^{28}Mg and the isolated rumen technique clearly showed the asymmetry of J_{ms} and J_{sm} (table1).

Table 1: Transport rates of Mg across the rumen epithelium were determined *in vitro* and *in vivo* and compared with calculated passive flux rates according to the Ussing equation. The transport rates (*in vitro*) were related to the dry matter of the incubated epithelium (g). Modified from Martens (1985a).

In vitro	**J_{ms}** **µmol·g^{-1}·h^{-1}**	**J_{sm}** **µmol·g^{-1}·h^{-1}**	**J_{net}** **µmol·g^{-1}·h^{-1}**	**Ratio[a]**	**PD_t** **mV**	**Flux Ratio** **Calculated[b]**	**Observed**
Control	13.50	1.51	11.59	1	7.4	0.58	8.94
DNP	2.81	3.06	-0.25	1	0	1	0.92
In vivo[d]	mmol·h^{-1}	mmol·h^{-1}	mmol·h^{-1}				
	1.49	0.22	1.27	5.21[c]	25	0.81	6.77

a: Ratio: Mg lumen versus Mg blood side in mmol·l^{-1}: 2.5 mmol·l^{-1} in both compartments. b: Calculated with the Ussing equation (4). c: Assuming that 60 % of the blood Mg (0.8 mmol·l^{-1}) is ionized. The lumen Mg is 2.5 mmol·l^{-1} and total ionization is assumed. d: Mg absorption is measured from the isolated rumen with an artificial rumen fluid.

Furthermore, it was excluded that bulk flow could explain the J_{net} Mg (Martens and Harmeyer, 1978) as shown by Behar (1974) in rat colon. Net flow of water across the rumen wall was induced by either hypo- or hypertonic intraruminal buffer solution. Neither secretion nor absorption of water influenced the Mg absorption from the rumen and rejected Mg transport by bulk flow (Martens, 1985b; Gäbel et al., 1987).
The various observations about Mg transport *in vitro* or *in vivo* as $J_{ms} > J_{sm}$ (table 1), its sensitivity against reduced temperature, its inhibition by oubain or blockade of ATP synthesis (DNP), and its saturation and absence of bulk flow are typical characteristics of an active and transcellular transport mechanism; this has been further confirmed by the exclusion of passive Mg movement according the Ussing equation (5). Active Mg transport was generally a new observation at that time and had been described neither in other tissues nor in the intestine of monogastric animals (Ross, 1961 and 1962; Hendrix et al., 1963; Aldor and Moore, 1970). The only mechanistic proposal for intestinal Mg transport at that time was the assumption of Mg absorption by bulk flow in rat ileum and colon (Behar, 1974).

3.3. Pathways of active Mg transport across the rumen epithelium

3.3.1. The black box: The demonstrated active movement of Mg across the rumen epithelium *in vitro* and *in vivo* requires transcellular transport and hence includes a) uptake across the apical membrane, b) the passage of Mg from one layer into the other of the multilayered epithelium until the stratum basale, and c) release across the basolateral membrane. The absence of a transport model of Mg across cell membranes or epithelia hindered early suggestions about points a – c, because of the lack of knowledge of Mg carriers or channels and the respective driving forces. The movement of Mg across the apical and the basolateral membrane of the rumen epithelium was considered a "black box".

An early indication for possible transport mechanisms (a – c) came from the well-known negative effect of K on Mg absorption from the rumen (Tomas and Potter, 1976b; Martens and Blume, 1986). An increase of K intake causes a linear increase of ruminal K concentration, which is accompanied by a decrease of Na concentration (Lang and Martens, 1999) and a rise of PD_t (Ferreira et al., 1966; Martens and Blume, 1986). These variations are related to a decline of Mg absorption from the rumen (fig. 3).

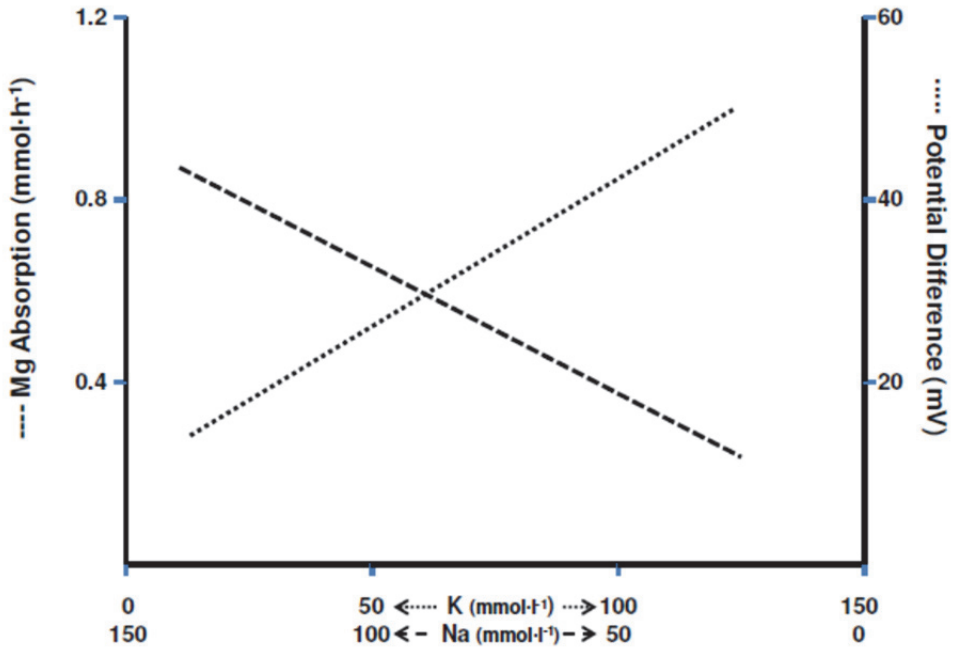


Figure 3: Schematic relationship between the reciprocal ruminal K and Na concentration, increase of PD_t, and reduced Mg absorption. Redrawn of data from Martens and Blume (1986).

Because three ruminal parameters (K ↑, PD_t ↑,Na ↓) changed at the same time, the causal reason of reduced Mg absorption could not be deduced from these studies.
A first indication about the possible electrophysiological effects of PD_t on Mg transport was deduced from an *in vitro* study with altered K concentrations and PD_t. Martens et al. (1987a) simulated, *in vitro*, the *in vivo* situation in Ussing chambers. A high luminal (rumen) K (70 $mmol \cdot l^{-1}$) concentration caused an increase of PD_t of 25.1 mV (blood side positive) under open circuit conditions (table 2). Unidirectional Mg flux rates were determined under open and short-circuit conditions (0 mV PD_t). This change of electrophysiology caused significant alterations of all flux rates (table 2). J_{ms} and J_{net} Mg were significant higher, and J_{sm} was lower, at PD_t 0.

Table 2: Mg transport rates across the isolated rumen epithelium of sheep. PD_t = mV; blood side positive; J_{ms}, J_{sm} and J_{net}: $nmol \cdot cm^{-2} \cdot h^{-1}$ (Modified from Martens et al., 1987a).

Group	PD_t	J_{ms}	J_{sm}	J_{net}
K	25.1*	52.2^a	11.6^a	40.6^a
K	0**	76.1^b	8.0^b	68.1^b
Control	26.1	47.4^a	12.2^a	35.2^a
Control	0	72.1^b	6.5^b	65.6^b

*Open circuit conditions; ** Short-circuit conditions; a, b: different superscripts denote significant differences.

In the two control groups, the electrophysiological changes were simulated at constant low luminal K (5 $mmol \cdot l^{-1}$). The clamped potential differences caused almost the same variations as in the K groups: a high PD_t (caused by mucosal K or external current) reduced J_{ms} Mg, slightly increased J_{sm} Mg, and hence decreased J_{net} Mg (table 2). This slight augmentation of J_{sm} Mg is in agreement with results obtained by Care et al. (1984) in studies with the rumen pouch of sheep and assumes a passive J_{sm}. This passive flow of Mg traverses the shunt pathway in both directions, and the passive efflux and influx have the same magnitude in the absence of electrical and chemical gradients (under short-circuit conditions): J_{sm} = passive J_{ms}, where passive J_{ms} is the passive part of J_{ms}. An electrical gradient (PD_t) acts as a driving force for both passive fluxes of Mg, J_{sm}, and passive J_{ms}. The passive flow is enhanced in one direction and reduced in the other. The PD_t has been shown to increased J_{sm} (table 2) from 8.00 at zero PD_t to 11.6 $nmol \cdot cm^{-2} \cdot h^{-1}$ at 25.1 mV (mucosal side negative). Clearly, passive J_{ms} in the opposite direction must be reduced under these circumstances, as can be calculated with the aid of the Ussing equation (Ussing,

1949). Passive J_{ms} decreases from 8.00 at zero PD_t to 1.8 nmol·cm^{-2}·h^{-1} at a PD_t of 25.1 mV. As can be seen from Table 2, the reduction of (total) J_{ms} from 76.1 to 52.2 nmol·cm^{-2}·h^{-1} cannot be explained by the small decline of passive J_{ms}. The drop of passive J_{ms} (6.2 nmol·cm^{-2}·h^{-1}) accounts only for 25.9 % of the total decrease of J_{ms}. This conclusion agrees with the data of Grace et al. (1988). The effect of K on net Mg transport occurs via a reduction of absorptive Mg flux (J_{ms}), rather than by increased secretion (J_{sm}) into the rumen. The results of these model studies *in vitro* (Martens et al., 1987a) or *in vivo* (Grace et al., 1988) confirm the effect of K on Mg digestibility in the balance study of sheep. The K-dependent depression of Mg digestibility is caused by decreased absorption and not by increased endogenous secretion (Newton et al., 1972).

Therefore, the transcellular active part of J_{ms} has been suggested to be depressed by high mucosal K concentrations under open-circuit conditions. Chemical interaction between K and Mg at the luminal membrane is not very likely because the effect of high mucosal K concentration is not observed under short-circuit conditions. There is no reason to assume that a possible chemical effect of K can be abolished by an external current. Furthermore, a similar decrease of the transcellular active transport of Mg can be observed when a transepithelial PD_t is created by an external current (table 2). Thus, the change of PD_t appears to be an important parameter disturbing the active transcellular transport of Mg, but the manner in which the change of PD_t is created is unimportant.

The important effect of PD_t has been demonstrated by compilation of data from *in vivo* and *in vitro* studies (Leonhard-Marek et al., 1998) (fig. 4). Although various methods (rumen pouch, isolated rumen or isolated rumen tissue) have been used, the relative decrease of J_{net} Mg is independent of the *in vivo* or *in vitro* method and is linearly related to the change of PD_t.

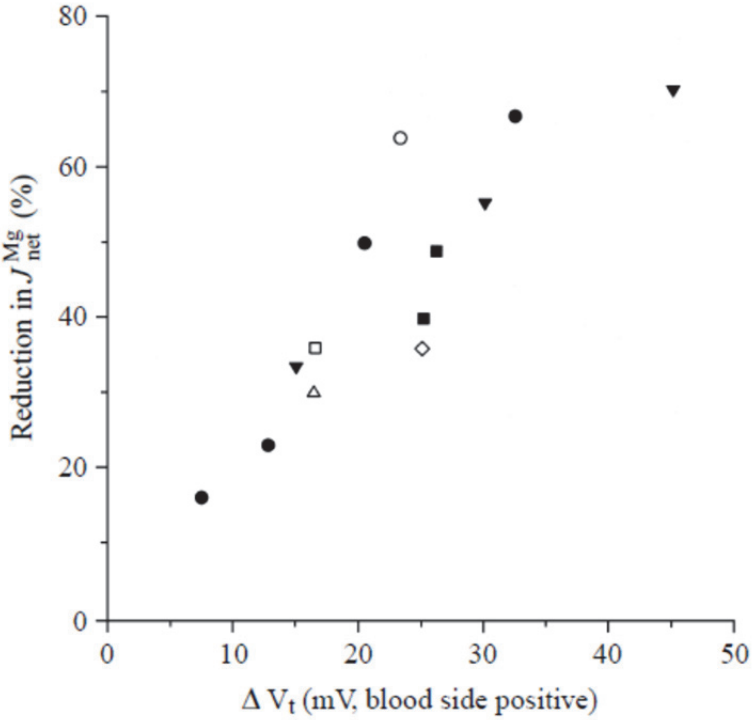


Figure 4: Effect of ΔV_t (ΔPD_t) on net Mg transport (% reduction of J_{net} Mg) according to various studies. ●: Martens and Blume 1986 – *in vivo;* ○: Care et al. 1984 – *in vivo*; ◊: present study; □: Beardsworth et al. 1987 – *in vivo*; ■: Martens et al. 1987a – *in vitro* ; ▼: Martens, unpublished (from Leonhard- Marek et al., 1998).

3.3.2. PD-dependent Mg uptake: The close relationship between the transcellular transport of Mg and the PD_t has led to the suggestion that the transcellular transport of Mg at the apical and/or basolateral membrane depends on electrical gradients, because PD_t is the sum of the apical (PD_a) and basolateral potential difference, PD_b.

$$PD_t = PD_a - PD_b \quad (6)$$

Hence, a change of PD_t is caused by altered PD_a, PD_b, or both. Lang and Martens (1999) have shown that an increase of PD_t of 25 mV (blood side positive) *in vitro* by a current depolarizes PD_a by some 15 mV. Because high luminal K increases PD_t, K has been suggested to exert its effect mainly on changing PD_a. This conclusion is related to similar studies in other epithelia such as the gall-bladder of Necturus (Reuss, 1979) and the flounder small intestine (Halm et al., 1985). The underlying effects of K gradients on PD_t have been investigated in these epithelia. Important observations are that the apical membrane in both Necturus gall-bladder and flounder intestine is permeable to K ions, and that the PD_t of the apical membrane is dependent upon the distribution of K ions across it. A mucosal increase of K

concentration leads to the depolarization of PD_t across the apical membrane in these epithelia and to a concomitant augmentation of PD_t (Reuss, 1979; Halm et al. 1985). Evidence has been presented that the apical luminal membrane of the ruminal epithelium is permeable to K ions (K conductance). Unidirectional K fluxes across the ruminal epithelium provide evidence of the active transport of K from the serosal to mucosal side, whereby K is pumped by the Na/K-ATPase across the basolateral membrane into the cells of the epithelium, before leaving the cells through the K conductance in the apical membrane (Ferreira et al., 1972). These results support the assumption of active electrogenic transport of K from the serosal to the mucosal side. Under these circumstances, a mucosal increase of K might lead to similar consequences as those observed in Necturus gall-bladder (Reuss, 1979) and flounder small intestine (Halm et al. 1985), i.e., the depolarization of the apical membrane and consequently the augmentation of the PD_t. A decrease of PD_a across the apical membrane could reduce the driving force for the electrogenic uptake of Mg across this membrane and could consequently diminish the transcellular transport of Mg.

This suggestion of Mg transport includes PD_a as the driving force for the uptake of ionized Mg. The PD-dependent transport of ions has been studied in other epithelia, such as in rabbit ileum (Frizzell and Schultz, 1972), and a theoretical model has been proposed:

$$J = J_d \cdot \xi + J_m \quad (7)$$

J = Total transport of an ion
$J_d \cdot \xi$ = PD-dependent transport of an ion (slope of 7)
J_m = PD-independent transport of an ion (intercept at y-axis)

$$\xi = \frac{z \cdot F \cdot V/R \cdot T}{e\, z \cdot F \cdot V/R \cdot T - 1}$$

z = charge of the ion
V = potential difference across the epithelium
F, R, T = usual meanings

The equation simply assumes that an ion flux across an epithelium is influenced by the PD or not, and the corresponding flux rates can be differentiated between PD-dependent and PD-independent transport according to equation 7. Figure 5 is a schematic drawing of ruminal Mg transport *in vitro* according to this equation.

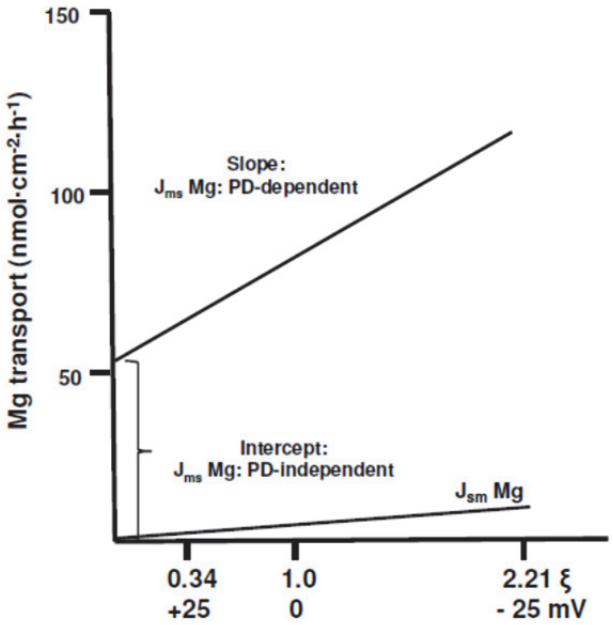


Figure 5: Scheme of PD-dependent and PD-independent Mg transport *in vitro* according to equation 7. The slope of J_{ms} Mg represents the PD-dependent J_{ms} Mg, and the intercept at the y-axis the PD-independent J_{ms} Mg. The shallow slope of J_{sm} Mg is solely PD-dependent and probably signifies paracellular and passive Mg transport. Modified from Leonhard-Marek and Martens, 1996.

The application of this model to J_{ms} Mg transport rates across the rumen epithelium of sheep showed a linear correlation between ξ and J_{ms} Mg within – 25 and + 25 mV (Leonhard-Marek and Martens, 1996), and the obtained slope confirmed the suggestion of PD-dependent J_{ms} Mg transport and Mg uptake as an ion. The applied PD_t of + 25 or – 25 mV caused a ΔPD_a of some 30 mV (Lang and Martens, 1999); this is a significant change of driving force of ionized Mg across the apical membrane. Two further observations are in agreement with this model. Leonhard-Marek and Martens (1996) have demonstrated a K conductance in the apical membrane, and the K gradient across the apical membrane predominantly contributes to PD_a. Impalement of sheep rumen epithelium from the mucosal side with a microelectrode has measured a PD_a under open circuit conditions of - 67.3 mV (cytosol negative), which is depolarized by the mucosal K concentration (PD_a = - 82.8 + 21.3 log K (mucosal) (Leonhard-Marek and Martens, 1996). Hence, the K-dependent increase of PD_t is mainly explained by a depolarization of PD_a (see equation 6; fig. 6).

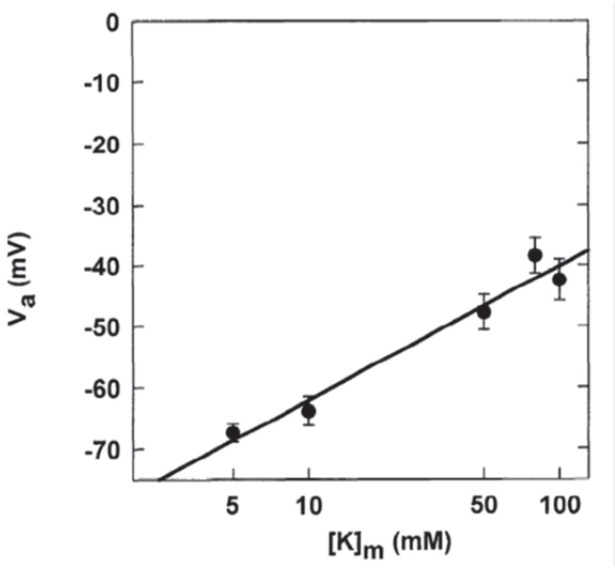


Figure 6: Correlation between mucosal K (lg) concentration and depolarization of V_a (PD_a). (from Leonhard-Marek and Martens, 1996).

The K-dependent decrease of PD_a diminishes the driving force for the PD-dependent uptake of Mg. This direct interaction has been demonstrated by Leonhard-Marek and Martens (1996). An increase of mucosal K concentration from 5 to 100 mmol·l^{-1} decreases J_{ms} Mg in a curvilinear manner (fig. 7), because the effect of mucosal K is small at high K concentrations (80 versus 100 mmol·l^{-1}). An increase of mucosal K decreases J_{ms} significantly, and this effect of K on PD_a is the most likely explanation for the reduced J_{ms} (Leonhard-Marek and Martens, 1996) and is in agreement with the proposed mechanism of PD-dependent Mg uptake.

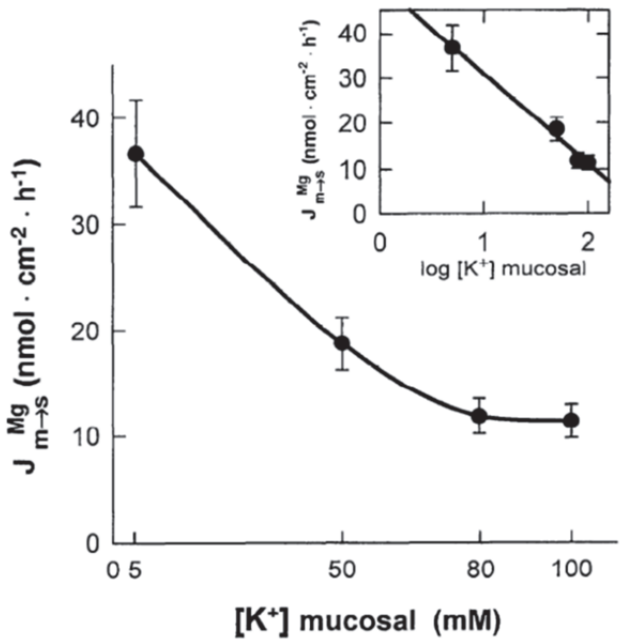


Figure 7: Correlation between mucosal K concentration and J_{ms} Mg. An increase in K depolarizes PD_a and reduces the driving force for Mg uptake. The interaction is curvilinear at normal concentration but is linear at log concentration (see inset). The effect of K is diminished at high concentration, because the further depolarization of PD_a is small at these concentrations (from Leonhard-Marek and Martens, 1996).

The driving force PD_a is augmented by the free intracellular Mg concentration, Mg_i, of 0.54 mmol·l^{-1}, which has been measured in isolated rumen epithelial cells (Schweigel et al., 1999), and which is lower than ruminal Mg of 3.75 mmol·l^{-1} at a Mg intake of 1.64 g/d in sheep (Ram et al., 1998). Hence, the combined electro-chemical gradient as a driving force across the luminal membrane permits effective Mg uptake.

Furthermore, mucosal verapamil (0.25 mmol·l^{-1}) significantly depolarizes PD_a and J_{ms} Mg (Leonhard-Marek and Martens, 1996). In agreement with these observations are the results of Schweigel et al. (1999) who have studied, in isolated rumen epithelial cells (REC), Mg influx and concentration. Membrane depolarization with high extracellular K (40, 80, or 140 mmol·l^{-1}) and the K channel blocker quinidine (50 and 100 µmol·l^{-1}) results in a decrease in intracellular Mg (Mg_i). Hyperpolarization in the presence of valinomycin induces a 15% increase of Mg_i.

The significant correlation between changes of PD_t, PD_a, and Mg transport has led to the suggestion of a Mg channel (Martens et al., 1987a). This type of Mg transport protein is now well established (Hoenderop and Bindels, 2007), and hypomagnesemia in man is now known to be caused by mutation of a channel of the transient receptor potential (TRP) gene family, TRPM6 (Konrad et al., 2004). TRPM channels belong to the ion channel superfamily TRP and are activated by multiple

stimuli (Touyz, 2008). TRPM proteins have 6 transmembrane domains and the region between domains 6 and 7 is believed to contribute to the cation pore as a homo- or hetero-tetramer (TRPM6 and TRPM7) (Dimke et al. 2011). Both amino and carboxy tails are located in the cytosol, and four subunits are assumed to be assembled into a functional channel (Cahalan, 2001). TRPM6 and TRPM7 are active as a C-terminal threonine-serine kinase, and these proteins are sometimes named chanzymes (Cahalan, 2001) (fig. 8).

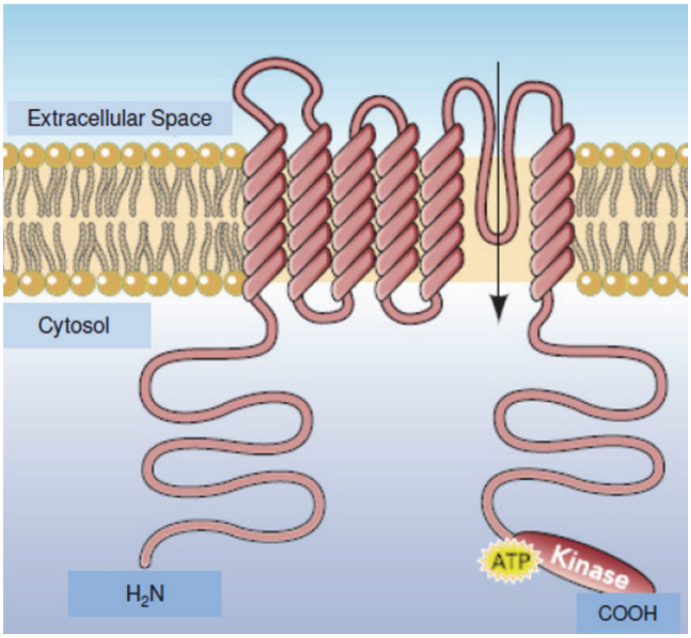


Figure 8: Scheme of a TRPM channel. The TRPM channels are both a cation channel and a kinase at the carboxyl-terminus. The channel protein has 6 transmembrane domains and the region between domains 6 and 7 is believed to contribute to the cation pore as a homo- or hetero-tetramer (Hoenderop and Bindels, 2007).

A member of this channel family has been found in REC, and the expression of mRNA and protein of TRMP7 has been shown to be modulated by Mg content of the incubation medium (Schweigel et al., 2008). Because TRPM 6 has not been detected in REC (Schweigel et al., 2008), but the used primer pairs had be designed against human TRPM6 because the ovine one is unknown (Schweigel et al., 2009). Some uncertainty remains, because TRPM7 is thought to be a Mg transporter primarily involved of intracellular Mg regulation rather than transepithelial Mg transport (Schmitz et al., 2003) and is suggested to be an intracellular Mg sensor (Demeuse, 2006). If TRPM7 represents the suggested Mg channel, its structure (channel and

kinase) suggests multiple mechanisms of regulation, but little is known for rumen Mg transport.

3.3.3. PD-independent (electroneutral) Mg uptake: Surprisingly for the authors, J_{ms} Mg also included a PD-independent transport (Leonhard-Marek and Martens, 1996) and, hence, an intercept at the y-axis according equation 7 (J_m) (fig. 5). Obviously, a PD-dependent and a PD-independent uptake mechanism exist in the apical membrane, and both work in parallel. The PD-independent transport is not influenced by PD_a, and clearly, the charge of Mg is compensated either by counter-transport of a respective cation or by co-transport with an anion.

The suggestion of counter-transport for the neutralization of the charge has been deduced from feeding experiments and the effects of fermentation products. The intake of high levels of readily fermentable carbohydrates (Giduck and Fontenot, 1987) increases Mg digestibility, and the enhancing influence of short chain fatty acids (SCFA) or CO_2 on Mg absorption from the isolated rumen of heifers (Martens et al., 1988) suggests effects of these fermentation products on Mg transport. Corresponding *in vitro* studies have shown a stimulation of J_{ms} Mg by both SCFA and CO_2 at unchanged J_{sm} (Leonhard-Marek et al., 1998). Furthermore, the luminal addition of SCFA does not influence PD_a and, hence, is not the driving force for PD-dependent Mg uptake. Rather, the PD-independent part of J_{ms} is stimulated, and $Mg^{2+}/2H^+$ exchange has been proposed to represent this transport mechanism (Leonhard-Marek et al., 1998). This suggestion has been explained by the uptake of undissociated SCFA and the release protons or the production of protons from CO_2 in the cytosol by the activity of the carboanhydrase. These protons are used for $Mg^{2+}/2H^+$ exchange, which has been described for Mg efflux from mitochondria (Jung and Brierly, 1994) or uptake in rat jejunum (Scharrer and Lutz, 1990).

However, further studies of PD-independent Mg transport have not supported the assumption of a $Mg^{2+}/2H^+$ exchange. Schweigel et al. (2000) have studied, in isolated REC of sheep, Mg uptake und intracellular pH (pH_i) under a variety of conditions. HCO_3^- and butyrate stimulate Mg uptake, but the authors have found no experimental evidence for $Mg^{2+}/2H^+$ exchange and suggest a co-transport of Mg with an anion as HCO_3^- or Cl^- (Schweigel and Martens, 2003). This part of Mg transport requires further studies for clarification, because the activity of the vacuolar H^+-ATPase modulates Mg transport (Schweigel and Martens, 2003).

The PD-independent uptake relies on the chemical gradients of the involved ions. The apical gradient for Mg facilitates Mg uptake, because the ruminal Mg concentration is normally larger than Mg_i. Because uncertainties exist about the involved anion, an exact approximation of the net driving forces for this uptake mechanism is not possible. The molecular characteristic of this Mg transporter is still unclear. In plants, AtMHX is an *Arabidopsis thaliana* tonoplast transporter that can exchange protons with Mg and Zn ions (Gaash et al., 2013) and shows the highest similarity to mammalian Na/Ca exchange (NCX) transporters. Because Mg transport studies in REC do not support an exchange mechanism with protons (Schweigel et al., 2000), molecular identity is awaiting clarification.

3.3.4. Mg transport within the epithelium: The rumen epithelium is a squamous multilayered epithelium, like frog skin. Hence, any transcellular transport of a substance from the apical to the basolateral membrane has to pass from one layer into the other: Uptake occurs across the apical membrane of the Stratum corneum → Stratum granulosum → Stratum spinosum → extrusion across the basolateral membrane of the Stratum basale. Henrikson (1971) proposed, very early on, connections between the cell layers for ruminal transcellular transport of Na. These connections have now been verified by Graham and Simmons (2005).

Plasma membrane connexin 43 has been demonstrated by immunostaining at the stratum granulosum and decreases in intensity through the stratum spinosum and stratum basale. Connexin 43 belongs to the family of gap junction proteins that allow cell-cell coupling. Hexameric structures form channels in the relevant membrane of adjacent cells, and these combine to a low resistance intercellular pathway and to permit the passage of small molecules, second messenger, and electrical signals (Saez et al., 2003). Ions such as Mg or Na are assumed to pass through the connexins (gap junctions) by simple diffusion and to traverse the whole epithelium from the apical to the basolateral membrane. Hence, the rumen epithelium is a functional syncytium and can be simplified to one intracellular transport compartment. Up to now, no evidence exists for a Mg-binding protein like calbindin for Ca (Hoenderop and Bindels, 2007).

3.3.5. Basolateral Mg extrusion: Contrary to passive uptake mechanisms, the extrusion of Mg across the basolateral membranes occurs against a steep electrical gradient of PD_b, which is slightly higher than PD_a. The transport of the Mg ion out of the cell against a positive PD requires energy, and the early observations support this

conclusion. Ouabain almost completely inhibits (Martens and Harmeyer, 1978) and DNP abolishes Mg transport (Martens, 1985) supporting the clear dependence on ATP for this uphill transport. A direct link to Mg extrusion cannot be deduced from these observations, and indirect interaction with the Na/K-ATPase has been assumed. Based on the well-established Ca transport via the Na/Ca exchanger, a similar mechanism has been suggested. The first experimental approach for testing this hypothesis reinforced this model. Reduction of serosal Na reduced J_{ms} Mg (Leonhard-Marek and Martens, 1994), and in REC cells, the release or uptake of Mg depended on the direction of the Na gradient (Schweigel et al., 2000). These Mg fluxes can best be explained by the proposed Na/Mg exchange. This conclusion has further been corroborated by the application of imipramine, an inhibitor of Na/Mg exchange (Schweigel et al., 2000; Leonhard-Marek et al., 2005), which reduces Mg transport. Further, cAMP stimulates Na/Mg exchanger (Leonhard-Marek et al., 2005; Schweigel et al., 2006). The anti-Na/Mg exchanger antibody mAb inhibits Mg extrusion and has detected a 70-kDa immunoreactive band in protein lysate of ovine REC (Schweigel et al., 2006). Western blot experiments with REC have shown that the expression of the Na/Mg exchanger protein is modulated by the Mg concentration of the incubation medium (Schweigel et al., 2008, 2009). The further characterization of Na/Mg exchange in HEK cells has revealed that the human gene SLC41A1 encodes for this Mg transport protein (Kolisek et al., 2012; Schweigel-Röntgen and Kolisek, 2014). It should be mentioned that the proposed Na/Mg exchanger is a well-characterized Mg transporter of membranes in various tissues (Günther and Vormann, 1995) and in mouse model basolateral extrusion of Mg in the intestine was mediated by CNNM4, an electroneutral Na/Mg exchanger (Yamakazi et al., 2013) (CNNM4: Ancient conserved domain protein/cyclin M 4 is an evolutionarily conserved Mg^{2+} transporter).

Therefore, transepithelial Mg transport is accomplished by this type of transporter. The basolateral “downhill” influx of Na down its electrochemical gradient mediates the “uphill” efflux of Mg, and the Na/K-ATPase4 ensures the transport of Na out of the cell maintaining the Na gradient as the driving force. The Na/K-ATPase consequently energizes Mg transport indirectly, and this explains the early observation of the inhibition by ouabain (Martens and Harmeyer, 1978) and DNP (Martens, 1985) (fig. 9). Hence, Mg is transported by a secondary active process, and ruminants are the first

species with a known epithelial Mg transport and its essential site of absorption in the GIT.

The intention of this review is not to include Mg transport in other cells or epithelia. Corresponding data can be found in publications of Flatman (1991), Schlingmann and Gudermann (2005), Hoenderop and Bindels (2007), Bindels (2010), Quamme (2010), and Schweigel-Röntgen and Kolisek (2014).

3.4. Passive paracellular Mg transport.

Under short-circuit conditions, passive J_{ms} equals J_{sm}; these flux rates are assumed to represent the paracelluar and passive transport rates. This assumption is underlined by the effects of PD_t on J_{sm} (fig. 5). J_{sm} Mg exhibits a PD-dependent transport with a very shallow slope and no intercept (fig. 5; equation 7); this is in agreement with the assumption of the passive and paracellular flow of ionized Mg. J_{sm} is in the range of 6 – 10 nmol·cm^{-2}·h^{-1} at 2 mmol·l^{-1} Mg in the serosal solution. On the assumption of a serosal ionized Mg concentration of 0.6 mmol·l^{-1}, J_{sm} is extremely small and approximately 2 – 4 nmol·cm^{-2}·h^{-1}. The permeability for this low passive and paracellular flow of Mg, P_{Mg}, can be calculated according to

$$P_{Mg} = Flux_{Mg}/Mg_{Conc} \quad (8)$$

P_{Mg} = cm·s^{-1}
$Flux_{Mg}$ = nmol·cm^{-2}·h^{-1}
Mg_{Conc} = µmol·cm^{-3}

This backflow corresponds to a Mg permeability of 1 x 10^{-6} cm·s^{-1}, which is almost one hundred times smaller than the permeability for Na in the small intestine of mice (Wada et al., 2013) but within the range of 0.60 x 10^{-6} cm·s^{-1} measured for the paracellular flux in Caco-2 monolayers (Thongon and Krishnamra, 2011). This low passive flow rate limits the passive Mg movement (secretion or absorption), when $PD_t \neq PD_e$ (fig. 2). Notably, all manipulations such as the application of SCFA, CO_2, or a carboanhydrase inhibitor cause large changes in J_{ms} Mg but do not influence J_{sm} (Leonhard-Marek et al., 1998), a finding that again supports the assumption of passive transport. Passive J_{ms} Mg accounts indeed for the total J_{ms} (table 2) but only for 10 % under short-circuit conditions and is probably extremely small and negligible *in vivo*.

4. MODEL OF RUMINAL MAGNESIUM TRANSPORT

4.1. Mechanisms and pathways

The *in vitro* data demonstrate two direct uptake and one efflux mechanisms for ruminal Mg transport; these are schematically shown in figure 9.

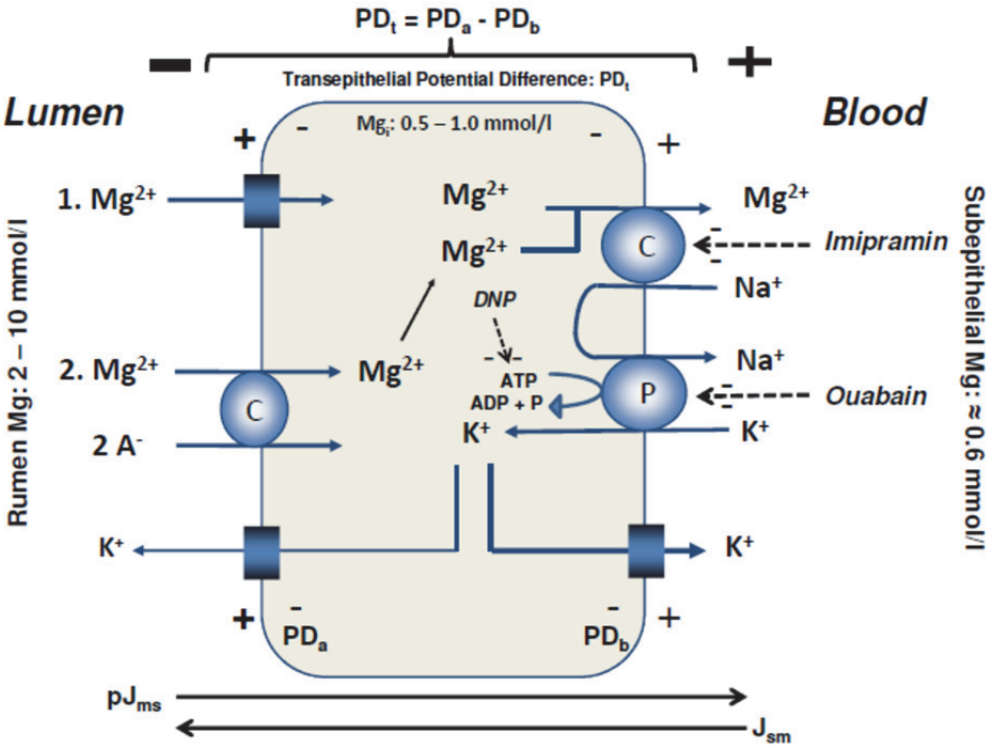


Figure 9: Representation of transepithelial ruminal Mg transport. The multi-layered epithelium is simplified to one compartment. Passive Mg uptake is driven (1) mainly by the potential difference, PD_a, or (2) by the chemical gradient of involved ions. The PD-dependent uptake (1) is thought to be mediated by the epithelial channel TRPM7: transient receptor potential melastatin subtype 7 (see text above for discussion). The molecular identity of PD-independent (2) uptake is unknown. The efflux of the intracellular uptake is mediated via Na/Mg exchange, and the molecular candidate is the *SLC41A1* (solute carrier family 41 member 1) Na/Mg exchanger. Mg_i: intracellular ionized Mg. The negative effects of inhibitors (-) on various steps of Mg transport are printed in *italics.* pJ_{ms} and J_{sm} represent the passive flow through the paracellular pathway. C = carrier; P = Na/K-ATPase (pump). The cylindrical scheme represents a channel.
Example for PD_t (+ 15 mV) = PD_a (- 45 mv) - PD_b (- 60 mV). Depolarization of PD_a by an increase of ruminal K increases PD_t.

Ionized Mg uptake (as an ion through a channel) is driven by the potential difference of the apical membrane, PD_a. This mechanism is called PD-dependent or K-sensitive

Mg uptake, because PD_a is mainly modulated by the ruminal K concentration (Leonhard-Marek and Martens, 1996).

The crucial role of PD_a for transepithelial Mg transport has been shown in the distal collecting tubule (DCT) of the kidney. A mutation of the corresponding K channel (KCNA1) causes autosomal dominant hypomagnesemia. KCNA1 is co-localized with the Mg-transporting TRPM6 in the DCT, and its impaired function causes urinary waste of Mg (Bindels, 2010). In the rumen, PD-dependent Mg transport is thought to be mediated by the epithelial TRPM7: transient receptor potential melastatin subtype 7 (Schweigel et al., 2008; see text above for discussion).

The second uptake mechanism is obviously a co-transport together with an anion, which neutralizes the charge of Mg. The driving force for this uptake is the gradient of the involved ions. Hence, it is called the PD-independent or K-insensitive mechanism. The molecular basis is still unknown, and surprisingly, even in new and recent reviews about Mg transport, an electroneutral uptake mechanism has not been described (Romani, 2011; Schweigel-Röntgen and Kolisek, 2014).

Finally, the basolateral extrusion of Mg is mediated by Na/Mg exchange. Na influx is driven by the Na gradient (extracellular >> intracellular) and used for Mg extrusion. The best molecular candidate is the *SLC41A1* (solute carrier family 41 member 1) Na/Mg exchanger (Kolisek et al., 2012; Schweigel-Röntgen and Kolisek, 2014) although in the intestine the Na/Mg exchanger CNN4 (see above) has been demonstrated.

The evidence for two separate uptake mechanisms was unexpected and immediately raised questions about their physiological significance. Because the rumen is an essential site of absorption for Mg homeostasis, Mg transport must be secured at all Mg concentrations. Therefore, the two uptake mechanisms were proposed to work in parallel and to exhib job sharing: the PD-dependent and K-sensitive mechanism has the capability of *high* affinity and *low* capacity for Mg uptake at low Mg concentrations. The conclusion was derived from early observations of Greene et al. (1983a) in balance experiments with sheep and Care et al. (1984) in studies of Mg absorption from a rumen pouch of sheep. The authors showed that high ruminal K intake reduced Mg absorption to higher extend a low Mg intake (Greene et al., 1983), and high K concentrations in a rumen pouch of sheep depressed Mg absorption, particularly at low Mg concentrations (Care et al., 1984).

An approximation of the driving forces by Martens and Schweigel (2000) supported the proposed function of the PD-dependent Mg transport: “The electrochemical gradient ($\Delta\mu Mg$) for PD-dependent Mg uptake is high and consequently permits Mg uptake even at very low ruminal Mg concentrations. Assuming a PD_a of - 50 mV (intracellular negative), an intracellular Mg concentration of 1 $mmol \cdot l^{-1}$ and a ruminal Mg concentration of 3 $mmol \cdot l^{-1}$, $\Delta\mu Mg$ is 65 mV and constitutes a large driving force of Mg uptake across the luminal membrane. Under this condition the chemical gradient is equivalent to 15 mV and accounts for only 23 % of $\Delta\mu Mg$. Furthermore, the ruminal Mg concentration at equilibrium would be 0.024 $mmol \cdot l^{-1}$ (PD_a – 50 mV and intracellular Mg of 1 $mmol \cdot l^{-1}$). Hence the PD-dependent Mg uptake mechanism would allow Mg uptake at all ruminal Mg concentrations > 0.024 $mmol \cdot l^{-1}$. It is obvious from this example, that the PD-dependent uptake mechanism has the advantage and the capability to absorb Mg from the rumen at low Mg concentrations and consequently, ensures Mg absorption at low Mg intake.”

The PD-dependent Mg uptake correlates with PD_a (Leonhard-Marek and Martens, 1996), and the magnitude of PD_a is mainly determined by the K concentration in the rumen. Consequently, a possible negative effect of K intake will be pronounced at high K and low Mg concentration.

Vice versa, the PD-independent and K-insensitive mechanism has a *high* capacity and *low* affinity at high Mg concentrations. This uptake mechanism relies on the gradients of the involved ions. Because the accompanying anion is not known, a corresponding calculation of $\Delta\mu Mg$ is not possible. However, the driving forces are readily demonstrable to increase on raising the Mg concentration, and the proposed transport functions can be recognized similar to those mentioned above. Table 3 summarized the major characteristics of ruminal Mg transport.

Table 3: Characteristics of Mg transport across the rumen epithelium.

	Passive Luminal Mg Uptake			**Active Mg**
Ions	**Driving Force**	**Properties**	**Nomenclature**	**Extrusion[a]**
Mg^{2+}	PD_a	High affinity Low capacity	PD-dependent K-sensitive	Na/Mg Exchanger
Mg^{2+} + Anions	Chemical Gradient	Low affinity High capacity	PD-independent K-insensitive	Na/Mg Exchanger

a: Extrusion of Mg across the basolateral membrane via Na/Mg exchange is linked to Na/K-ATPase and a secondary active transport.

The proposed properties (table 3) of Mg transport were suggestions about the physiological meaning of the unforeseen two uptake mechanisms (Leonhard-Marek and Martens, 1996).

4.2. Saturation of Mg transport

Mg transport is saturated *in vitro* (Martens et al., 1987a) and in studies *in vivo* with isolated rumen epithelium of sheep (Martens, 1979) and calves (Martens, 1983) or rumen pouch of sheep (Care et al., 1984). This apparent saturation probably includes the combined transport capacities of both uptake mechanisms, because the single properties (K_m and V_{max}) of PD-dependent and PD–independent mechanisms are unknown. However, this saturation has, to the knowledge of the author, never been observed in conventional balance studies. Martens (1981) summarized that Mg absorption occurred before the duodenum in sheep and cows and found a linear correlation between Mg intake and absorption. Weiss (2004) and Schonewille et al. (2008) analyzed Mg intake and digestibility and found, in cows, a linear correlation between Mg intake and apparent (Weiss, 2004) or true Mg absorption (Schonewille et al., 2008) over a wide range of Mg intake. Mg absorption in sheep also increased almost linearly at a rumen concentration up to 9.71 $mmol \cdot l^{-1}$ (Ram et al., 1998), which is significant higher than the observed saturation in studies *in vivo* with the isolated rumen (Martens, 1979) or pouch of sheep (Care et el. 1984).

Two possible reasons can be proposed for the discrepancy. First, the observed saturation under experimental conditions simulated, but very likely did not represent the real *in vivo* conditions. Leonhard-Marek et al. (1988) have demonstrated the stimulation of ruminal Mg transport by CO_2 and SCFA, and the effects of these fermentation products can increase V_{max} under normal *in vivo* conditions. Second, Mg was probably ionized in the experimental model studies (Martens, 1979; Care et al., 1984) and was totally available for transport. Indeed, chelating Mg by EDTA severely depresses Mg transport *in vitro* across the rumen epithelium (Leonhard et al., 1990). Therefore, a significant part of total Mg is probably not ionized in the normal rumen fluid and, hence, is not available for Mg transport (see below ruminal pH).

5. MODULATION OF RUMINAL Mg TRANSPORT

In one of his first publications about tetany in cows, Sjollema (1932) reported the composition of tetany-prone grass, which exhibited high concentrations of K and nitrogen, low concentrations of Na, and moderate levels of Mg. Hence, simple Mg deficiency appeared unlikely to be the major reason for hypomagnesemic tetany, and this disease “does not arise by inadequate intake of Mg” (Head and Rook, 1955). It also occurs after a change of diet from forage and concentrate to young grass when the diet is isomagnesemic (Care et al., 1967), and a decrease of blood Mg was even observed in cows despite an increase of Mg intake from 16 g/d to 23 g/d (Johnson et al., 1988). Today, dietary factors are well known to interfere with Mg absorption, and the changes of grass composition reported by Sjollema (1932) have a physiological background on Mg transport in the gut.

5.1. The classical implications of K

The knowledge about interactions between K and Mg has been developed over many decades and includes four steps.

5.1.1. Early suggestions: The early studies and reports go back to the finding of Sjollema (1932) concerning the high K content of young tetany-prone grass. First observations about possible interactions between K and Mg led to conflicting results. Pearson et al. (1949) and Odell et al. (1952) did not detect alterations of blood Mg in sheep at high K intake. By contrast, Kunkel et al. (1953) measured significant lower blood Mg concentrations in sheep at a high K diet. These contradictory observations are nowadays not surprising in the light of equation (1): As long as Mg influx is larger than efflux including the excretion of surplus in urine, blood Mg varies within the physiological level despite lower influx, even when the influx is reduced by high K intake.

5.1.2. Reduced digestibility: The suggested interaction was substantiated by the studies of Fontenot et al. (1960). High K intake significantly reduced blood Mg and furthermore decreased Mg digestibility and consequently urinary excretion in sheep (Fontenot et al., 1960) and cows (Kemp et al., 1961) (see equation 1). The reduced Mg digestibility was caused by a decrease of absorption and not by an increase of endogenous Mg loss (Newton et al., 1972). This effect of K on the reduced Mg absorption has been demonstrated by many studies in sheep and is summarized by

Fontenot et al. (1973). In general, the results of these experiments in sheep were confirmed by Schonewille et al. (1999) in cows, but the apparent digestibility of Mg appears to be lower in this species. Further, the effect of K appears to be dose-dependent (Greene et al.. 1983a; Rahnema and Fontenot, 1986). A dose response curve between K intake and Mg digestibility has revealed that an increase of K between 1 – 3 % of DM firmly reduces Mg digestibility, with minor effects above this concentration. In agreement with this conclusion are the results in cows of Schonewille et al. (1997a). The authors have not found a correlation between Mg digestibility and K content of the diet within the range of 29 g (2.9 %) to 44 g (4.4 %) K/kg dry matter. Notably, Martens et al. (1988) have observed, in studies with heifers, that the absorption of Mg from the temporarily isolated rumen dramatically decreases between 25 and 75 $mmol \cdot l^{-1}$ K concentration in the artificial rumen fluid, whereas 100 and 120 $mmol \cdot l^{-1}$ K do not further depress Mg absorption.

5.1.3. Site of K effect: A further step for a better understanding of the negative effect of K intake on Mg absorption is represented by the results of Tomas and Potter (1976b). A higher K intake reduced Mg absorption from the forestomachs. This reduction of absorption was not compensated by the small or large intestine. Furthermore, K infusion into the rumen had no effect on Mg solubility (Rahnema and Fontenot, 1986) but depressed Mg absorption, whereas the infusion of K into the abomasum or ileum did not affect Mg absorption (Wylie et al., 1985). Again, the effect of K is also restricted to forestomachs in cows (Greene et al., 1983b).

5.1.4. K and Mg: The effect of concentrations: The alterations of increasing K intake within the rumen are well known (fig. 3). The concentration of K and the PD_t is increased, the absorption of Mg is reduced (Tomas and Potter, 1976b; Greene et al., 1983b, Martens and Blume, 1986), and that of Na is stimulated (Stacy and Warner, 1966; Lang and Martens, 1999). The improved knowledge about the effects of K and the proposed model of Mg transport mechanisms (job sharing table 3; fig. 9) allows conclusions and suggestions that the effect of K depends on both ruminal K *and* Mg concentration.

The inhibition of Mg absorption is pronounced between 1 and 3 % K (see above) and is attenuated at higher K intake and concentrations in the rumen fluid (Greene et al., 1983a; Rahnema and Fontenot, 1986; Martens et al., 1988). The K-concentration-dependent inhibition is in agreement with theoretical estimations of changes driving forces for PD-dependent Mg uptake. Martens and Schweigel (2000) using the linear

correlation between ruminal (log) K and PD_a = 21.3 (log) K – 82.3 (Leonhard-Marek and Martens, 1996) remark: “This relationship leads to the consequence that an increase of the ruminal K from 20 to 60 mmol·l^{-1} have more severe effects on Mg absorption than an increase from 80 to 120 mmol·l^{-1}, although the ΔK is 40 mmol·l^{-1} in both cases. Increasing the ruminal K from 20 to 60 mmol·l^{-1} depolarized PD_a from – 55 to – 44 mV (ΔPD_a = 11 mV). In contrast, an increase of K from 80 to 120 mmol·l^{-1} causes only a decrease of PD_a from – 41 to – 38 mV ((ΔPD_a = 3 mV)“. The change of the driving forces agrees with the statement of Rahnema and Fontenot (1986): “Also it is possible that the effect of increasing K above 3.2 % on Mg absorption may be limited”.

Further, the proposed model of job sharing (table 3) of the two uptake mechanisms also suggests that the effect of K depends on the Mg concentration. The reduction by K must be higher if Mg is mainly transported via the K-sensitive mechanism at low Mg concentration and vice versa. The *in vivo* experimental proof of the Mg-concentration-dependent effect of K was obtained in the Netherlands in balance studies by the group of A. van Klooster. Ram et al. (1998) fed sheep increasing amounts Mg (1.64, 3.13 and 4.66 g/d) at two levels of K intake (10 and 36 g/kg DM). The obtained results exactly confirmed these suggestions (fig. 10).

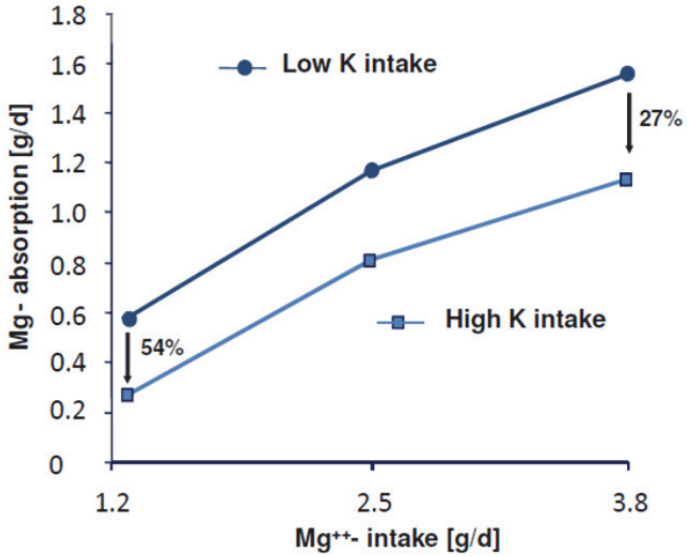


Figure 10: Mg absorption of sheep fed with increasing Mg at two levels of K (1 and 3.6 % DM). The inhibition of Mg absorption is much higher at a high K intake, but the relative effect is diminished at high Mg intake. Redrawn from Ram et al. (1998).

Mg absorption was reduced by 54 % at low Mg intake and by 27 % at high Mg intake. Further, the reduction of Mg absorption was 100 mg/d at all levels of Mg intake thereby supporting the assumption that the PD-dependent and K-sensitive Mg transport was probably saturated at all Mg intakes. The relative depression of Mg absorption was more pronounced at high K and low Mg intake and agreed with the findings of Dalley et al. (1997). Jittakhott et al. (2004) confirmed this effect of K on Mg absorption in cows.

5.2. Meta-analysis of Mg digestibility: reduction by K

The negative effect of K intake on Mg digestibility can be explained qualitatively by the proposed and experimentally established Mg transport model (see figures 9 and 10). The quantity of the effect of K on Mg has been analyzed in a meta-analysis by Weiss (2004) in cows:

Digestible Mg (g/d) = 4.5 (± 4.0) (g/d) + 0.24 (± 0.07) x Mg intake (g/d)
– 4.4 (± 2.2) (g/d) K (9)

K = % K in DM (number without dimension)
± = standard error
39 diets, 162 cows

Two pieces of information are evident from this equation: the effect of K is cancelled out at a level of K of 1 %, and the effect of K is more pronounced at a low Mg intake (table 4). At low Mg and high K intake (3 %), Mg digestibility becomes negative!

Table 4: Mg digestibility of a cow at increasing K content depends on Mg intake. Calculated with equation (9).

Mg Intake (g/d)	K % DM	Mg Absorption (g/d)	Mg Digestibility (%)
30	1	7.3	24.3
30	2	2.9	9.7
30	3	-0.5	-1.7
60	1	14.5	24.3
60	2	10.1	17.6
60	3	5.7	5.7

Hence, the data of the meta-analysis confirmed the Mg transport model and the studies of Ram et al. (1997) (fig. 10).

Schonewille et al. (2008) continued this meta-analysis with a larger number of diets and cows:

Mg true absorption (g/d) = 3.6 (± 0.67)(g/d) + 0.2 (± 0.01) x Mg-intake (g/d) – 0.08 (± 0.014) (g/d) x K (10)
K in g/kg DM[1]
± = standard error
68 diets, 323 cows

True absorption can be transferred to apparent absorption by correction for endogenous Mg secretion (700 kg BW x 4 mg/d = 2.8 g/d):

Mg apparent absorption (g/d) = 3.6 (g/d) – 2.8 (g/d) + 0.2 x Mg-intake (g/d) – 0.08 (g/d) x K (11)
K in g/kg DM[1]

Digestible Mg (g/d) = 0.8 (g/d) + 0.2 x Mg-intake (g/d) – 0.08 (g/d) x K (12)
K in g/kg DM[1]

This equation again shows, as in the meta-analysis of Weiss (2004) that the effect of K is cancelled out at a level of 1 % K in the dry matter. At this K concentration, the apparent Mg digestibility is slightly lower (20 %) than in the calculation of Weiss (2004) (24 %). However, agreement is observed regarding the negative effect of K. Mg digestibility is more depressed at low Mg intake (table 5). However, the effect of K is less pronounced (table 5).

Table 5: Variation of K and Mg intake. Mg digestibility is more reduced at low Mg and high K intake. Calculated with equation (12).

Mg Intake (g/d)	K % DM	Mg Absorption(g/d)	Mg Digestibility (%)
30	1	6.0	20.0
30	2	5.2	17.3
30	3	4.4	14.7
60	1	12.0	20.0
60	2	11.2	18.7
60	3	10.4	17.3

This quantification of the reduction of Mg digestibility by K permits an approach for the calculation of required Mg intake (see below).

The linear reduction of K on Mg digestibility (equations 9 and 12) is in contradiction to the discussed diminished effects of K at a higher intake (Greene et al., 1983a; Rahnema and Fontenot, 1986; Martens et al., 1988). The major reason for this discrepancy is probably the experimental design. The experiments of Greene et al.

[1] K/kg DM as number without dimension: 1 % K/DM = 10

(1983a), Rahnema and Fontenot (1986), and Martens et al. (1988) were performed under identical conditions. Equations 9 and 10 are the result of meta-analyses of many studies. The decrease of Mg absorption from the rumen depends on the K concentration and exhibits a wide variation, even at the same K intake (see compilation of data about K intake und ruminal K concentration in sheep; Schlüsing, 2000). The high standard error of the K coefficient in equations (9) and (10) supports this suggestion.

5.3. The role of Na

Na is the major cation in saliva and rumen fluid, and insufficient Na intake causes reciprocal changes of these two ions: Na decreases, and K increases in both saliva and rumen fluid (Bailey, 1961; Denton, 1956). The reciprocal changes are caused by the secretion of aldosterone at low Na intake and mediates a 1 : 1 exchange between Na (out) and K (in) in saliva (Traysen and Tarding (1974). Indeed, Martens et al. (1987b) fed artificial young grass with insufficient Na content (0.025 % of dry matter) to sheep and observed a decrease of Na in saliva and rumen fluid, an increase of K in both liquids, and an enhanced PD_t of the rumen epithelium. Mg absorption from the rumen decreased. All these changes were abolished by repletion of Na. Hence, Na deficiency causes the same alterations as K intake (table 6), as clearly shown by a study of Charlton and Armstrong (1989). Aldosterone intravenous infusion causes, within 4 h, an increase of K and a decrease of Na concentration in the rumen fluid. Mg concentration was raised because of impaired absorption by high K, and blood Mg declines (Charlton and Armstrong, 1989). However, aldosterone does not change Mg absorption from the rumen of sheep (Martens and Hammer, 1981).

Of note, the replacement of Na by Li in studies concerning Mg absorption from the isolated rumen in sheep did not alter Mg transport (Martens and Blume, 1986). Rumen Na concentration was not related to Mg absorption.

Table 6: Na deficiency and K intake change the same rumen parameter and have identical effects on Mg absorption. ↑: Increase; ↓: Decrease

	RUMEN			
	K $mmol \cdot l^{-1}$	**Na** $mmol \cdot l^{-1}$	**PD_t** mV	**Mg Absorption** g/h
K Intake	↑	↓	↑	↓
Na Deficiency	↑	↓	↑	↓

The risk of the disturbed Mg absorption during Na deficiency can easily be learnt from an estimation of saliva flow and the load of K to the rumen. A K concentration in saliva of 100 $mmol \cdot l^{-1}$ at Na deficiency and a daily flow rate of 200 l/d in high-producing cows lead to a total influx of K of some 780 g/d K, which is equal to a dry matter intake (DMI) of 26 kg with 3 % K in the DM. Hence, severe Na deficiency is a significant risk for reduced Mg absorption, because the flow of K into the rumen is as high as at high K intake. The possible danger is easily overlooked, because overt clinic signs of Na deficiency are missing. Furthermore, the large Na pool in the rumen at 200 g Na can be mobilized and can cover deficiency for a long time (Kemp and Geurink, 1966). However, this ruminal Na buffer capacity is markedly reduced at high K intake, because K concentration in the rumen fluid increases and stimulates Na absorption from the rumen (Scott, 1967; Lang and Martens, 1999). The absorbed Na cannot be stored and is rapidly excreted via urine (Dobson et al., 1966).

Young spring grass contains, in many cases, extremely low concentrations of Na that do not cover the requirement of grazing ruminants (Morris and Gartner, 1975) and particularly not of lactating cows (Kemp and Geurink, 1978). Notably, Sjollema (1932) mentioned Na deficiency in tetany-prone grass. Moreover, it was suggested as a risk factor as early as 1968 by Metson et al.: “If low sodium is confirmed as yet another stress factor in the development of hypomagenesaemia most of the present analyses (grass - the author) would undoubtedly qualify as tetany prone”. This suggestion is in agreement with the observation of Butler (1963) who found a negative relationship between low Na content of grass (Na deficiency – the author) and the incidence of tetany. Vice versa, grass tetany was prevented by supplementation of cows with NaCl (Paterson and Crichton, 1960).

These observations and suggestions can now be explained by the improved understand of alterations caused by Na deficiency (table 6; Martens et al., 1987b). Furthermore, modern milk production relies on supplementation of concentrate with adequate amounts of Na and Mg, and consequently, Na deficiency is avoided. However, in countries such as Ireland and New Zealand, where milk is mainly produced by grazing cows, sufficient Na supplementation is urgently required. Further, the classical risk of grass tetany is still given in beef cattle if they are suddenly moved from barn to young grassland in spring without sufficient supplementation (see below).

5.4. Nitrogen and ammonia

Tetany-prone young grass in spring exhibits a high concentration of crude protein (Sjollema, 1932), which is associated with the incidence of grass tetany (Kemp, 1960). The high crude protein content immediately causes a rapid increase of up to some 70 $mmol \cdot l^{-1}$ ruminal ammonia[2] on turning out sheep to grass (Annison et al., 1959). Various assumptions for the association between ammonia and tetany have been discussed as the toxicity of ammonia (Meyer and Scholz, 1973), formation of the complex magnesium-ammonium-phosphate (Wilcox and Hoff, 1974), or disturbed Mg absorption by ammonia (Head and Rook, 1955). Relationships between ammonia and Mg absorption have been tested in many experiments with contradictory results. Two reactions have been reported: the inhibition of Mg absorption and no effect on Mg digestibility by high ruminal ammonia. The obvious discrepancy has led to the suggestion that the experimental conditions might have determined the results. The negative effect of ammonia is time.dependent and is compensated by adaptation.

Indeed, sudden and acute increases of ruminal NH_4^+ concentrations decrease Mg absorption. After an intraruminal application of large amounts of ammonium acetate in cows, Head and Rook (1955) observed a decrease of blood Mg concentration and urinary Mg excretion. Martens and Rayssiguier (1980) found in studies with sheep and Martens et al. (1988) detected in young heifers that the absorption of Mg from the temporarily isolated rumen was severely reduced with increasing NH_4^+ concentrations from 0 – 40 $mmol \cdot l^{-1}$. This decrease agrees with the observation of Care et al. (1984) in studies with a rumen pouch in sheep. Mg absorption was decreased by ammonia in acute experiments, and this effect was additive to the known depression of K.

However, alterations of Mg metabolism were not observed in chronic experiments with a delay in sampling after raising ruminal NH_4^+ concentrations by two levels of crude protein or non-protein-nitrogen (urea) (Moore et al., 1972). Fontenot et al. (1973) further observed no effect of high protein intake or high ruminal NH_4^+ concentration on apparent Mg digestibility or urinary Mg excretion in conventional balance studies. These experiments were conducted after a pre-feeding period of two

[2]: The term ammonia is used without discrimination between NH_3 and NH_4^+. Chemical symbols are used when a specification is required.

or three weeks (Fontenot et al., 1973) or with ruminal NH_4^+ concentration being raised very slowly (Wilson, 1963).

These observations led to the hypothesis that an acute increase of ruminal NH_4^+ reduces Mg absorption and that chronic elevation of ruminal NH_4^+ induces adaptation to this fermentation product. Gäbel and Martens (1986) tested this hypothesis in studies with sheep und performed two series of experiments. In an acute experiment with the washed rumen of sheep, 40 $mmol \cdot l^{-1}$ NH_4^+ significantly reduced Mg absorption from 1.55 mmol/h to 1.05 mmol/h (fig. 11) and increased PD_t from + 25.4 mV to + 29.8 mV.

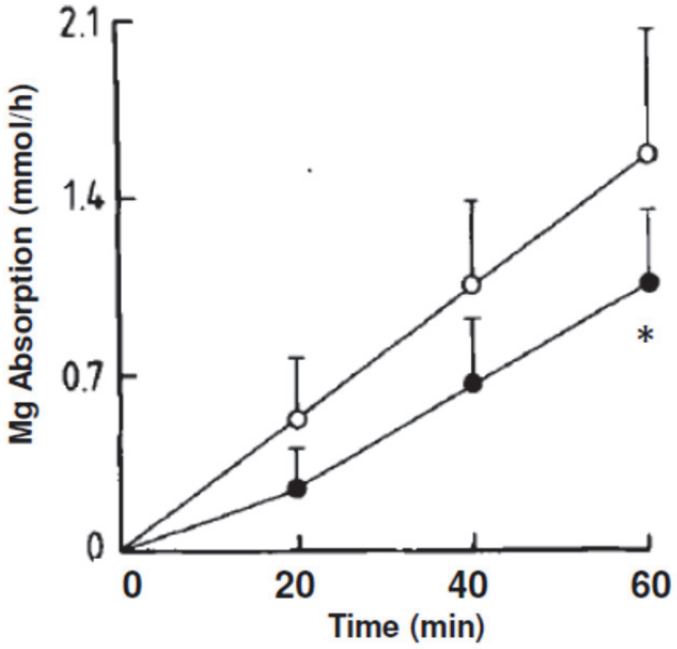


Figure 11: Mg absorption from the rumen of sheep without (O) or with 40 $mmol \cdot l^{-1}$ ammonia. Ammonia significantly reduced Mg absorption from the isolated rumen in an acute experiment with sheep (from Gäbel and Martens, 1986).

In a second experiment, ruminal NH_4^+ was suddenly increased from 4.81 ± 0.18 to 47.9 ± 0.3.1 $mmol \cdot l^{-1}$ within 1 day by intraruminal infusion of urea. Mg excretion in urine transiently decreased from 385 mg/d to 255 mg/d over two days after the start of urea infusion, but at the 3rd day, urinary Mg increased and was not significant different at the 4th day from control, despite elevated ruminal NH_4^+ (36.1 ± 4.8 $mmol \cdot l^{-1}$) (fig. 12). These results are in agreement with the proposed working hypothesis and lead to the conclusion that a sudden change in nitrogen intake and NH_4^+ concentration should be avoided (see Prophylaxis).

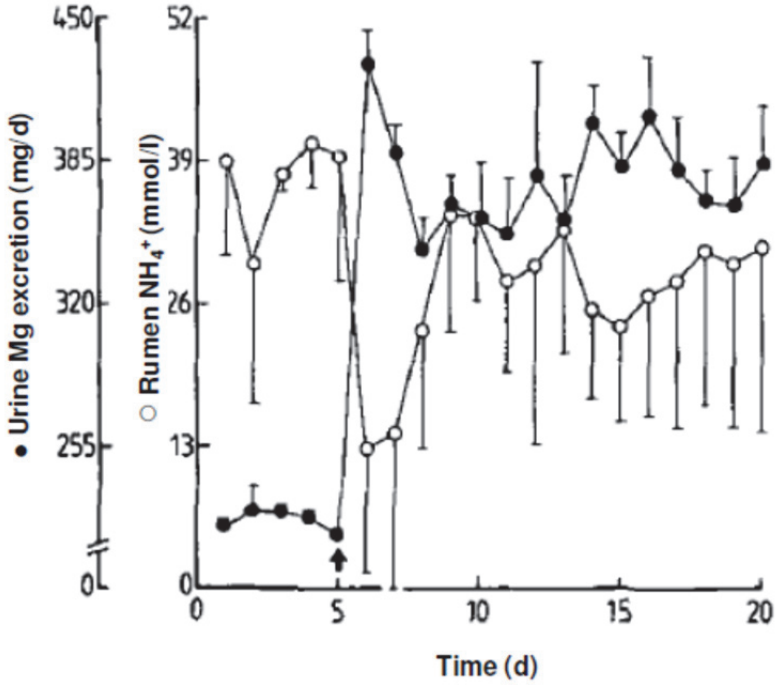


Figure 12: Time course of the rumen ammonia (●) concentration in the rumen fluid and Mg excretion in urine (O). Rumen ammonia concentration was raised by urea infusion into the rumen (↑). (from Gäbel and Martens, 1986).

The reason(s) for the temporary depressive effect of NH_4^+ on Mg absorption have not been studied. However, ruminal transport of ammonia causes distinct epithelial alterations and suggests interactions with Mg transport. Ammonia is transported across the rumen epithelium as NH_3 and NH_4^+ depending on the pH (Abdoun et al., 2005). At a pH of < 7.0, NH_4^+ is predominantly transported across a cation channel in the apical membrane (Abdoun et al., 2005); it decreases PD_a by a few mV (Lu et al., 2014) and increases PD_t (Gäbel and Martens, 1986). In the cytosol, NH_4^+ will dissociate, and the released proton decreases the pH_i (Abdoun et al., 2010), which is immediately recycled via the Na/H exchanger (NHE) and stimulates Na transport (Abdoun et al., 2005).

These intraepithelial alterations of PD_a and pH_i, offer some suggestions concerning possible effects of NH_4^+. The small effect on PD_a is unlikely to have a major influence on Mg uptake (ΔPD_t of some 1 mV by an increase of 10 mmol·l^{-1} ammonia; Gäbel and Martens, 1986). Direct interactions between pH_i and PD-independent Mg transport have not been reported, but urea feeding and enhanced NH_4^+ uptake activate NHE activity in sheep rumen epithelium (Abdoun et al., 2003). Because a feeding-dependent increase of NHE activity has been observed within a few days

(Etschmann et al., 2009), the observed adaptation to high ruminal ammonia (Gäbel and Martens, 1986) hints at possible direct or indirect effects of NHE activity and pH_i on presumably PD-independent Mg transport.

5.5. Ruminal pH

The presence of Mg in solution in the ruminal fluid is a precondition for Mg transport as an ion, because chelating Mg by EDTA reduces Mg transport (Leonhard-Marek et al., 1990). The range of soluble forms of ruminal Mg varies from 34 % to 77 % (Storry, 1961a; Grace et al., 1977; Rahnema and Fontenot, 1986; Grace et al., 1988; Dalley et al., 1997), depends on the diet (Johnson et al., 1988; Johnson and Jones, 1989; Dalley et al., 1997), and the size of supplemented MgO (Xin et al., 1989). However, the major determinant factor of Mg solubility in the rumen is the pH (Dalley et al., 1997). The curvilinear relationship between rumen pH and Mg solubility exhibits a steep slope between pH 5 and 7 (fig. 13), which varies with diet (Horn and Smith, 1978; Johnson and Jones, 1989; Dalley et al., 1997)

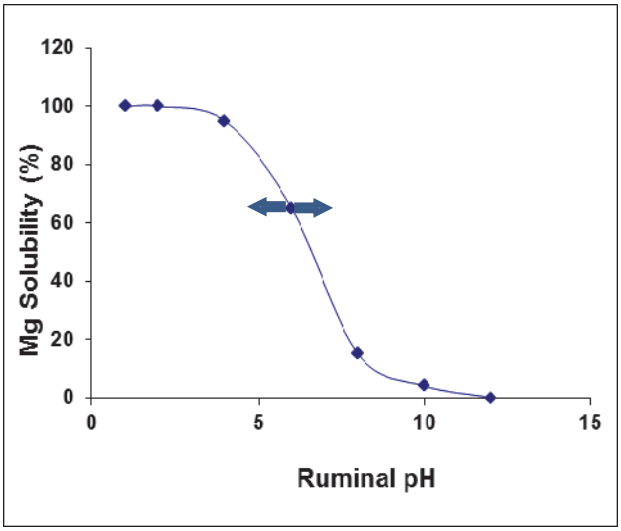


Figure 13: Scheme of Mg solubility in rumen fluid (redrawn from Dalley et al., 1997). The slope of Mg solubility between pH 5 and 7 is influenced by diet (symbol arrow).

A potential effect of ruminal pH for Mg digestibility was suggested early on by Wilcox & Hoff (1974), and a probable mechanism to explain such an association might be the reduced solubility of Mg in rumen content at higher pH values (fig. 13). Horn and Smith (1978) found a close and negative relationship between rumen pH and Mg absorption before the duodenum, and Johnson and Jones (1989) noted a correlation

between ruminal pH and apparent availability of Mg. A causal relationship cannot be deduced from these studies, but they clearly show the significance of pH and Mg solubility in the rumen with regard to Mg absorption. Furthermore, the putative Mg channel (TRPM7) exhibits a strong relationship to pH and is activated a pH of < 7.0 in patch clamp studies (Li et al., 2007).

The possible role of ruminal pH in the etiology of grass tetany is not clear, because both a higher (Horn and Smith, 1978; Johnson and Jones, 1989) or lower pH (Balch and Rowland, 1957; Johnson et al., 1988) has been reported in sheep or cows on a grass diet. However, the close correlation between ruminal pH and Mg absorption before the duodenum (Horn and Smith, 1978) and ruminal K (Na:K ratio) plus pH and Mg availability (Johnson and Jones, 1989) suggests a physiological significance, which could become significant in cold weather, or as a consequence of subclinical low Mg blood and at low dry matter intake.

5.6. Mg Absorption and fermentable carbohydrates (FC)

A reduced content of FC in tetany-prone grass has been suggested to be able to decrease Mg availability (Metson et al., 1966). Vice versa, the drenching of grazing dairy cattle with a starch solution increased blood Mg concentration (Wilson et al., 1969) and the apparent digestibility of Mg (Giduck and Fontenot, 1987; Giduck et al., 1987) but did not consistently improve Mg absorption (House and Mayland, 1976; Madsen et al., 1976). In the ruminal fluid, the addition of FC causes (a) an increase in the concentration of SCFA (Giduck et al., 1988), (b) a decrease in pH (Giduck et al., 1988), which (c) enhances Mg solubility (Dalley et al., 1997), (d) a decrease in NH_4^+ concentration, and (e) an increase of the number and size of rumen papilla (see review Martens et al., 2012) and hence the absorptive area for Mg (Gäbel et al., 1987). Therefore, an improvement of apparent Mg digestibility by the addition of FC is not surprising, but the underlying mechanisms cannot be deduced from conventional balance studies because of the simultaneous changes of parameters mentioned above. The exact mechanism of the stimulation of Mg transport by SCFA or HCO_3^-/CO_2 is not clear (Leonhard-Marek, 1999), but the PD-independent Mg transport is probably enhanced, because Schonewille et al. (1997b) have demonstrated, in studies with goats, that the depressive effect of K can be compensated by the addition of FC.

5.7. Mg intake, blood Mg, and Mg digestibility

The meta-analyses of Weiss (2004) and Schonewille et al. (2008) have demonstrated a linear correlation between Mg intake and digestibility suggesting a constant rate of Mg absorption according intake and no adaptation. However, McAlleese al. (1961) orally dosed ^{28}Mg in sheep and observed a higher ^{28}Mg absorption at deficient Mg intake. In line with this finding are the results of Schweigel et al. (2008, 2009): incubation of isolated rumen epithelial cells in a low or high Mg medium did not change the intracellular Mg concentration, but caused a corresponding increase or decrease of in- and efflux mechanisms of Mg. The expression of Na/Mg exchanger and the Mg channel MagT1 was increased at low Mg incubation and vice versa. In contrast, pre-incubation in media with diverse Mg concentrations only slightly increased the expression of TRPM7, but the activity of this channel changed with Mg concentration (Schweigel et al., 2008). Both experiments support the assumption of the adaptation of Mg transport at low Mg: uptake mainly by TRPM7 and efflux via imipramine sensitive Na/Mg exchanger.

Furthermore, Allsop and Rook (1979) have suggested a suppressed Mg absorption from the gut at high blood Mg concentration after increasing blood Mg by intravenous infusion. As a probable explanation, they conclude “that at high plasma magnesium concentration, the absorption from the gut is depressed” and “If plasma magnesium does affect the absorption of dietary magnesium, the most probable major site of action is therefore on the uptake of magnesium from the reticulorumen”.

Martens and Stössel (1988) have tested this hypothesis and measured the Mg absorption from the isolated rumen in sheep at various plasma magnesium concentrations. The plasma magnesium concentration was acutely raised from 0.89 ± 0.11 to 1.97 ± 0.27 $mmol \cdot l^{-1}$ by intravenous infusion of $MgCl_2$ immediately before the measurement of magnesium absorption or 20 h after the beginning of a continuous intravenous infusion of magnesium, which led to hypermagnesemic levels of 2.78 ± 0.59 $mmol \cdot l^{-1}$. Neither acute nor delayed hypermagnesemia changed the net absorption of magnesium. Furthermore, Mg absorption from the rumen was not changed, even after 5 weeks of hypomagnesemia, when the blood Mg concentration was decreased by a Mg-deficient diet from 0.96 ± 0.06 to 0.50 ± 0.06 $mmol \cdot l^{-1}$. Obviously, the active transport of magnesium from the rumen was not influenced by the plasma magnesium concentration or by the deficient Mg intake, and the passive

paracellular flow did not contribute to net absorption, despite highly different Mg gradients (blood 0.50 – 2.78 mmol·l^{-1}). Notably, the blood Mg concentration of Mg-deficient sheep increased when the Mg absorption from the isolated rumen was studied (Martens and Stössel, 1988).

The results of McAlleese et al. (1961) concerning enhanced Mg digestibility at Mg deficiency can probably be explained by the method used. Sheep on a Mg-deficient diet were orally dosed with ^{28}Mg, and the appearance of the isotope in blood was taken as Mg absorption. The specific radioactivity of ^{28}Mg was probably much higher in the rumen of Mg-deficient sheep and consequently more ^{28}Mg (not Mg) was absorbed compared with control sheep on a normal Mg intake and lower ruminal specific radioactivity. However, absorption from the rumen was not known at that time.

Allsop and Rook (1979) suggested the reticulorumen as the site of adaptation (see above), but in earlier studies in sheep on an artificial “Mg-free” diet, fecal Mg excretion was related to the blood Mg concentration, and this positive correlation was explained by endogenous secretion (Allsop and Rook, 1970). This assumption appears to be likely, because Care (1960) observed a decrease of Mg in the bile of hypomagnesemic sheep. Because Mg is secreted into the gut in various fluids such as saliva, gastric and pancreatic juice, and bile (Storry, 1961b), a correlation with blood Mg seems to be a probable explanation and agrees with the conclusion of Martens (1981) who suggested, from data of the literature, a linear correlation between blood Mg and secretion into the small intestine.

Hence, active Mg transport in the rumen remains unchanged at various blood Mg concentrations *in vivo* and also in sheep on deficient Mg intake for 5 weeks. Endogenous secretion is obviously not constant, probably depends on blood Mg (see below), and affects Mg digestibility to a small extent. The results of Schweigel et al. (2008; 2009) were obtained after incubation of rumen epithelial cells after 24 h incubation in a medium with low or high Mg. The results clearly show that the Mg transporting proteins (TRPM7, MagT1 and Na/Mg exchanger) are sensitive to Mg concentrations, but should be further characterized regarding their *in vivo* significance.

5.8. Endogenous Mg secretion

The amount of Mg in the extracellular space is relatively small (see figure 1). On the assumption of a blood Mg concentration of 1 mmol·l^{-1} and a blood volume of 5 % of

BW (700 kg), total blood Mg accounts for some 840 mg. The ultrafiltrable form of blood Mg (60 %) equilibrates with the extracellular fluid, and the Mg content of this space (15 % of body weight) is some 2500 mg Mg (calculation according to Storry 1961a). Hence, the total Mg content of the ECS is 3 – 4 g. This relatively small Mg pool is readily challenge by endogenous secretion into the gut. Storry (1961a) has made an estimation of Mg secretion in various secretions of sheep (saliva, gastric juice, bile etc.) and estimated a total daily secretion of 8 $mmol \cdot d^{-1}$ (192 $mg \cdot d^{-1}$) in a 40-kg sheep or 4.8 $mg \cdot kg^{-1}$. This amount is higher than the endogenous secretion of 0.4 - 1.4 $mg \cdot kg^{-1}$ in sheep on an artificial diet (Allsop and Rook, 1979), but within the range of 3.4 (Field, 1959) or 5.04 $mg \cdot kg^{-1}$ (Care, 1960). Dua and Care (1995) have pointed out that Mg in saliva significantly contributes to this amount, and approximately 40 % of the Mg in the ECS of sheep is secreted daily.

Schonewille and Beynen (2005) have summarized data from cows (range from 1.5 – 6.0 $mg \cdot kg^{-1}$) and proposed 4 $mg \cdot kg^{-1}$ for this species, a value that is also used by the (German) Gesellschaft für Ernährungsphysiologie (GfE 1978). Hence, the recommended amount of endogenous Mg is within the range of determined secretion and probably represents a realistic value. Possible small variations of endogenous secretion, as mentioned above, appear to be too small to influence total absorption, particularly at high Mg intake and low digestibility.

5.9. Animal breeds and Mg absorption

The digestibility of Mg in cows is influenced by the animal breed (Greene et al., 1989), and Mg absorption from the rumen of sheep of different breeds exhibit differences (Leonhard-Marek et al., 1998). Greene et al. (1989) have shown that Mg absorption is greater in Brahman than in Jersey, Holstein, and Hereford cows. Leonhard-Marek et al. (1998) measured the net Mg transport *in vitro* across isolated rumen epithelium of four species of sheep (Merino, Schwarzkopf, Skudde, and Heidschnukke) all of which had been kept on the same diet for 3 weeks before the experiment. Skudde transported significantly less Mg under short-circuit conditions. Considering the different breeding programs of these sheep, Heidschnucke and Skudde sheep had probably been kept under harsh conditions and fed with diets low in Mg over several generations, whereas the Merino and Schwarzkopf sheep would have been supplied with diets high in energy, protein, and minerals for fast meat production. Obviously, the different Mg intakes in previous generations did not influence the Mg transport

systems, indicating that the genetic information for the Mg transport proteins has been kept constant for a long time. The wide variation of Mg digestibility might also have a genetic background, but to an unknown extent, despite the discussed factors (see above).

The significance of genetic information involving Mg transport proteins has been shown in man. Schlingmann and Gudermann T (2005) and Hoenderop and Bindels (2007) have documented the mutation of TRPM channels and its pathophysiological consequences on Mg transport in intestine and kidney and on Mg homeostasis. Corresponding data in cattle are rare, but a report of an autosomal recessive renal disorder in Japanese Black cattle has shown a lack of claudin-16 protein leading to interstitial nephritis and finally death within a few months after birth, because of failures in both glomerular filtration and tubular absorption (Hirano et al., 2000).

5.10. Vitamin D and Mg

PTH and vitamin D_3 are the principal regulators of Ca metabolism. Vitamin D_3 is metabolized in the liver to 25(OH) D_3 (25 hydroxycholechalciferol) and 25(OH) D_3 in the kidney to 1,25$(OH)_2D_3$ (dihydroxycholechalciferol or calcitriol), which together with PTH represent the signal cascade for the regulation of blood Ca by increased absorption from the intestine, decreased excretion via urine, and by bone formation or resorption (Martin-Tereso and Martens, 2014). Interactions between PTH and calcitriol and Mg are well established (Schneider et al., 1985; Goff et al., 1986; Moate et al., 1987; Care et al., 1989). However, the results are, in some cases, contradictory.

5.10.1. Calcitriol and epithelial Mg transport: In an early study, Schneider et al. (1985) observed, in sheep, after treatment with calcitriol and Levine et al. (1980) in vitamin D deficient rats augmented Mg absorption. This positive effect was also observed by Beardsworth (1987). Calcitriol increased Mg absorption from the rumen in sheep (Beardsworth, 1987). However, the application of calcitriol to cattle did not change fecal excretion of Mg suggesting no altered Mg digestibility (Moate et al., 1987) which agrees with more recent data of Lameris et al. (2015) in mice. Mg transport was not altered by vitamin D (Lameris et al., 2015).

The effect of 1,25$(OH)_2D_3$ on Mg transport in the kidney is controversial (see literature in Ritchie et al., 2001), but in isolated mouse, distal convoluted tubule (MDCT) cell line, treatment with 1,25$(OH)_2D_3$ caused an increased uptake of Mg, which was modulated by the Ca concentration.

5.10.2. Calcitriol and systemic effects: Moate et al. (1987) studied the effects of $1,25(OH)_2D_3$ on Mg metabolism in cows. They daily injected 25 ng/kg into cows for ten days and measured Mg in blood, milk, urine, and feces during days 6 - 10. Calcitriol increased blood Ca as expected but significantly decreased Mg. Mg in feces was not influenced, although a numerical decrease of Mg in urine (not significant) was observed. Mg in milk was not changed. The decline of blood Mg is in agreement with other studies (Sansom et al., 1976; Yano et al., 1984), but the mechanism is not clear. An uptake of Mg into soft tissue has been suggested (Yano et al., 1984, Moate et al., 1987). By contrast, Schneider et al. (1985) have observed an increase of blood Mg concentration in hypomagnesemic sheep and Lameris et al. (2015) in mice.

5.10.3. PTH and Mg: In the study of Goff et al. (1986), the infusion of bovine PTH in cows caused an increase of $1,25(OH)_2D_3$, Ca, and Mg in blood and a decrease of Mg in urine. This information indicates that PTH enhances Mg resorption in the kidney, an effect that disappears upon withdrawal of the PTH infusion.

All these data clearly show significant interactions between the PTH and $1,25(OH)_2D_3$ axis and Mg metabolism. However, the physiological significance of this interaction is not clear. The effect of $1,25(OH)_2D_3$ on epithelial Ca transport is related to the activation of TRPV5 and TRPV6 channels, which are Ca channels, although not absolutely specific for Ca (Hoenderop and Bindels, 2007). Mg transport appears to be possible under some circumstances. Hence, the stimulation of Mg transport by $1,25(OH)_2D_3$ should be considered as a side effect and not an effect directed to the regulation of Mg homeostasis. PTH and $1,25(OH)_2D_3$ are indeed probably not related to the pathogenesis of hypomagnesemia. The modulation of Mg transport and excretion in the kidney by PTH thus deserves further attention.

6. URINARY Mg EXCRETION VIA KIDNEYS

Sufficient Mg influx (absorption) is a precondition for Mg homeostasis with renal excretion (efflux of surplus Mg) being a precondition for the regulation of physiological blood concentration (fig. 1). Mg absorption from the rumen is to the best knowledge of the author, is not regulated according to requirement (see above) and hence, Mg influx rarely equals Mg efflux. Additional mechanisms are necessary for adjustment of possible differences. This fine tuning is controlled by the kidneys and renal handling of Mg is, in addition to absorption from the rumen the second major component of Mg homeostasis.

The kidneys compensate the difference between influx > efflux and are responsible for Mg balance. However, kidneys can only effectively compensate for Mg surplus. If Mg influx is lower than Mg efflux then urinary Mg excretion becomes extremely small (< 1.0 $mmol \cdot l^{-1}$) and hypomagnesaemia might occur. Hence, the acute adjustment of renal Mg transport permits the re-absorption of almost all filtered Mg (Rook and Balch, 1958) or a rapid excretion of Mg surplus (Schonewille et al., 2000). The adaptation of renal handling to actual Mg requirement (influx ≠ efflux) includes two steps: filtration and re-absorption according to actual requirement.

6.1. Mg Filtration

Plasma Mg varies from 0.8 – 1.2 $mmol \cdot l^{-1}$. Approximately 40 % is bound to albumin and globulin and some 60 % is ultrafiltrable. Hence, 0.48 - 0.72 $mmol \cdot l^{-1}$ Mg are the concentration of in the urine of the glomerular filtration rate (GFR) is 0.48 – 0.72 $mmol \cdot l^{-1}$, which accounts for some 22 – 32 g/d filtered Mg in a cow of 650 kg BW (calculated with GFR data from Murayama et al., 2013). The GFR of cows is influenced by high Na intake (Bailey, 1978), but not by protein in the diet (Bailey, 1978; Maltz and Silanikove, 1996) or, by age (Deetz et al., 1982) and possible effects on Mg filtration are not known.

6.2. Re-Absorption of Mg

Most of the filtered Mg (22 – 32 g/d) is not excreted with urine, although excretion higher than filtered load has been observed under experimental conditions (Carney et al.,1980). Three mechanisms have been described for the re-absorption of Mg in the nephron (fig. 14):

6.2.1. Proximal tubule: In the proximal tubule 20% to 30% of the filtered Mg is reabsorbed, probably passively, together with paracellular flow of water and this transport is probably not regulated. The transport of water (some 60%) exceeds the transport of Mg in the proximal tubule and consequently, the Mg concentration is higher at the end of the proximal tubule (fig. 14).

6.2.2. Ascending limb of Henle: Most Mg (60%–70%) is reclaimed in the thick ascending limb of Henle (TAL) (fig. 14). The paracellular and passive transport in TAL is mainly driven by the transepithelial potential difference (lumen positive) and mediated by the tight junctional channel protein, claudin-16 (paracellin-1), which has been postulated to interact with claudin-19 to form a cation-selective channel (Günzel and Yu, 2013; Konrad et al., 2006; Hoenderop and Bindels, 2007; Hou et al. 2013). Mg transport in TAL is stimulated by PTH (Sharegi and Agus, 1982) as has been found *in vivo* in cows (Goff et al., 1986). The passive transport across this pathway is regulated by Mg availability (Efrati et al., 2010). Hypomagnesaemia increases both claudin-16 protein and mRNA abundance and Mg loaded animals (mice) down-regulated claudin-16 (Efrati et al., 2010). Further studies of this group have shown that the expression of the claudin-16 channel is inhibited by calcitriol by a mechanism sensitive to the Ca receptor (CaSR) (Kladnitsky et al., in press). Because Mg transport across claudin-16 is further influenced by a variety of hormones such as glucagon, insulin, calcitonin, vasopressin or isoproterenol (Quamme, 1997) a systemic estimate of these effects and their actual physiological significance *in vivo* is difficult to evaluate. In line with the effects of these hormones is the observation of Ikari et al. (2006) that the regulation of claudin-16 is mediated by the cAMP/PKA-dependent signal cascade.

Furthermore, Ca transport via claudin-16 is reduced by Mg (Ikari et al., 2004) and might be the reason for interactions of Ca and Mg in TAL: “A competitive transport of Mg and Ca via the common paracellular route in TAL could explain the coupling between Mg and Ca excretion” (Ferrè et al., 2012) (see below).

The remarkable meaning of claudin-16 for Mg homeostasis has been learnt from mutation in man. Magnesium homeostasis is severely impaired by a mutation of the claudin-16 gene (Simon et al., 1999; Kausalaya et al., 2006; San-Cristobal et al., 2010). Patients with this autosomal recessive disorder suffer from hypomagnesaemia, hypermagnesuria and hypercalciuria. A similar genetic disorder has been reported in Japanese black cattle and is caused by the homozygous

deletion (not mutation) of the claudin-16 gene (Ohba et al. 2000; Hirano et al., 2000). Disturbances of blood Mg in these cattle have not been reported (Ohba et al., 2000), but renal Mg clearance and reabsorption are significant lower in affected cattle (Ohba et al., 2002). The general lack of claudin-16 in cattle leads to interstitial nephritis, cystically dilated tubules, atrophy, sclerosis and hence renal failure (Kobayashi et al., 2000).

6.2.3. Distal tubule: Approximately 5 – 10 % of the filtered Mg is reabsorbed in the distal convoluted tubule (DCT) by an active transport mechanism (fig. 14) and Mg absorption in this part of the nephron finally controls the Mg concentration in the blood. Luminal Mg uptake is mediated by TRPM6 and the major driving force for this uptake is PD_a (San-Cristobal et al., 2010). Renal TRPM6 is regulated by epidermal growth factor (EGF) which is considered as the first autocrine/paracrine magnesiotropic hormone (Groenestege et al., 2007). Magnesium deficit increases the mRNA and for protein in kidneys of mice (Groenestege et al., 2006) like claudin-16 in TAL (Efrati et al., 2010). Neither PTH nor $1,25(OH)_2D_3$ stimulated TRPM6 expression in the kidney (Groenestege et al., 2006). Interestingly, TRPM6 expression is influenced by the acid-base status of the animal. Metabolic acidosis decreases renal TRPM6 expression and increases Mg excretion, whereas metabolic alkalosis led to the opposite effects (Nijenhuis et al., 2006). However, a pH of < 7.00 activates TRPM6 in patch clamp studies (Li et al., 2006). Activation of TRPM6 by pH (Li et al., 2007) and the increased urinary Mg excretion at metabolic acidosis appears only partly to be compensated by the reduced expression of TRPM6.

The tight control of Mg transport by TRPM6 has led to the conclusion that TRPM6 functions as a gatekeeper of Mg handling in the kidney. This transport mechanism is likely to limit Mg excretion and determines the low renal threshold of Mg.

The efflux mechanism across the basolateral membrane is still uncertain, although such a mechanism (Na/Mg exchanger CNNM4) has been demonstrated in the intestine (Yamazaki et al., 2013)

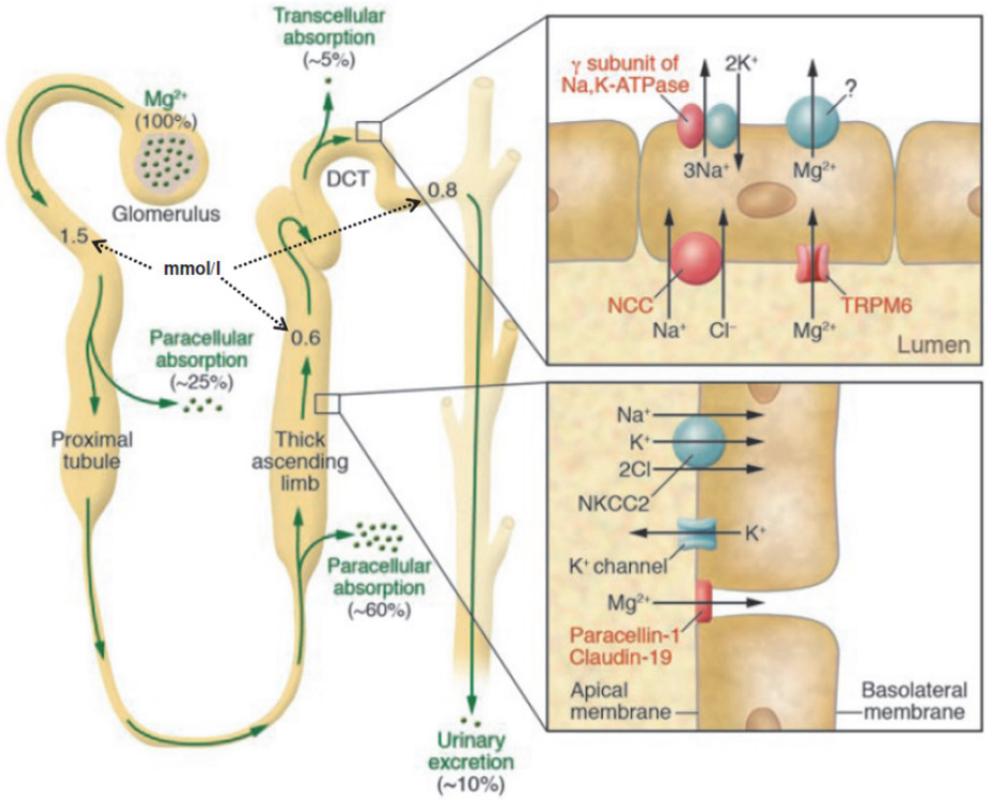


Figure 14: Presentation of the nephron and Mg transport. Ca. 25 % of the filtered Mg is paracellular absorbed in the proximal tubule probably together with water. This passive absorption is also observed in the thick ascending limb of the loop of Henle. The paracellular transport is driven by the serosal negative potential difference and is mediated in the paracellular pathway by claudin-16 (paracellulin-1) and claudin-19. In the distal convolute tubule (DCT) 5 - 10 % Mg is absorbed by an active mechanism via TRPM6. The mechanism of basolateral extrusion is still uncertain. (from Muallem and Moe, 2007)

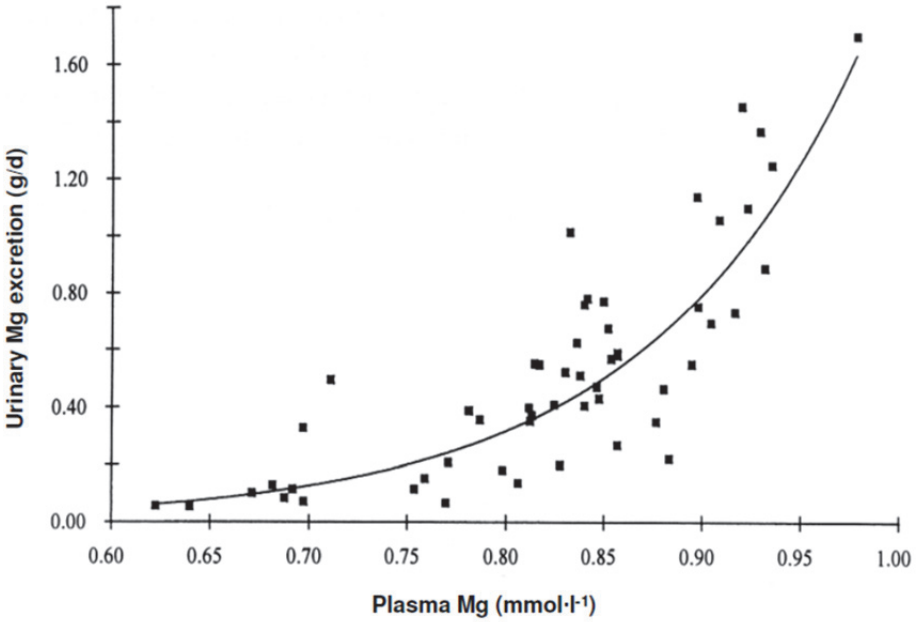
The adaptation of Mg transport in TAL and DCT according to influx > efflux has raised questions regarding the possible sensing mechanism and the involved signal cascade. Particularly intriguing is the rapid adaptation of Mg excretion by the re-absorption of almost all filtered Mg under low Mg intake with tiny alterations of blood Mg concentration (fig. 16). Ferrè et al. (2012) concluded that “CaSR affects many molecular players in paracellular and transcellular reabsorption of Ca and Mg in the kidney. Moreover, it provides crucial basolateral- and luminal sensing mechanisms...” Furthermore, Cole and Quamme (2000) showed that mutation of CaSR caused disturbances of Mg homeostasis in man. More recently, Stuiver et al. (2011) have identified a protein (CNNM2), that is located in the basolateral membrane of TAL and DCT, and that is upregulated under Mg deficiency; its mutation causes a disturbance of Mg homeostasis. The authors suggest that CNNM2 “might contribute to a Mg sensing mechanism rather than transporting Mg itself”. Sensing Mg and the signal

cascade of induction of changed Mg transport are obligatory subjects of research in the future.

6.3. Urinary excretion

6.3.1. Magnesium: The regulation of TRPM6 and claudin-16 (Efrati et al., 2010; San-Cristobal et al., 2010) explains the renal handling of Mg surplus (Mg influx > Mg efflux) and the adapted change of renal Mg transport activity has been illustrated by Schonewille et al. (2000) (fig. 15A), who describe the correlation between urinary Mg and blood Mg. The exponential correlation clearly shows a turning point in blood Mg between 0.8 and 0.9 mmol·l^{-1}. A similar correlation has been found by Holtenius et al. (2008) (fig. 15B), despite considerably higher excretion in urine.

A



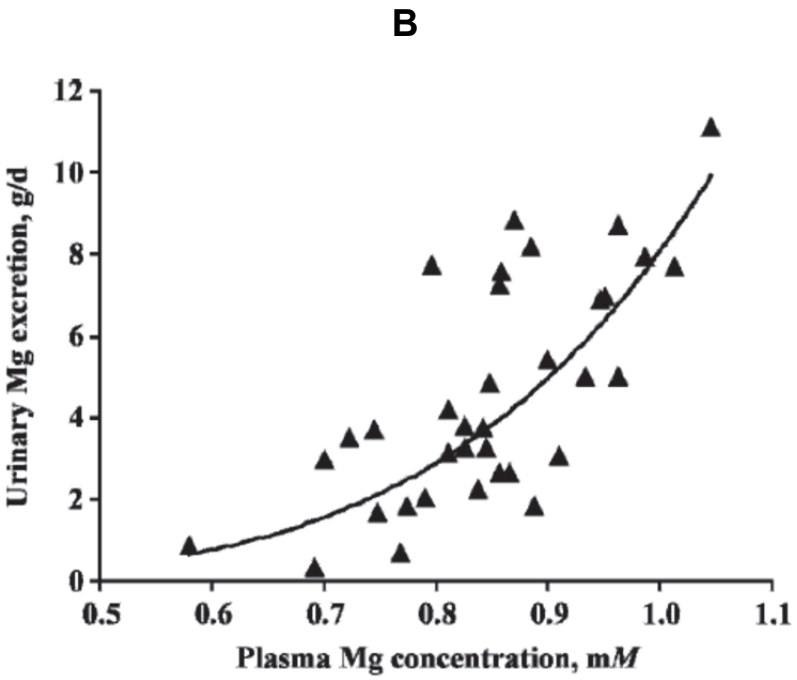


Figure 15: Relation between plasma Mg concentration and renal Mg excretion in cows (A: Schonewille et al., 2000; B: Holtenius et al., 2008). Both curvilinear relationships exhibit a turning point between plasma Mg concentrations of 0.80 – 0.95 mmol·l^{-1}, although the renal Mg excretion was much higher in B. This means that the same blood Mg concentration can indicate a markedly different Mg status.

The tight control of Mg transport activity in TAL (Efrati et al., 2010) and DCT (San-Cristobal et al., 2010) suggests that the Mg concentration in urine is a more sensitive parameter of Mg status than blood Mg. This is indeed the case; Rook and Balch (1958) suddenly changed the diet of cows from a winter ration to cut herbage (fig. 16) and observed a much more pronounced decline of Mg in urine than in blood (fig. 16).

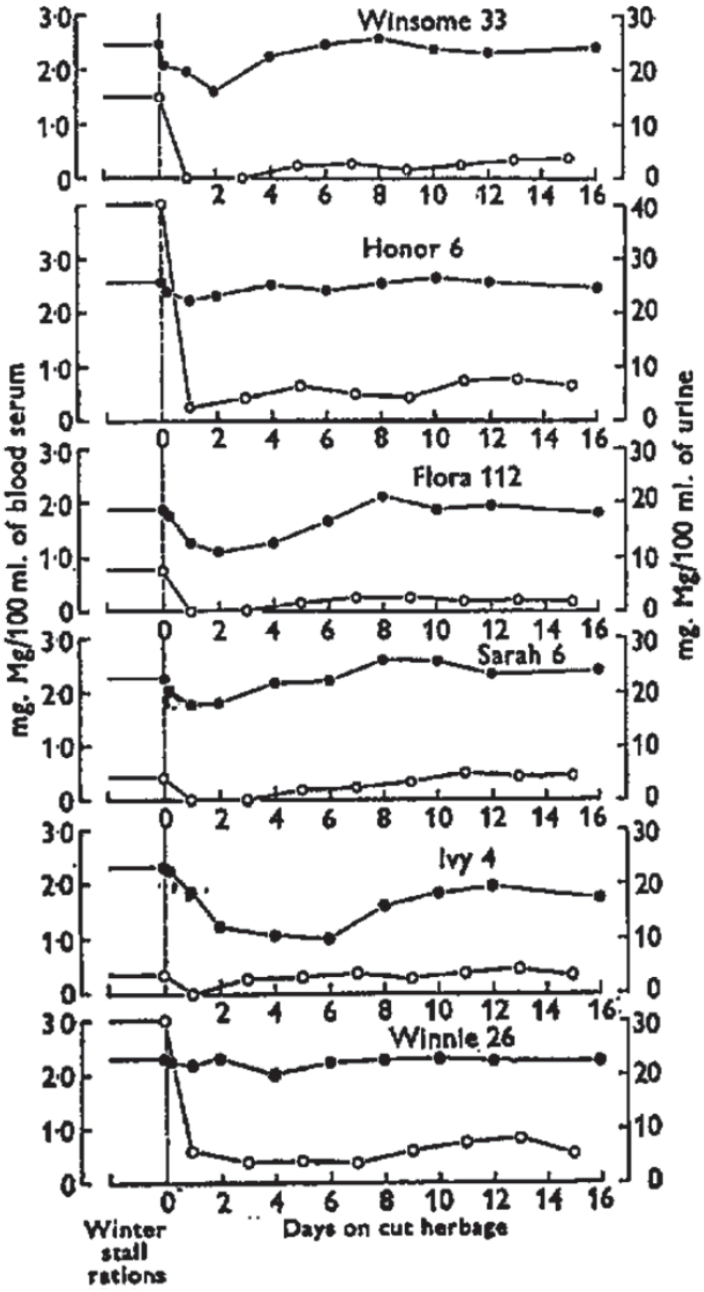


Figure 16: Time course of Mg concentration in blood and urine of six cows before and after a change of diet from winter stall ration to cut herbage. The blood Mg concentration (●) declined after the change of diet within 2 – 4 days, but this decrease varied between almost zero (Winnie 26) and some 60 % (Ivy 4). By contrast, the Mg concentration in urine (**O**) immediately decreased in all cases within one day by more than 70 % (from Rook and Balch, 1958).

This difference in blood and urine concentrations under a challenge of diet change and probably under altered Mg absorption (influx) (fig. 16) confirms the principle of Mg homeostasis (fig. 1). The Mg concentration in the ECF and blood is “buffered” by the rapid adaptation of renal Mg excretion which fluctuates according the difference between influx and efflux. Hence, Mg in urine can be used as a diagnostic tool. Kemp

(1983) suggests a shortage in Mg uptake at urinary Mg < 0.87 mmol·l^{-1} (table 7). Urinary Mg < 1 mmol·l^{-1} is probably a reliable indicator of insufficient intake/absorption. According to the data of Kemp (1983) Mg uptake is sufficient at urinary Mg > 4.4 mmol·l^{-1} and the range of 0.87 – 4.4 mmol·l^{-1} might indicate at risk of Mg shortage (table 7).

Table 7: Mg in urine and Mg status (Kemp, 1983).

Mg mg/l	Mg mmol·l^{-1}	Comment
> 100	> 4.40	Sufficient Mg intake (absorption)
20 - 100	0.87 – 4.40	Marginal Mg intake; risk of shortage
< 20	< 0.87	Mg deficiency, risk of tetany

Figure 15A and 15B and table 7 show that both the Mg concentration in blood (0.8 – 0.9 mmol·l^{-1}) and in urine 0.87 – 4.4 mmol·l^{-1}) exhibits a range of concentrations with some uncertainties about the exact determination of Mg status. The quotient (blood/urine) should be calculated in future studies with aim of giving a better evaluation of Mg status. The data of figure 16 support this assumption.

6.3.2. Interaction of Magnesium and calcium: The effects of Ca on renal functions are well known. Mutual interaction of transport between these two cations has been suggested and competition for net transport might result (Massry and Coburn, 1973). Quamme (1982) has designed experiments in rats to determine the location of these effects within the nephron. Hypercalcaemia causes a reduction of Mg re-absorption within the loop of Henle and a large increase in urinary Mg. The interaction between Ca and Mg occurs in TAL, and the paracellular transport is mediated by claudin-16.

The mutual interaction of Ca and Mg probably also takes place in cows. Waterman et al. (1991) have observed that urinary Ca excretion in cows was higher for diets supplemented with Mg. Paquay et al. (1968) and Roche et al. (2002) found a strong relationship between Ca and Mg in the urine of cows.

In agreement with this observation are the results of Kallfelz et al. (1987). High Mg intake (1.4 %) in calves decreases blood Ca concentration. Because balance data have not been measured both impaired absorption and/or increased excretion of Ca in urine have been suggested as possible reasons.

7. ASSESSMENT OF Mg REQUIREMENT

In a meta-analysis Weiss (2004) determined dietary factors influencing the apparent absorption of Mg in dairy cows. The predominant reason for low Mg digestibility was the K concentration of the diet (Weiss, 2004) (see equation 10). Schonewille et al. (2008) extended this meta-analysis (see equation 11). Correction of true Mg absorption by endogenous Mg secretion led to the following equation (see also equation 11 and 12):

Apparent Mg absorption (g/d) = 0.8 (g/d) + 0.2 x Mg intake (g/d) - 0.08 (g/d) x dietary K (g/kg DM) (13) (identical with 12)
(K = g/kg DM)[3]

Apparent Mg absorption must cover the Mg requirement for milk, endogenous secretion, and tissue uptake (soft tissue, bone, pregnancy). In an adult, Mg in the non-pregnant cow is used for milk production and endogenous secretion. Mg uptake in tissues and bones is assumed to be zero in the absence of growth and when no fetal Mg is required.

Apparent Mg absorption = Milk (0.12 g/l) + endogenous secretion (2.8 g/d) (14)

Equation (14) can be rearranged to:

Milk (0.12 g/l) + end. secretion (2.8 g/d) = 0.8 (g/d) + 0.2 x Mg intake (g/d) - 0.08 (g/d) x dietary K (g/kg DM)^2 (15)

Equation (15) permits an approach for the assessment of Mg intake according milk production and K content (Martens and Stumpff, 2011):

Mg intake (g/d) = 10 g/d + Milk (l/d) x 0.6 (g/l) + 0.4 (g/d) x dietary K (g/kg DM)^2 (16)

[3] K/kg DM as number without dimension: 1 % K/DM = 10

This equation (16) was used to calculate Mg intake at increasing milk production and K content (table 8). At low Mg requirement (milk 0 l/d), the necessary increase of Mg intake was 57 % to counteract the effect of dietary K from 1 to 4 %. This additional amount of Mg was only 24 % at high Mg intake for 60 l/d. Again, the effect of K is pronounced at low Mg and high K intake. Mg absorption is calculated according equation (16) and covers the Mg requirement (table 8). As mentioned by Schonewille et al. (2008), Mg intake must be increased by 4 g Mg when the K concentration of the diet is raised by 1 % or 10 g k/DM above 1 % K.

Table 8: Mg intake, increasing milk yield and dietary K by using equation (16). Maint. = maintenance, Requir. = requirement. Absorption was calculated by equation (12). 12 mg/l milk and endogenous secretion 700 kg BW x 4 mg/kg (2.8 g/d). The absorption of Mg covers at all K intake Mg requirement.

Milk Yield (l/d)	Mg Milk (g/d)	Mg (g/d)		Mg Intake (g/d			Mg (g/d)
		Maint.	Requir.	1% K	2 % K	4 % K	Absorption
0	**0**	**2.8**	**2.8**	**14.0**	**18.0**	**26.0**	***2.8***
20	**2.4**	**2.8**	**5.2**	**26.0**	**30.0**	**38.0**	***5.2***
40	**4.8**	**2.8**	**7.6**	**38.0**	**42.0**	**50.0**	***7.6***
60	**7.2**	**2.8**	**10.0**	**50.0**	**54.0**	**62.0**	***10.0***

However, this calculation uses the mean values and the mean of requirements for milk and endogenous secretion. The variation of these values (Weiss, 2004; Schonewille et al., 2008) is great, and a safety margin should be included. Schonewille and Beynen (2005) have proposed a factor of 1.6.

The correction of Mg intake of 4 g per 1 % of K by Schonewille et a. (2008) is much lower than the amount of 18 g proposed by Weiss (2004). The possible reasons for this discrepancy have been discussed in detail by Schonewille et al. (2008) and includes the differences of K content in the data set of the meta-analysis, the cows (lactating and not lactating), and differences of the DMI and diet composition. Schonewille et al. (2008) conclude that the more pronounced effect of K in the study of Weiss (2004) can be explained by the higher K intake and the corn silage (USA) instead of grass feeds (Europe) as a main source of rouphage and suggest an overestimation of the effect of K. Hence, Schonewille et al. (2008) conclude, for the typical European diet, a Mg absorption of 20 % with a correction of 4 g per 1 % increase of K above 1 % (see table 8).

8. MAGNESIUM AND DISEASES

8.1. Pathogenesis of tetany

The classic clinical signs of hypomagnesemia in cows such as ataxia, recumbency, convulsions, and finally tetanic muscle spasms were known before Sjollema (1930) demonstrated the relationship between the clinical symptoms and hypomagnesemia. However, the Mg concentration in blood exhibits some variation (table 9), and the observed nervous disturbances are not closely related to the blood Mg concentration (Halse, 1979).

Table 9: Status of Mg metabolism and blood Mg concentration.

Mg Status	**Blood Mg** ($mmol \cdot l^{-1}$, mg/100 ml)
1. Normal Mg	0.9 – 1.2 (2.19 – 2.92)
2. Uncertainty	0.8 – 0.9 (1.95 – 2.19)*
3. Suspicion of hypomagnesemia	0.7 – 0.8 (1.70 – 1.95)
4. Hypomagnesemia	< 0.7 (< 1.70)

*See table 7

A blood concentration of < 0.9 $mmol \cdot l^{-1}$ does not permit the safe adjustment of Mg status as this value could mean adequate Mg or even a Mg concentration at the risk of hypomagnesemia (see below). Nevertheless, a low blood Mg concentration is a precondition of the clinical symptoms, which are probably produced in two steps: a) hypomagnesemia and b) impaired function of the central nervous system (CNS).

8.1.1. Clinical hypomagnesemia: Classic tetany was originally observed after a few days once cows had been let out onto grass in the spring. Older cows are more susceptible and hypomagnesemia is not linked with parturition as is milk fever (Blaxter and McGill, 1956).

The predominant reason of the pathogenesis of hypomagnesemia is the small amount of Mg of 3 - 4 g (fig. 1) which is present in the extracellular fluid, and which depends on an undisturbed ratio between influx > efflux. As mentioned above, hypomagnesemia “does not arise by inadequate intake of Mg” (Head and Rook, 1955) and even occurs after a change of diet when the diet is isomagnesemic (Care et al., 1967) or despite an increase of Mg intake from 16 g/d to 23 g/d (Johnson et al., 1988). Nevertheless, the Mg absorption (influx) from the diet was reduced.

Mg in the ECF is further challenged by transport of Mg into milk (12 mg/l). The Mg requirement for milk lowers the blood Mg concentration, and Baker et al. (1986) have suggested that the speed of blood Mg decline promotes the onset of clinical signs. Reduced influx at identical efflux leads to hypomagnesemia and cannot be compensated by Mg mobilization from the large pools in bone or soft tissue (Blaxter and McGill, 1956) or only to a negligible small extent (Storry and Rook, 1963).

***8.1.2. Impaired function of the central nervous system (CNS)*:** Hypomagnesemia was originally suggested to be caused by the uncontrolled activation of muscles by impaired synaptic transmission at the motoric endplate (Hemmingway and Ritchie, 1965). This hypothesis was not confirmed in the experiments of Todd and Horvath (1970), and the involuntary activation of muscles was considered unlikel to occur primarily in the periphery. The possible involvement of the central nervous system was discussed by Chutkow and Meyers (1968) when they found decreased Mg concentrations in the cerebrospinal fluid (CSF) of Mg-deficient rats. The hypothesis of the decreased Mg concentration in CSF as a reason for clinical signs such as ataxia and tetany was tested by Meyer and Scholz (1972) in Mg-deficient sheep by measuring Mg concentration in blood and CSF, together with the registration of clinical signs. They found that the Mg concentration in the CSF is kept constant over a wide range of blood Mg. Mg in the CSF tends to decrease at a blood Mg value of < 0.5 mM and decreases almost linearly at a blood Mg value of < 0.25 mM. Clinical signs of Mg deficiency are absent at > 0.7 mM Mg in the CSF but are frequently observed below < 0.7 mM. Allsop and Pauli (1975) further tested the discussed causal correlation between Mg in CSF and clinical signs by ventriculolumbar perfusion of the CSF space with artificial CSF. Mg concentrations of < 0.25 mM in this solution produced episodes of tetany that were abolished by higher Mg concentrations (Allsop and Pauli, 1975). Because these effects were not accompanied by changes in blood parameters, the clinical symptoms were considered to be caused by the non-controlled activation of muscles by the central nervous system (CNS). Mg is a well-known modulator and physiological antagonist of Ca-induced transmitter release at synapses (Thompson, 1986), and low Mg in the CSF might facilitate Ca-dependent transmitter release and the excitation of CNS neurons that, amongst others, activate muscles. The approved treatment of hypomagnesemia by intravenous (Mayer and Busse, 1975) or rectal (Reynolds et al., 1984) infusion of Mg salts increases CSF Mg levels and further supports the

conclusion that the clinical symptoms of hypomagnesemia are caused by malfunctions of the CNS. Of interest, Reynolds et al. (1984) and Allsop and Pauli (1975) have observed, in addition to diminished Mg, lower Ca concentrations in the CSF in sheep or calves with episodes of tetany.

8.2. Mg and milk fever

Parturition is a challenge for both the energy and the mineral metabolism of the cow. Intrauterine nutrition is replaced by milk secretion. Mineral requirement at the end of pregnancy accounts for some 10 g/d Ca and 0.2 g/d Mg for the calf (House and Bell, 1993). These amounts are higher for Ca and Mg in colostrum, and this rapid increase of Ca requirement is one of the major reasons for hypocalcemia and milk fever (Martin-Tereso and Martens, 2014).

However, insufficient Mg intake impairs Ca homeostasis and might contribute to the pathogenesis of hypocalcemia. Payne et al. (1973) have reported an association between subclinical hypomagnesemia and a high incidence of milk fever. A possible deficiency of Mg (and risk of milk fever) can be deduced from observations of Ward and Parker (1999). They found, in a field study of 10,200 cows a.p., a critical blood Mg concentration of < 0.74 $mmol \cdot l^{-1}$ in some 9 % of the cows.

Evidence has accumulated for many years that hypomagnesemia impairs both the release of PTH (Anast et al., 1972; Rayssiguier et al., 1977) and the effects of PTH at the target organ (MacManus et al., 1971; Goff, 2008). These results are in agreement with the observations of Payne et al. (1970). Both vitamin D_3 and 1α-hydroxycholecalciferol D_3 are ineffective in hypomagnesemic cows (Payne et al., 1970), and the conversion of vitamin D_3 to 25-hydroxycholecalciferol requires Mg (Horsting et al., 1969). Consequently, hypomagnesemia causes secondary hypocalcemia (Rayssiguier et al., 1977; Littledike and Cox, 1979) (fig. 17).

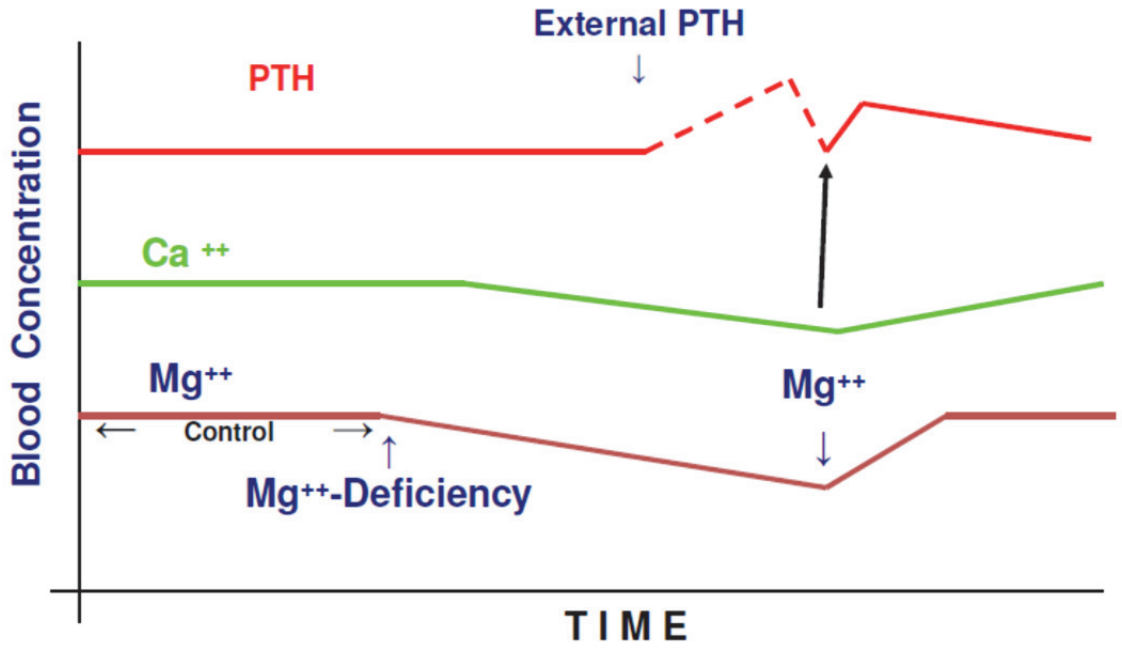


Figure 17: Representation of the effects of Mg deficiency on Ca metabolism and PTH. A Mg-deficient diet causes first hypomagnesemia and, with some delay, secondary hypocalcemia, which does not elicit a release of PTH. Further, the external addition of PTH does not change blood Ca. Hence, the release and effects of PTH depend on normal blood Mg. The time course of these effects probably varies with the magnitude of difference between Mg intake and requirement (= degree of deficiency).

This hypocalcemia was particularly pronounced in the studies of Baker et al. (1979). The diet of the sheep was changed from oaten chaff to an infusion of milk into the abomasum at low Mg intake. The blood Mg decreased almost immediately and, with some delay, Ca also declined, although the intake of 2.6 g/d was sufficient. Interestingly, the blood P concentration increased, which further supports the assumption of impaired effect of PTH on P excretion in urine. The impaired function of PTH on the Ca concentration was also evident in the studies of Reynolds et al. (1984). Rectal infusion of $MgCl_2$ increased blood Mg within 5 minutes and blood Ca after a delay of 120 minutes in calves fed a low Mg and high K diet. However, the function of $1,25(OH)_2D_3$ is not influenced in normal or hypomagnesemic cows (Moate et al., 1987). Hence, the impairment of hypomagnesemia is restricted to the proximal part of the PTH and vitamin D_3 axis.

Hypomagnesemia impairs the periphal function of PTH at osteocytes and reduces the capacity for Ca mobilization from the bone. This impairment could possibly increase the risk of milk fever. Indeed, Samson et al. (1983) have demonstrated, in cows, that hypomagnesemia significantly reduces Mg mobilization (table 10).

Table 10: Effect of hypomagnesaemia on Ca mobilization from bone in cows. A decrease of blood Mg concentration causes a reduction of Ca mobilization* (Samson et al., 1983).

Cows	Control		Mg Deficiency	
	Mg $mmol \cdot l^{-1}$	Ca-Mobil. $mmol \cdot min^{-1}$	Mg $mmol \cdot l^{-1}$	Ca-Mobil. $mmol \cdot min^{-1}$
Lactation	1.10	0.41	0.54	0.26*
Pregnancy	0.96	0.54	0.76	0.39*

The effect of Mg intake on Ca mobilization from bone has also been demonstrated by van de Braak et al. (1987). After parturition, two cows (from 9) in a low Mg group showed clinical signs of hypocalcemia, but none of the high Mg group (10 cows) exhibited these symptoms. Cows with a lower Mg intake had a lower mean rate of calcium mobilization (0.27 mmol min^{-1}) than those at high Mg intake (0.34 mmol min^{-1}). These rates are almost identical with the previous data of Sansom et al. (1983) (table 10). Interestingly, Terashima et al. (1988) have observed the lowest rate of Mg mobilization in Mg-deficient sheep at low Mg *and* high K intake (see above), which again confirms the conclusions about the risks of this combination of Mg and K intake. Indeed, “high amounts of K in the diet may increase the risk of milk fever linearly” (Kronqvist et al., 2012). However, differences of Ca mobilization have not been observed in dry and non-lactating cows at normal (0.2 %) or high (0.37 %) Mg intake (Wang et al., 1992). Hence, Mg deficiency is a precondition for impaired Ca mobilization from bone, which is not observed at the Mg intake above requirement.

The important role of sufficient Mg intake at parturition is underlined by the results of a meta-analysis of possible causal reasons of milk fever. Lean et al. (2006) concluded that “an increase in magnesium concentration from 0.3 to 0.4% of DM, while maintaining the other variables, would result in an approximate 62% decrease in milk fever risk“. This high Mg concentration is far above the German recommendation of 0.2 % but can easily be explained by a calculation of Mg intake and necessary requirement, which includes Mg for maintenance (2.8 g/d) + colostrum (3.6 g/d) (5 l x 0.733 mg/l) = 6.4 g/d). As is well known, DMI is severely reduced at parturition, which varies between 6 and 10 kg/d, and hence, the usual recommendation of 0.2 % is too low. A DMI of 6 – 10 kg/d is accompanied by an Mg intake of 18 – 30 g/d (Mg 0.3 %) or 24 – 40 g/d (Mg 0.4 %) (table 11). Because the Mg digestibility is generally low (Schonewille et al., 2008), the calculated Mg

digestibility for covering the requirement of 6.4 g/d shows (table 11) that the assumed Mg intake of 6 or 8 kg/d DMI is low, even at these high Mg concentrations of 0.3 or 0.4 % Mg (table 11). Indeed, in a field study, Kronqvist et al. (2012) have observed that “feeding less than 26 g Mg per day 3 weeks before calving … were associated with higher odds of high milk fever incidence”. Conversely, cows with a Mg intake of 34 – 52 g/d exhibited the lowest incidence of milk fever. This high amount of Mg intake is possible with a percentage of 0.4 % Mg in the dry matter (see table 11).

Table 11: Dry matter intake, Mg intake, and necessary Mg digestibility for Mg requirement at parturition

Dry Matter Intake kg/d	Mg % DMI	Mg intake g/d	Mg Requirement g/d	Necessary Mg Digestibility (%)
6	0.3	18	6.4	36*
8	0.3	24	6.4	27*
10	0.3	30	6.4	21
6	0.4	24	6.4	27*
8	0.4	32	6.4	20
10	0.4	40	6.4	16

*Unlikely high Mg digestibility for the necessary requirement

Hence, the conclusions of the meta-analysis of Lean et al. (2006) agree well with the field observation of Kronqvist et al. (2012) and confirm the recommendation of 0.35 – 0.40 % Mg in the diet of the close-up dry cow and early lactation of Goff (2008).

Notably, Mg absorption from a rumen pouch of sheep is challenged by a high ruminal Ca concentration (Care et al., 1984). Furthermore, Mg digestibility in cows is impaired at high Ca intake (Kronqvist et al., 2011) and probably also by an increase of ruminal pH and reduced Mg solubility (Dalley et al., 1997) because DMI (and fermentation) decreases before and at parturition (Peterson et al., 2005). The mentioned interaction between Ca and Mg should include the observation that low Ca intake increases Mg digestibility and vice versa (Verdaris and Evans, 1976).

An important interaction between Mg and Ca has recently been published by Oehlschlaeger et al. (2014). The authors study the effect of low DCAD on Ca absorption from the gut; this is reduced to –70 meq/kg by $MgCl_2$. The change in Mg content and DCAD alters pre-duodenal Ca absorption from 11.07 g/d to a net

secretion of 2.89 g/d. Total Ca absorption from the gut is numerically reduced from 27.37 g/d to 23.77 g/d and corresponds to the mentioned interaction of absorption between Ca and Mg. In agreement with these effects of Mg are the observations of Roche et al. (2002); the addition of $MgSO_4$ or $MgCl_2$ lowers the incidence of plasma Ca concentration (< 1.4 $mmol \cdot l^{-1}$) precalving, and the plasma concentration is greater postcalving.

Furthermore, lipolysis and the increase of NEFA caused by the intravenous infusion of adrenalin causes a drop of blood Mg (Rassiguier, 1977a). Lipolysis and a sharp increase of NEFA around parturition are well known, because the cows are in a negative energy balance (Martens, 2013), and the stimulus of lipolysis seems to be correlated with hypomagnesemia (Rayssiguier, 1977a). The specific metabolic situation of the dairy cow at parturition appears to impair Mg homeostasis, which supports the suggestion of additional Mg supplementation.

Low Mg intake, impaired absorption, and homeostasis before and at parturition is probably an overlooked factor in the pathogenesis of milk fever.

9. PREVENTION OF HYPOMAGNESAEMIA

The improved knowledge about the major reasons of impaired Mg absorption makes recommendations for prophylactic measures easier, but this depends on the specific situation.

9.1. Conventional dairy cows

The possible risk of classic grass tetany is generally low in dairy cattle fed with concentrate diet for milk production. Concentrates increase fermentation, and the low rumen pH with high Mg solubility and SCAF/CO_2 concentrations improves Mg absorption. Such diets are supplemented with minerals (Mg and Na) and dilute the K content of the total ration. Rapid changes of diet (N intake and high NH_4^+) are rare, and reports of hypomagnesemia are associated with single farms (Donovan, 2004; Urdaz et al., 2003). In both publications, hypomagnesemia was observed, despite there being 0.28 % Mg in the dry matter of the diet, a level that complies with the National Research Council's recommendation, and that is even higher than the German recommendation of 0.2 %. Obviously, the Mg digestibility was low and, in one case, was related to the particle size of MgO (Urdaz et al., 2003). An increase of Mg intake abolished the clinical cases of affected or dead cows.

9.2. Dairy cows on pasture

In New Zealand and in Ireland, milk is traditionally produced by dairy cows on pasture. Hence, the traditional risks for hypomagnesemia exist. McCoy et al. (1996) have reported a survey of the incidence of hypomagnesemia in Northern Ireland; it included 377 dairy (3626 cows) and 722 suckler herds (6664 cows). Overall, 8 % and 2 % of dairy cows were found with marginal (0.6 – 0.8 $mmol \cdot l^{-1}$) or deficient (< 0.6 $mmol \cdot l^{-1}$) levels of Mg, respectively. The risk of tetany is still high under these feeding conditions, and dairy farmers used high Mg concentrates as a prophylaxis. This supplementation reduced the percentage of marginal and deficient blood Mg concentrations.

The percentage of hypomagnesemia was higher in suckler herds: 22.1 % marginal and 7.3 % deficient. Surprisingly, Mg blocks provided by farmers of suckler herds were obviously not successful, because no differences between farms with or without supplementation (Mg blocks) were found (McCoy et al., 1996).

Other methods of Mg supplementation, such as Mg shaken over silage or meal or Mg with molasses, were little used (McCoy et al., 1996).

Beef cattle are probably still under the risk of hypomagnesemia as suckler herds after a rapid change of diet from barn feeding to young spring grass, because all risk factors (high K, insufficient Na, high nitrogen) are typical and should be avoided.

In all cases of risk of hypomagnesemia, supplementation with hay, straw, or good silage with low K should be offered in addition to Mg and Na.

10. CONCLUSIONS AND PERSPECTIVES

Studies of Mg metabolism in cows were initiated because of the correlation between blood Mg concentration and a disease termed grass staggers or grass tetany described 80 years ago. As was found very early, hypomagnesaemia was not caused by Mg deficiency. However, the availability of Mg in the diet was obviously reduced. This observation led to studies about the site of Mg absorption, the mechanism of Mg transport, and the characterization of those factors that influence Mg transport and digestibility. The framework of these studies and the significantly improved knowledge permit the successful prophylaxis of classic hypomagnesemia. A possible risk is still present in countries with milk production on grass, as occurs in Ireland or New Zealand. If, however, reliable supplementation with Mg and Na is carried out, and if rapid changes of diet are avoided, hypomagnesemia can be prevented. Furthermore, an assessment of Mg intake according to milk production and K content of the diet is feasible.

A growing body of evidence indicates that Mg is involved in the pathogenesis of milk fever. In addition to established knowledge concerning the role of Mg in PTH release and of Mg-dependent PTH effects, the provision of an adequate Mg intake and the Mg status of farm animals around parturition deserve more attention. The antagonism of Mg on Ca absorption and urinary Ca excretion can be considered as a challenge for Ca homeostasis and might be one reason for the recommendation of higher Mg intake at parturition as a prophylaxis against milk fever. These topics should form the bases of further research, which should include the effects of Mg on Ca digestibility, urinary Mg excretion, and mechanisms of Ca homeostasis. In particular, renal excretion *in vivo* deserves further attention, because renal Mg transport is regulated according Mg surplus. A better understanding of this mechanism could lead to improved diagnosis of Mg status.

11. SUMMARY

Aspects of Magnesium Metabolism in Ruminants: Mg Absorption, its Modulation and Meaning for Assessment of Mg Intake.

Magnesium is an essential mineral that is not regulated by hormones. The blood Mg concentration under steady state conditions depends on Mg influx (absorption) > Mg efflux (growth, milk secretion, endogenous secretion). A surplus of Mg (influx > efflux) is excreted by the kidneys and is related to the difference between influx minus requirement.

The main and essential site of absorption for Mg (influx) homeostasis is the rumen, where Mg is absorbed by an active transport mechanism including an apical PD-dependent and a PD-independent uptake mechanism. This transport mechanism represents Mg influx and is probably not regulated but is influenced by a variety of factors such as high K concentration, sudden increase of ammonia, pH (solubility of Mg), and SCFA. Possible impaired Mg absorption in the rumen is not compensated by increased transport in the small or large intestine.

The kidney has the capability for fine tuning the surplus of Mg by regulated excretion with the urine adjusted to requirement. However, a shortage of Mg can hardly be compensated because Mg cannot be mobilized from bone or by increased absorption from the rumen.

Hypomagnesemia (influx < efflux) is causing the clinical complex tetany. A growing body of evidence indicates that a subclinical deficiency of Mg is involved in the pathogenesis of milk fever.

Meta-analysis of Mg digestibility permits the calculation of Mg requirements, and the characteristics of Mg transport explain the variations in this calculation.

The recommendations for Mg intake with diet are suggested to be too low.

The framework including the functions of Mg, the principles of Mg homeostasis, and the meta-analysis of Mg digestibility allows sufficient understanding for the proposal of reliable recommendations for an adequate Mg intake in the diet according to actual requirements and for the avoidance of possible disturbances.

12. ZUSAMMENFASSUNG

Aspekte des Mg Stoffwechsels bei Wiederkäuern: Absorption, dessen Beeinflussung und Bedeutung für die Abschätzung der Mg Aufnahme.

Magnesium ist ein essentieller Mineralstoff, der nicht hormonal geregelt wird. Die Mg Konzentration im Blut wird unter steady-state Bedingungen bestimmt durch die Mg Absorption (Influx) und den Mg-Bedarf (Efflux) für Wachstum einschließlich Trächtigkeit, Milchsekretion und endogene Verluste. Überschüssiges Mg (Influx > Efflux) wird über die Niere ausgeschieden und entspricht der Differenz zwischen Influx und Mg Bedarf (Efflux).

Mg wird primär im Pansen aktiv resorbiert. Diese Mg Resorption ist essentiell für die Mg Homeostase und wird offensichtlich nicht reguliert. Zwei Aufnahmemechanismen der luminalen Membran des Pansenepithels sind beschrieben worden: Ein PD-abhängiger und ein PD-unabhängiger Mechanismus. Diese Aufnahmemechanismen werden beeinflusst durch die K Konzentration, durch einen raschen Anstieg der Ammoniakkonzentration, durch den pH Wert (Mg Löslichkeit) und SCFA. Eine gestörte Mg Resorption aus dem Pansen wird nicht durch Mg Transport im Dünn- oder Dickdarm kompensiert.

Über die Nieren wird über den Bedarf resorbiertes Mg ausgeschieden und die Ausscheidung wird exakt dem Bedarf angepasst. Ein Mg Defizit kann jedoch nicht kompensiert werden, weil eine Mg Mobilisation aus dem Knochen oder eine erhöhte Resorption nicht möglich ist.

Hypomagnesämie (Influx < Efflux) verursacht die klinischen Symptome Tetanie. Es liegen Hinweise vor, dass eine subklinische Unterversorgung mit Mg an der Pathogenese der Gebärparese beteiligt ist.

Die vorliegenden Meta-Analysen über die Mg Verdauung bei der Milchkuh erlauben die Kalkulation der für den Bedarf erforderlichen Mg Menge und Kenntnisse über den Mg Transport eine Erklärung der möglichen Beeinflussung.

Die Empfehlungen für eine bedarfsgerechte Mg Aufnahme sollten überprüft werden. Es liegen Hinweise vor, dass aktuellen Empfehlungen zu niedrig sind.

Die Erkenntnisse über die Funktionen des Mg, die Prinzipien der Mg Homeostase und die Ergebnisse der Meta-Analysen ermöglichen Empfehlungen über eine bedarfsgerechte Mg Aufnahme mit dem Futter sowie eine Abschätzung der möglichen Beeinflussung.

13. REFERENCES

Abdoun K, Stumpff F, Rabbani I, Martens H (2010). Modulation of urea transport across sheep rumen epithelium *in vitro* by SCFA and CO_2. Am J Physiol 298, G190-G202.

Abdoun K, Stumpff F, Wolf K, Martens H (2005) Modulation of Na transport of sheep rumen epithelium by luminal ammonia. Am J Physiol 289, G508-G520.

Abdoun K, Wolf K, Martens H (2003) Effect of ammonia on Na^+ transport across isolated rumen epithelium of sheep is diet dependent. Br J Nutr 90, 751-758.

Aldor A, Moore E (1970) Magnesium absorption by everted sacs of rat intestine and colon. Gastroenterology 59, 745-753.

Allsop T, Pauli J (1975) Responses to the lowering of magnesium and calcium concentrations in the cerebrospinal fluid of anaesthetized seep. Aust J Biol Sci 28,475-481.

Allsop T, Rook J (1970) Influence of the diet on the relationship between endogenous faecal loss of magnesium in sheep. J Agric Sci 92, 403-408.

Allsop T, Rook J (1979) The effect of diet and blood-plasma concentration on the endogenous faecal loss of magnesium and plasma magnesium concentration in sheep. J Agric Sci 92, 403-408.

Anast CS, Mohs JM, Kaplan SL, Burns TW (1972) Evidence for parathyroid failure in magnesium deficiency. Science 177, 606-608.

Annison E, Lewis D, Lindsay B (1959) The metabolic changes which occur in sheep transferred to lush spring grass. I. Changes in blood and rumen constituents. J Agric Sci 53, 34-41.

Bailyey C (1961) Saliva secretion and its relation to concentration of sodium, potassium, chloride and inorganic phosphate in mixed saliva and rumen fluid. Br J Nutr 15, 498-498.

Bailey, C (1978) Renal function in cows given native grass hay containing added sodium chloride and rapseed meal, with particular reference to clearance of silicic acid. Can J Anim Sci 58, 651-657.

Baker R, Boston R, Boyes T, Leaver D (1979) Variations in the response of sheep to experimental magnesium deficiency. Res Vet Sci 36, 129-133.

Balch D, Rowland S (1957) Volatile fatty acids and lactic acid in the rumen of cows receiving a variety of diets. Br J Nutr 11, 288-298.

Beardsworth P (1987) Physiological factors involved in the aetiology hypomagesaemia in swheep. Ph. D. Thesis, University of Leeds.

Beardsworth L, Beardsworth P, Care A (1087) The effect of potassium concentration on the absorption of magnesium from the reticulo-rumen of conscious sheep. J Physiol 386, 89P.

Behar J (1974) Magnesium absorption by the rat ileum and rat colon. Am J Physiol 227, 334-340.

Bell M, Oluokun J, Ramsey N (1978) Magnesium treatment of cows for grass tetany. Feedstuffs 50, 24-25.

Bindels R (2010) 2009 Homer W. Smith award: Minerals in motion: From new ion transporters to new concepts. J Am Soc Nephrol 21, 1263-1269.

Blaxter KL, McGill RF (1956) Magnesium metabolism in cattle. Vet Rec Annotations 2, 35-55.
Butler E (1963) The mineral element content of spring pasture in relation to the occurrence of grass tetany and hypomagnesaemia in dairy cows. J Agric Sci 60, 329-340.
Cahalan M (2001) Cell biology. Channels as enzymes. Nature 411, 542-543.
Care, A (1960) The kinetic of magnesium distribution within sheep. Res Vet Sci 1, 338-349.
Care A, Beardsworth I, Beardsworth P, Breves B (1989) The absorption of calcium and phosphate from the rumen. Acta Vet Scand Suppl. 152-158.
Care A, Brown R, Farrar A, Pickard D (1984) Magnesium absorption from the digestive tract of sheep. Q J Exp Physiol 69, 577-587.
Care A, van't Klooster (1964) *In vivo* transport of magnesium and other cations across the wall of the gastrointestinal tract of sheep. J Physiol 177, 174-191.
Care A, Vowles L, Mann S, Ross D (1967) Factors affecting magnesium absorption in relation to the aetiology of acute hypomagnesaemia. J Agr Sci 68, 195-204.
Charlton J, Armstrong D (1989) The effect of intravenous infusion of aldosterone upon magnesium metabolism in the sheep. Q J Ex Physiol 74, 329-337.
Carney S, Wong N, Quamme G, Dirks J (1980) Effect of magnesium deficiency on renal magnesium and calcium transport in the rat. J Clin Invest 65,180-188.
Cole D, Quamme G (2000) Inherited disorders of renal magnesium handling. J Am Soc Nephrol 11, 1937-1947.
Chutkow J, Meyers S (1968) Chemical changes in the cerebrosoinal fluid and brain in magnesium deficiency. Neurol 18, 963-974.
Cowan J (2002) Structural and catalytic chemistry of magnesium-dependent enzymes. BioMetals 15, 225-235.
Dalley D, Isherwood P, Sykes A, Robson A (1997) Effect of intraruminal infusion of potassium on the site of magnesium absorption within the digestive tract of sheep. J Agricultural Sci 129, 99-105.
Dalley D, Isherwood P, Sykes A, Robson A (1997) Effect of *in vitro* manipulation of pH on magnesium solubility in ruminal and caecal digesta of sheep. J Agric Sci 129, 107-111.
Demeuse P, Penner P, Fleig A (2006) TRPM7 channel is regulated by magnesium nucleotides via its kinase domain. J Gen Physiol 127, 421-434.
Denton D (1956) The effect of Na^+ depletion on the Na^+-K^+ ratio of the parathyroid saliva in sheep. J Physiol 131,516-525.
Deetz L, McGee B, Tucker E, Terashima Y, Mitchell G (1982) Renal function and magnesium balance in young and old cows given sodium nitrate. Nutrition Reports International 25, 293-300.
Dillon J, Scott D (1979) Digesta flow and mineral absorption in lambs before and after weaning. J Agric Sci 92, 289-297.
Dimke H, Hoenderop J, Bindels R (2011) Molecular basis of epithelial Ca^{2+} and Mg^{2+} transport: insights from TRP channel family. J Physiol589, 1535-1542.
Dobson A, Scott D, Bruce JB (1966) Changes in sodium requirement of the sheep associated with changes of diet. Q J Exp Physiol 51, 311-323.
Donovan, G. A. (2004) Hypomagnesemia among cows in a confinement-house dairy system. JAVMA 224**,** 96-99.
Dua K, Care A (1995) Impaired absorption of magnesium in the aetiology of grass tetany. Br Vet J 151, 413-425.
Dua K, Leonhard S, Martens G, Abbas SB, Care AB (1994) Effect of parathyroid homerone-related protein on the rates of absorption of magnesium, sodium,

potassium and phosphate ions from the reticulo-rumen of sheep. Exp Physiol 79, 401-408.
Edrise B, Smith R (1979) Absorption and secretion in the omasum of the joung steer. Ann Rech Vet 10, 354-355.
Efrati E, Hirsch A, Kladnitsky O, Rozeneld J, Kaplan M, Zinder O, Zelikovic I (2010) transcriptional regulation of the claudin-16 gene by Mg^{2+} availability. Cell Physiol Biochem 25, 705-714.
Etschmann B, Suplie D, Martens H (2009) Change of ruminal Na transport in sheep during dietary adaptation. Arch Anim Nutrition 63, 26-38.
Ferrè S, Hoenderop G, Bindels R (2012) Sensing mechanisms involved in Ca^{2+} and Mg^{2+} homeostasis. Kidney International 82, 1157-1166.
Ferreira H, Harrison F, Keynes R, Nauss A (1966) Observations on the potential across the rumen in sheep. J Physiol 187, 615-630.
Ferreira H, Harrison F, Keynes R, Zurich, L (1972). Ion transport across an isolated preparation of sheep rumen epithelium. J Physiol 222, 77-93.
Field A (1959) Balance trials with magnesium-28 in sheep. Nature, 183, 983.
Field A (1961) Studies on magnesium ruminant nutrion.3. Distribution of ^{28}Mg in the gastro-intestinal tract and tissue of sheep. Br J Nutr 15, 349-359.
Field A, Munro C (1977) The effect of site and quantity on the extent on absorption of Mg infused into the gastrointestinal tract of sheep. J Agric Sci 89, 365-371.
Flatman P (1991) Mechanism of magnesium transport. Ann Rev Physiol 53, 259-274.
Fontenot J, Allen V, Bunce G, Goff J (1989) Factors influencing magnesium absorption and metabolism in ruminants. J Anim Sci 67, 3445-3455.
Fontenot J, Miller R, Whitelwas C, MacVicar R (1960) Effect of high-protein high-potassium ration on mineral metabolism of lambs. J Anim Sci 19, 127-133.
Fontenot J, Wise M, Webb K (1973) Interrelationship of potassium, nitrogen, and magnesium in ruminants. Fed Proc 32, 1925-1978.
Funato Y, Yamazaki D, Mizukami S, Du L, Kikuchi K, Miki H (2014) Membrane protein CNNM4-dependent Mg^{2+} efflux suppresses tumor progression. J Clin Invest 124, 5398-5410.
Frizzell R, Schultz S (1972) Ionic conductances of extracellular shunt pathway in rabbit ileum. Influence of shunt on transmural sodium transport and electrical potential differences. J Gen Physiol. 59, 318-346.
Gaash R, Elazar M, Mizrahi K, Avramov-Mor M, Berezin I, Shaul O (2013) Phylogeny and structural model of plant MHX transporters. Plant Biol 13, 75-
Gäbel G, Martens H (1986) The effect of ammonia on magnesium metabolism in sheep. Z. Tierphysiol Tierernähr Futtermittelkd 55, 278-287.
Gaebel G, Martens H, Suendermann M, Galfi P (1987) The effect of diet, intraruminal pH and osmolarity on sodium, chloride and magnesium absorption from the temporarily isolated and washed reticulo-rumen of sheep. Q J Exp Physiol 72, 501-511.
Gesellschaft für Ernährungsphysiologie (1978) Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere Nr. 1. Empfehlungen zur Mineralstoffversorgung.
Giduck S, Fontenot J (1987) Utilization of magnesium and other macrominerals in sheep supplemented with different ready-fermentable carbohydrates. J Anim Sci 65, 1667-1673.
Giduck S, Fontenot J, Rahnema S (1988) Effect of ruminal infusion of glucose, volatile fatty acids and hydrochloric acid on mineral metabolism in sheep. J Anim Sci 66, 532-542.
Goff J (2008) The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet J 176, 50-57.

Goff J, Littledike R, Horst R (1986) Effect of synthetic bovine parathyroid hormone in dairy cows: Prevention of hypocalcemic parturient paresis. J Dairy Sci 69, 2278-2289.
Grace N, Caple I, Care A (1988) Studies on sheep on the absorption of magnesium from low molecular weight fraction of the reticulo-rumen contents. Br J Nutr 49, 93-108.
Grace N, Davies E, Monro J (1977) Association of Mg, Ca, P and K with various fractions in the diet, digesta and feces of sheep fed fresh pasture. N Z J Agric Res 20, 441-454.
Graham C, Simmons N (2005). Functional organization of the bovine rumen epithelium. Am J Physiol Regul Integr Comp Physiol 288, R173–R181.
Greene L, Baker J, Hardt P (1989) Use of animal breeds and breeding to overcome the incidence of grass tetany: A review. J Anim Sci 67,3463-3469.
Greene L, Fontenot J, Webb K (1983a) Effect of dietary potassium on absorption of magnesium and other macroelements in sheep fed different levels of magnesium. J Anim Sci 56, 1208-1213.
Greene L, Fontenot J, Webb K (1983b) Site of magnesium and other macromineral absorption in steers fed high levels of potassium. J Anim Sci 57, 503-510.
Groenestege W, Thébault St, Wijst J, van den Berg D, Janssen R, Tejpar S, van den Heuvel L, van Cutsem E, Hoenderop J, Knoers N, Bindels R (2007) Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesaemia. J Clin Invest 117, 2260-2267.
Groenestege W, Hoenderop J, van den Heuvel L, Knoers N, Bindels R (2007) The epithelial Mg^{2+} channel transient receptor potential melastatin 6 is regulated by dietary Mg^{2+} content and estrogens. J Am Sod Nephr 17, 1035-1043.
Günther T (1977) Stoffwechsel und Wirkungen des intrazellulären Magnesium. J Clin Chem Clin Biochem 15*,* 433-438.
Günther T (1981) Biochemistry and pathobiochemistry of magnesium. Artery 9, 167-181.
Günther T (2010) The biochemical function of Mg^{2+} in insulin secretion, insulin transduction and insulin resistance. Magnesium Research 23, 5-18.
Günther T, Ising H, Mohr-Nawroth F, Chahoud I, Merker H (1981) Embryotoxic effects of magnesium deficiency and stress on rats and mice. Teratology 24, 225-233.
Günther T, Vormann J (1995) Reversibilty of Na^{+}/Mg^{2+} antiport in rat erythrocytes. Biochim Biohys Acta 1234, 105-110.
Graham C, Simmons N (2005) Functional organization of the bovine rumen epithelium. Am J Physiol - Regulatory, Integrative and Comparative Physiology 288, R173-R181.
Günzel D, Yu A (2013) Claudins and the modulation of the tight junction permeability. Phys Rev 93, 525-569.
Halm D, Krasny, E, Frizzell R (1985). Electrophysiology of flounder intestinal mucosa. I. Conductance properties of the cellular and paracellular pathway. J Gen Physiol 85, 843-864.
Halse K (1970) Individual variation in blood magnesium and susceptibility to hypomagnesaemia in cows. Acta Vet Scand 11, 394-414.
Harrison F (1963) Absorption and excretion of lithium and magnesium in sheep. Bioch. J. 89, 99-100P.
Head J, Rook J (1955) Hypomagnesaemia in dairy cattle and its relation to ruminal ammonia production. Nature 176, 262-263.
Hemingway R, Ritchie N (1965) The importance of hypocalcemia in the development of hypomagnesaemic tetany. Proc Nutr Soc 24, 54-63.

Henrikson R (1971) Mechanism of sodium transport across ruminal epithelium and histochemical localization of ATPase. Exp Cell Res 68, 456-458.
Hirano T, Kobayshi N, Itoh T, Takasuga A, Nakamaru T, Hirotsune S, Sugimoto Y (2000) Null mutation of PCLN-1/claudin-16 results in bovine chronic interstitial nephritis. Genome Res 10, 659-663.
Hoenderop J, Bindels R (2008) Calciotropic and magnesiotropic TRP channels. Physiology 23, 32-40.
Holtenius K, Kronqvist, Briland E, Sörndy R (2008) Magnesium absorption by lactating cows on grass silage-based diet supplied with different potassium and magnesium level. J Dairy Sci 91, 743-748.
Horn J, Smith R (1978) Magnesium absorption by the steer. Br J Nutr 40, 473-484.
Horsting M, De Luca HF (1969) *In vitro* production of 25-hydroxycholecalciferol. Biochem Biophys Res Communication 36, 251-256.
Hou J, Rajagopal M, Yu A (2013) Claudins and the Kidney Volume 75: Annual Review of Physiology. Ann Rev Physiol 75, 479-491.
House, W, Bell, A (1993) Mineral accretion in the fetus and adnexa during late gestation in Holstein cows. J Dairy Sci 76, 2299-2310.
House W, Mayland H (1976) Magnesium utilization in wethers fed diets with varying ratios of nitrogen to readily fermentable carbohydrate. J Anim Sci 43, 842-849.
Ikari A, Hirai N, Shiroma M, Harada H, Sakai H, Hayashi H, Suzuki Y, Degawa M, Takagi K. (2004) Association of paracellin-1 with ZO-1 augments the reabsorption of divalent cations in renal epithelial cells. J Biol Chem 279, 54826-54832.
Ikari A, Matsumoto S, Harada H, Takagi K, Hayashi H, Suzuki Y, Degawa M, Miwa M (2006) Phosphorylation of paracellin-1 at Ser217 by protein kinase A is essential for localization in tight junctions. J Cell Sci 119, 1781-179.
Jittakhot S, Schonewille J, Wouterse H, Yuangklang C, Beynen A (2004) Apparent magnesium absorption in dry cows fed 3 levels of potassium and 2 levels of magnesium intake. J Dairy Sci 87, 379-385.
Johnson C, Helliwell S, Jones A (1988) Magnesium metabolism in the rumen of lactating cows fed on spring grass. Q J Exp Physiol 73, 23-31.
Johnson C, Jones A (1989) Effect of change of diet on the mineral composition of rumen fluid, on magnesium metabolism and water balance in sheep. Br J Nutr 61, 583-594.
Jung D, Brierley G (1994) Magnesium transport by mitochondria. J Bioenerg Biomembr 26, 527-35.
Kausalaya P, Amasheh S, Günzel D, Wurps H, Müller D, Fromm M, Hunziker W (2006) Disease-associated mutation affect intracellular traffic and paracellular Mg^{2+} transport function of claudin-16. J Clin Invest 116,878-891.
Kallfelz F, Ahmed A, Wallace, Sasangka, Warner R (1987) Dietary magnesium and urolithiasis in growing calves. Cornell Veterinaria 77, 33-45.
Kemp A (1960) Influence of fertiliser treatment of grassland on the incidence of hypomagnesaemic tetany (grass tetany in milking cows). Neth J Agric Sci 8, 281-304.
Kemp A. (1983): The effect of fertilizer treatment of grassland on the biological availability of magnesium to ruminants. . In: J.P. Fontenot, G.E. Bunce, K.E. Webb, V. Allen (eds), Proc. of John Lee Pratt international symposium on the role of magnesium in animal nutrition. Virginia Polytechnic Institute and State University Blacksburg, Virginia, 143-157.
Kemp A, Geurink J (1966) Further information on sodium requirement and sodium supply of lactating cows. Tydschrift Voor Diergeneskunde 91, 580-613.
Kemp A, Deijs W, Hemkes O (1961) Hypomagnesaemia in milking cows: intake and utilization of magnesium from herbage by lactating cows. Neth J agric Sci 9, 134-149.

Kemp A, Geurink J (1978) Grassland farming and minerals in cattle. Neth J Agric Sci 26, 161-169.
Kladnitsky O, Rozenfeld J, Azulay-Debby H, Efrati E, Zelikovic I (2015) The claudin-16 channel gene is transcriptionally inhibited by $1,25(OH)_2D_3$ VitD. Exp Physiol (in press).
Kobayashi N, Hirano T, Maruyama S, Matsuno H, Mukoujima H, Morimoto H, Noike H, Tomimatsu H, Hara K, Imakawa K, Nakayama H, Nakamaru T, Sugimoto Y (2000) Genetic mapping of a locus associated with bovine chronic interstitial nephritis to chromosome 1. Animal Genetics 31, 91-95.
Kolisek M, Nestler A, Vormann J, Schweigel-Röntgen M (2012) Human gene SLC41A1 encodes for Na^+/Mg^{2+} exchanger. Am J Physiol Cell Physiol 302, C318-C326.
Konrad, M, Schlingmann K, Gudermann T (2004) Insights into the molecular nature of magnesium homeostasis. Am J Physiol Renal Physiol 286, F599-F605.
Konrad M, Schaller A, Seelow D, Pandey AV, Waldegger S, Lesslauer A, Vitzthum H, Suzuki Y, Luk JM, Becker C, Schlingmann KP, Schmid M, Rodriguez-Soriano J, Ariceta G, Cano F, Enriquez R, Juppner H, Bakkaloglu SA, Hediger MA, Gallati S, Neuhauss SC, Nurnberg P, Weber S (2006) Mutations in the tight-junction gene claudin 19 (CLDN19) are associated with renal magnesium wasting, renal failure, and severe ocular involvement. Am J Hum Genet 79, 949-57.
Kronqvist C, Emanuelson U, Tråvén M, Spörndly, Holtenius K (2012) Relationship between incidence of milk fever and feeding minerals during the last 3 weeks of gestation. Animal 6, 1316-1321.
Kronqvist C, Emanuelson U, Spörndly, Holtenius K (2011) Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows. J Dairy Sci 94, 1365-1373.
Kunkel H, Barns K, Camp, B (1953) A study of sheep fed high levels of potassium bicarbonate with particular reference to induced hypomagnesemia. J Anim Sci 12, 451-458.
Lamb G, Stephenson D (1994) Effects of intracellular pH and Mg^{2+} on excitation-contraction coupling in skeletal muscle fibres of the rat. J Physiol 478, 331-339.
Lameris A, Nevalainen P, Reijnen D, Simons E, Eygensteyn J, Monnens L, Bindels RJ, Hoenderop J (2014) Segmental transport of Ca^{2+} and Mg^{2+} along the gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol doi: 10.1152/ajpgi.00093.2014. Epub ahead of print.
Lang I, Martens H (1999) Na transport in sheep rumen is modulated by a voltage-dependent cation conductance in the luminal membrane. Am J Physiol 277, G609 – G617.
Lean I, DeGaris P, McNeil D, Block E (2006) Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. J Dairy Sci 89, 669-684.
Leonhard-Marek S (1999) Do forestomach epithelia exhibit a $Mg^{2+}/2H^+$- exchanger? Magnesium Research 12, 99-108.
Leonhard-Marek S, Gäbel G, Martens H (1998) Effect of short chain fatty acids and carbon dioxide on magnesium transport across rumen epithelium. Exp Physiol 83, 155-164.
Leonhard-Marek S, Marek M, Martens H (1998) Effect of transmural potential difference on Mg transport across rumen epithelium from different breeds of sheep. J Agric Sci 130, 24 1-247.
Leonhard-Marek S, Martens H (1994) Influences of Na on magnesium transport across sheep rumen epithelium. Proc Soc Nutr Physiol 3, 88 (Abstract).

Leonhard-Marek S, Martens H (1996) Effects of potassium on magnesium transport across rumen epithelium. Am J Physiol 271,G1034-G1038.
Leonhard S, Smith E, Martens H, Gäbel G, Ganzoni E 1990: Transport of magnesium across an isolated preparation of sheep rumen: A comparison of $MgCl_2$, Mg Aspartate, Mg Pidolate, and Mg-EGTA. Magnesium Trace Element 9, 265-271.
Leonhard-Marek S, Stumpff F, Brinkmann I, Breves G, Martens H (2005). Basolateral Mg^{2+}/Na^{+} exchange regulates apical nonselective cation channel in sheep rumen epithelium via cytosolic Mg^{2+}. Am J Physiol 288, 630-645.
Leroy J (1926) Nécessite du magnesium pour la croissance de la souris. Comptes Rendus des Séances de la Société de Biologie 94, 432-433..
Li F, Chaigne-Delalande B, Kanellopoulou C, Davis JC, Metthews H, Douek, D, Cohen J, Uzel, G, S HC, Lenardo M (2011) Second messenger role for Mg^{2+} revealed by human T-Cell immunodeficiency. Nature 475, 471-476.
Li M, Jiang J, Yue L (2006) Functional characterization of homo- and heteromeric channel kinase TRPM6 and TRPM7. J Gen Physiol 127, 525-537.
Li M, Du J, Jiang J, Ratzan W, Su LT, Runnels LW, Yue L (2007) Molecular determinants of Mg^{2+} and Ca^{2+} permeability and pH sensitivity inTRPM6 and TRPM7. J Biol Chemistry 282, 25817-25830.
Littledike E, Cox P (1979) Clinical, minerals, and endocrine interrelationships in hypomagnesemic tetany. In: V.V. Randig and V.L. Grunes (Ed.) Grass TetanyAm Soc Agron Special Publ. 35. Madison, Wi. pp 1-49.
Levine B, Brautpar N, Walling M, Lee D, Coburn J (1980) Effects of vitamin D and diet magnesium on magnesium metabolism. Am J Physiol 239, E515-523.
Lüthi D, Güntzel D, McGuigan J (1999). Mg-ATP: Its modification by spermine, the relevance of cytosolic Mg^{2+} buffering, changes in intracellular ionized Mg^{2+} concentration and the estimation of Mg^{2+} by 31P-NMR. Exp Physiol 84, 233-352.
MacManus, J, Heaton, F, Lucas, P (1971) A decreased response to parathyroid hormone in magnesium deficiency. J Endocrinol **49,** 253-258.
Madsen F, Lentz D, Miller J, Lowrey-Harnden D, Hansen S (1976) Dietary carbohydrate effects upon magnesium metabolism in sheep. J Anim Sci 42,1316-1322.
Maltz E, Silanikove N (1996) Kidney function an nitrogen balance of high yielding dairy cows at the onset of lactation. J Dairy Sci 79, 1621-1626.
Marongiu A (1971) Assorbimento del Magnesio nei diversi segmenti del tubo digerente nell'ovino. Bolletino della Societa Italiana di Biologica Sperimentale 47, 768-770.
Martens H (1978) Lokalisation und Mechanismus der Magnesiumresorption bei Wiederkäuern. Übers Tierernähr 6, 75-90.
Martens H (1979) In vivo Untersuchungen über die Absorption von Magnesium aus dem Pansen von Schafen. Eine Abschätzung der maximalen Absorptionskapazität des Pansens. Berl Münch Tierärztl Wschr 92, 152-155.
Martens H (1981) Neue Erkenntnisse über den Magnesiumstoffwechsel bei Wiederkäuern. Übers Tierernährung 9, 233-258.
Martens H (1983) Saturation kinetic of magnesium efflux across the rumen wall in heifers. Br J Nutr 49, 153-158.
Martens H (1985a) The effect of dinitrophenol on magnesium transport across the isolated preparation of sheep rumen epithelium. Q J Exp Physiol 70, 567-563.
Martens H (1985b) Magnesium absorption from the temporarily isolated rumen of sheep. The effect of water absorption and osmotic pressure. Zbl Vet Med 32, 631-635.

Martens H (2013) Erkrankungen von Milchkühen in der frühen Laktationsphase – Risikofaktor negative Energiebilanz und Hyperketonämie. Tierärztliche Umschau 68, 463-476.
Martens H, Blume I (1986) Effect of intraruminal sodium and potassium concentrations and of the transmural potential difference on magnesium absorption from the temporarily isolated rumen of sheep. Q J Exp Physiol 71, 409-415.
Martens H, Gäbel G, Strozyk H (1987a) The effect of potassium and the transmural potential difference on magnesium transport across an isolated preparation of sheep rumen epithelium. Q J Exp Phys 72, 181-188.
Martens H, Hammer U (1981) Resorption von Natrium und Magnesium aus dem vorübergehend isolierten Pansen von Schafen während intravenöser Infusion von Aldosteron. Dtsch Tierärztl Wschr 88, 404-407.
Martens H, Harmeyer J (1978) Magnesium transport by isolated rumen epithelium of sheep. Res Vet Sci 24, 161-168.
Martens H, Heggemann G, Regier K (1988) Studies on the effect of K, Na, NH_4^+, VFA and CO_2 on the net absorption of magnesium from the temporarily isolated rumen of heifers. J Vet Med *A* 35, 73-80.
Martens H, Harmeyer J, Breves G, Scholz H (1976) Magnesiumabsorption *in vitro* durch die Pansenschleimhaut von Schafen. Z Tierphysiol Tierernähr Futtermittelkde 37, 44-52.
Martens H, Kubel OW, Gäbel G, Honig H (1987b) Effects of low sodium intake on magnesium metabolism in sheep. J Agric Sci 108, 237-243.
Martens H, Rabbani I, Zanming S, Stumpff F, Deiner D. (2012) Changes in rumen absorption processes during transition. Animal Feed Science and Technology (2012) 172, 95-102.
Martens H, Rayssiguier Y (1980) Magnesium metabolism and hypomagnesaemia. In Ruckebusch Y, Thivend P (eds): Digestive Physiology and Metabolism in Ruminants. MTP Press, p 447-466.
Martens H, Schweigel M (2000) Pathophysiology of grass tetany and others hypomagnesaemias. Vet Clin North America. Food Animal Practice 16, 339-368.
Martens H, Stössel EM (1988) Magnesium absorption from the temporarily isolated rumen of sheep: no effect of hyper- or hypomagnesaemia. Quart J Exp Physiol 73, 217-223.
Martens H, Stumpff F (2011) Ruminal Mg transport and assessment of Mg intake in dairy cows.Two sides of one coin (Abstract). J Dairy Sci 94 (Suppl. 1) 510.
Martin-Tereso Y, Martens H (2014) Ca and Mg physiology and nutrition in relation to the prevention of milk fever and tetany (Dietary management of macrominerals in preventing disease). Vet Clin North America. Food Animal Practice 30, 643-670.
Massry S, Coburn J (1973) The hormonal and non-hormonal control of renal excretion of calcium and magnesium. Nephron 10, 66-112.
McAlleese D, Bell M, Forbes R (1961) Magnesium-28 studies in lambs. J Nutr 74, 505-514.
McCoy M, Goodall, Kennedy D (1996) Incidence of bovine hypomagnesaemia in Northern Ireland and method of supplementation. Vet Rec 138, 41-43.
McGuigan J, Kay J, Elder H, Lüthi D (2007) Comparison between measured and calculated ionised concentrations in Mg^{2+}/ATP, Mg^{2+}/EDTA and Ca^{2+}/EGTA buffers; influence of changes in temperature, pH and pipetting errors on the ionised concentrations. Magnesium Research 20, 72-81.
Metson A, Saunders W, Collie T, Graham Y (1966) Chemical composition of pastures in relation to grass tetany in beef breeding cows. N Z J Agric Res 9, 410-436.

Meyer H, Busse F (1975) Zur rektalen Absorption von Magnesium bei Wiederkäuern. Dtsch Tierärztl Wschr 82, 140-141.
Meyer H, Scholz H (1972) Research into the pathogenesis of hypomagnesaemic tetany: 1. Relationship between the magnesium content of the blood and the cerebrospinal fluid in sheep. Dtsch Tierärztl Wschr 80, 55-61.
Meyer H, Scholz H (1973) Untersuchungen zur Pathogenese der hypomagnesämischen Tetanie. III. Toleranz von Schafen mit unterschiedlicher Mg-Versorgung gegenüber parenteralen NH_3-Gabe. Dtsch Tierärztl Wschr 80, 441-468.
Meyer T, Verwoert G, Hwang S, Glazer N, Smith A, van Rooij F, Ehret G, Boerwinkle E, Felix J, Leak T, Harris T, Yang Q, Dehghan A, Aspelund T, Katz R, Homuth G, Kocher T, Rettig R, Ried J, Gieger C, Prucha H, Pfeufer A, Meitinger T, Coresh J, Hofman A, Sarnak M, Chen Y, Uitterlinden A, Chakravarti A, Psaty B, van Duijn C, Kao W, Witteman J, Gudnason V, Siscovick D, Fox C, Köttgen A (2010) Genome-wide association studies of serum magnesium, potassium, and sodium concentrations identify six Loci influencing serum magnesium levels. PLOS Genetics 6, e1001045.
Miller J, Madsen F, Lentz D, Wong W, Ramsey N, Tysinger C, Hansard S (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, 1073-1079.
Moate P, Schneider K, Leaver D, Morris D (1987) Effect of 1,25 dihydroxyvitamin D_3 on the calcium and magnesium metabolism of lactating cows. Austr Vet J 64, 73-75.
Moore W, Fontenot J, Webb K (1972) Effect of form and level of nitrogen on magnesium utilization. J Anim Sci 35, 1043-1052.
Morris JG, Gartner RJW (1975) The effect of potassium on the sodium requirements of growing steers with and without α-tocopherol supplementation. Brit J Nutr 34, 1-14.
Muallem S, Moe O (2007) When EGF is offside, magnesium is wasted. J Cin Invest 117, 2086-2089.
Murayama J, Miyano A, Susaki Y, Kimura A, Sato S, Furumaha K (2013) Glomerular filtration rate in Holstein dairy cows estimated from a single blood sample using iodixanol. J Dairy Sci 96, 5120-5128.
National Research Council. Nutrient requirement of dairy cattle (2001) 7th revised ed. Washington,DC. Academy Press 128-131.
Newton G, Fontenot J, Tucker R, Polan C (1972) Effects of high dietary potassium intake on magnesium metabolism in sheep. J Anim Sci 35, 440-445.
Nijenhuis T, Renkema K, Hoenderop J, Bindels R (2006) Acid-base status determines the renal expression of Ca^{2+} and Mg^{2+} transport proteins. J Am Soc Nephr 17, 617-626.
Odell D, Waterfield E, Strewberry W, Gibson W, MacVicar R (1952) Effect of high dietary potassium on sheep. J Anim Sci 11, 790-791.
Oehlschleger V, Wilkens M, Schroeder B, Daenicke S, Breves G (2014) Effects of 25-hydroxyvitanim D_3 on localization and extent of gastrointestinal calcium absorption in cattle. Animal Prod Sci 54, 1394-1398.
Ohba Y, Kitagawa H, Kitoh K, Sasaki Y, Takami M, Shinkai Y, Kunieda T (2000) A deletion of the paracellin-1 gene is responsible for renal tubular dysplasia in cattle. Genomics 68, 229-238.
Ohba Y, Nakamura H, Okuda H, Kunieda T, Sasaki Y, Kitagawa H (2002) Renal reabsorption of magnesium and calcium by cattle with renal tubular dyplasia. Vet Rec 151, 384-387.
Paquay R, Lomba F, Lousse A, Bienfet V (1968) Statistical research on the fate of dietary mineral elements in dry and lactating cows. J Agric Sci 71, 173-180.

Paterson R, Crichton C (1960) Grass staggers in large scale dairying on grass. J Br Grassland Soc 15, 100-105.
Payne JM, Dew SM, Manston R, Faulks M (1970) The use of metabolic profile test in dairy herds. Vet Rec 129, 150-158.
Payne J, Rowlands G, Manston R, Dew S (1973) A statistical appraisal of the results of metabolic profile tests on 75 dairy herds. Br Vet J 129, 370- 381
Pearson P, Gray J, Reiser R (1949) The calcium, magnesium and potassium content of the serum of cows fed high levels of potassium. J. Anim Sci 8, 52-56.
Pearson P, Luthman J (1974) The effect of insulin, glucose and potassium content on some blood minerals in sheep. Acta Vet Scand 15, 519-532.
Perry S, Creigle R, Miller J (1967) Effect of ration upon the intestinal distribution of Ca, Mg, N, K and N in claves. J Nutr 93, 283-290.
Peterson A, Orth M, Goff J, Beed D (2005) Periparturient responses of multiparous Holstein cows fed different dietary phosphorus concentrations prepartum. J Dairy Sci 88, 3582-3594.
Pfeffer E, Rahman K (1974) Untersuchungen zur Lokalisierung der Magnesium-Absorption beim Wiederkäuer. Z Tierphys Tierern Futtmk 33, 209 (Abstract).
Phillipson A, Storry J (1965) The absorption of calcium and magnesium from the rumen and small intestine in sheep. J Physiol 181, 130-150.
Poutainen E (1971) Major mineral elements in the bovine rumen fluid II. The input, flow and absorption of calcium, magnesium, phosphorus and chloride. Annales Agriculturae Fenniae 10, 14-24.
Quamme G (1997) Renal magnesium handling: New insights in understanding old problems. Kidney International 52, 1180-1195.
Quamme G (1982) Effect of hypercalcemia on renal tubular handling of calcium and magnesium. Can J Physiol Pharmacol 60, 1275-1280.
Quamme G (2010) Molecular identification of ancient and modern mammalian magnesium transporters. Am J Physiol Cell Physiol 298, C407-C429.
Rahnema S, Fontenot J (1986) Effect of potassium on association of minerals with various fractions of digesta and feces of sheep fed hay. J Anim Sci 63, 1491-1501.
Ram L, Schoneville T, van‘t Klooster, A, Martens H, Beynen AC (1998) The decrease in the amount of magnesium absorbed in weathers fed potassium bicarbonate is independent of the dietary magnesium concentration. J Dairy Sci 81**,** 2485-2492.
Reuss L (1979). Mechanisms of sodium and chloride transport by gallbladder epithelium. Fed Proc 38, 2733-2738.
Rayssiguier Y (1977a) Hypomagnesaemia resulting from adrenaline infusion in sheep: Its relation to lipolysis. Horm Metab Res 9, 309-314.
Rayssiguier Y (1977b) The influence of thyroid and parathyroid glands on the variations in magnesemia in fed and fasted rats. Horm Metab Res 9, 161-163.
Rayssiguier Y, Garel, J, Davicco M, Barlet J (1977) Parathyroid hormone and calcitonin in magnesium-deficient calves. Horm Metabol Res 9, 438-439.
Rayssiguier Y, Libako P, Nowacki W, Rock E (2010) Magnesium deficiency and metabolic syndrome: stress and inflammation my reflect calcium activation. Magnesium Res 23, 73-80.
Reynolds C, Bell M, Sims M (1984) Changes in plasma, red blood cells and cerebrospinal fluid mineral concentrations in calves during magnesium depletion followed by repletion with rectally infused magnesium chloride. J Nutr114, 1334-1341.
Ritchie G, Kerstan D, Dai L, Kang H, Canaff L, Hendy G, Quamme G (2001) $1,25(OH)_2D_3$ stimulates Mg^{2+} uptake into MDCT cells: modulation by extracellular Ca^{2+} and Mg^{2+}. Am J Physiol Renal Physiol. 280, F868-878.

Roche J, Morton J, Kolver E (2002) Sulfur and clorine paly a non-acid base role in perparturient calcium homeostasis. J Dairy Sci 85, 3444-3453.
Rogers P, van't Klooster A (1969) The fate of Na, K, Ca, Mg and P in the digesta. Mededelingen Landbouwhogeschool 69, 26-39.
Romani A (2011) Cellular magnesium homeostasis. Arch Biochem Biophys 511, 1-23.
Romani AM (2012) Modulation of Mg^{2+} content in cardiac cells by α1-adrenoreceptor stimulation and anti-arrhythmic agents. Recent Pat Biotechnol 6, 212-222.
Romani M, Scarpa A (2000) Regulation of cellular magnesium. Front Biosci 5, d720-d734.
Rook J, Balch C (1958) Magnesium metabolism in the dairy cow. II. Metabolism during the spring grazing season. J Agric Sci 51, 199-207.
Ross D (1961) Influence of sodium on the transport of magnesium across the intestinal wall of the rat *in vitro*. Nature 189, 840-841.
Ross D (1962) *in vitro* studies on the magnesium transport across the intestinal wall of the rat. J Physiol160, 417-428.
Saez J, Berthoud V, Branes M, Martinez A, Beyer E (2003) Plasma membrane channels formed by connexins: their regulation and functions. Physiol Rev 83, 1359–1400.
Sansom B, Manston R, Vagg M (1983) Magnesium and milk fever. Vet Rec 112, 447-449.
San-Cristobal P, Dimke H, Hoenderop J, Bindels R (2010) Novel molecular pathways in renal Mg^{2+} transport: a guided tour along the nephron. Curr Opin Nephrol Hypertens 19, 456-462.
Sansom B, Allen W, Davies D, Hoare M, Stenton J, Vagg M (1976) Use of 1alpha-OH cholecalciferol in preventing post parturient hypocalcaemia and its potential value for the prevention of milk fever in dairy cows. Vet Rec 99, 310-312.
Scharrer E, Lutz T (1990) Effects of short chain fatty acids and K on absorption of Mg and other cations by the colon and caecum. Z Ernährungswiss 29, 162-168.
Schlingmann K, Gudermann T (2005) A critical role or TRPM channel-kinase for human magnesium transport. J Physiol 566, 301-308.
Schlüsing H (2000) Der Einfluss der Kaliumaufnahme auf die Magnesiumabsorption im Pansen von Schafen. Thesis – Freie Universität Berlin.
Schmitz C, Perraud A, Johnson C, Smith M, Penner R, Kurosaki T, Feig A, Scharenberg A (2003) Regulation of vertebrate cellular Mg^{2+} homeostasis bei TRPM7. Cell 114, 191-200.
Schneider K, Parkinson G, Houston J, Leaver D (1985) 1,25-Dihydroxyvitamin D_3 increases plasma magnesium and calcium in sheep fed liquid diets low in calcium and magnesium. Austr Vet J 62, 82-85.
Schonewille J, Beynen A (2005) Reviews on the mineral provision in ruminants (III): Magnesium Metabolism and Requirement in Ruminants. In CVB Documention Report Nr. 35. Central Veevoederbureau Lelystad. P. 13-26.
Schonewille J, Everta H, Jittakof S, Beynen A (2008) Quantitative prediction of magnesium absorption in dairy cows. J Dairy Sci 91, 271-278.
Schonewille J, Ram L, van't Kloster A, Wouterse H, Beynen A (1997a) Intrinsic potassium in grass silage and magnesium absorption in dry cows. Livest Prod Sci 48, 99-110.
Schonewille J, Ram L, van't Klooster A, Wouterse H, Beynen A (1997b) Native corn starch versus either cellulose or glucose in the diet and the effects on apparent magnesium absorption in goats. J Dairy Sci 80, 1738-1743.

Schonewille J, van ‘t Klooster A, Wouterse H, Beynen A (2000) Time courses of plasma magnesium concentrations and urinary magnesium excretion in cows subjected to acute changes in potassium intake. Veterinary Quarterly 22, 136-140.
Schonewille J, van’t Kloster A, Wouterse H, Beynen A (1999) Effect of intrinsic potassium in artificial dried grass and supplemental potassium bicarbonate on apparent magnesium absorption in dry cows. J Dairy Sci 82, 1824-1830.
Schweigel M, Kolisek M, Nikolic Z, Kuzinski J (2008) Expression and functional activity of the Na/Mg exchanger, TRPM7 and MagT1 are changed to regulate Mg homeostasis and transport in rumen epithelial cells. Magnesium Res 21, 1-6.
Schweigel M, Kuzinski J, Deiner C, Kolisek M (2009) Rumen epithelial cells adapt magnesium transport to high and low extracellular magnesium conditions. Magnesium Res 22, 1-18.
Schweigel M, Martens H (2003) Anion-dependent Mg^{2+} influx and a role for a vacular H^{+}-ATPase in sheep rumen epithelial cells. Am J Physiol 285, G45-G53.
Schweigel M, Lang I, Martens H (1999) Mg^{2+} transport in sheep rumen epithelium: evidence for an electrodiffusive uptake mechanism. Am J Physiol 277, G976-G982.
Schweigel M, Park H, Etschmann B, Martens H (2006) Characterization of the Na^{+}-dependent Mg^{2+} transport in sheep ruminal epithelial cells. Am J Physiol 290, G56-G65.
Schweigel-Röntgen M, Kolisek M (2014) SLC41 Transporters – Molecular identification and functional role. Current Topics in Membranes 73, 383–410.
Schweigel M, Voigt J, Mohr E (2009) Indication of intracellular magnesium deficiency in lactating cows revealed by magnesium loading and renal fractional excretion. J Anim Physiol Nutr 93, 105-112.
Schweigel M, Vormann J, Martens H (2000) Mechanisms of Mg^{2+} transport in cultured ruminal epithelial cells. Am J Physiol 278, G400-G4008.
Scott D (1965) Factors influencing the secretion and absorption of calcium and magnesium in the small intestine of sheep. Q J Exp Physiol 50, 312-329.
Scott D (1967) The effect of potassium supplement upon absorption of potassium and sodium from the sheep rumen. Q J Exp Physiol 51, 382-391.
Shareghi G, Agus Z (1982) Magnesium transport in the cortical thick ascending limb of Henle’s loop of the mouse. J Clin Invest 69, 759-769.
Simon D, Lu Y, Choate K, Velazquez H, Al-Sabban E, Praga M, Casari G, Bettinelli A, Colussi G, Rodriguez-Soriano J, McCredie D, Milford D, Sanjad S, Lifton R (1999) Paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption. Science 285, 103-106.
Sjollema B (1930) On the nature and therapy of grass staggers. Vet Rec 10, 425-430.
Sjollema B (1932) Untersuchungen über die Ursachen der Grastetanie und der großen Frequenzzunahme der Krankheit. Dtsch Tierärztl Wochenschr 40, 225-250.
Smith R (1959) The development and function of the rumen in milk-fed calves. J Agric Sci 52, 72-78.
Smith R, (1963) Net exchange of certain inorganic ions and water in the alimentary tract of the milk fed calves. Biochem 83, 151-163.
Stacy B, Warner A (1966) Balance of water and sodium in the rumen during feeding osmotic stimulation of sodium absorption in the sheep. Q J Exp Physiol 51, 79-93.
Steward J, Moodie E (1956) The absorption of magnesium from the alimentary tract of sheep. J Comp Path 66, 10-20.
Storry J (1961a) Studies on calcium and magnesium in the alimentary tract of sheep. 1. The distribution of calcium and magnesium in the contents taken from various parts of the alimentary tract. J Agric Sci 57, 97-102.

Storry J (1961b) Calcium and magnesium content of various secretions entering the digestive tract of sheep. Nature 190, 1197-1198.
Storry J, Rook, JAF (1963) Magnesium metabolism in dairy cow. V. Experimental observations with purified diet low in magnesium. J Agric Sci 61, 167-171.
Stuiver M, Lainez S, Will C, Terryn S, Günzel D, Debaix H, Sommer K, Kopplin K, Thumfart J, Kampik NB, Querfeld U, Willnow TE, Němec V, Wagner CA, Hoenderop J, Devuyst O, Knoers N, Bindels R, Meij I, Müller D (2011) CNNM2, encoding a basolateral protein required for renal Mg2+ handling, is mutated in dominant hypomagnesemia. Am J Hum Genet *88,* 333-343.
Stumpff F, Martens H (2007) The rumen and potassium homeostasis: A model. J AnimFeed Sci 16 (Suppl. 2) 436-441.
Terashima Y, Matsunobu S, Yanagisawa, Itoh H (1988) Calcium mobilization in hypomagnesemic whthers on a low magnesium and/or high potassium diet. Japan J Zootech Sci 59, 75-81.
Thomson A (1986) A magnesium-sensitive post-synaptic potential in rat cerebral cortex resembles neuronal responses to *N*-methylasparate. J Physiol 370, 531-549.
Thongon N, Krishnamra N (2011) Omeprazole decreases magnesium transport across Caco-2 monolayers. World J Gastroenterology 17, 1574-1583.
Todd J, Horvath D (1970) Magnesium and neuromuscular irritability in calves, with particular reference to hypomagnesaemic tetany. Br Vet J 126, 333-346.
Tomas F, Potter B (1976a) The site of Mg absorption from the ruminant stomach. Br J Nutr 36, 37-45.
Tomas F, Potter B (1976b) The effect and site of action of potassium upon magnesium absorption in sheep. Aust J Agric Res 27, 873-880.
Traysen J, Tarding F (1974) Ductual reabsorption of Na in 1:1 exchange for K in sheep parotid gland. In: Secretory Mechanism of Exocrine Glands (ED. N. A. Thorn and O. H. Petersen), pp. 464-472. Copenhagen Munksgaard.
Touyz R (2008) Transient receptor potential melastin 6 and 7 channels, magnesium transport, and vascular biology: implications in hypertension. Am J Physiol Heart Circ Physiol 294, H1103-H1118.
Urdaz J, Santos J, Jardon P, Overton M (2003) Importance of appropriate amounts of magnesium in rations for dairy cows. JAVMA 222, 1518-1523.
Van de Braak AE, van't Klooster AT, Malestein A (1987) Influence of a deficient supply of magnesium during the dry period on the rate of calcium mobilization by dairy cows at parturition. Res Vet Sci 42, 101-108.
Van der Wijst J, Hoenderop G, Bindels R (2000) Epithelial Mg^{2+} channel TRPM6: inside into molecular regulation. Magnesium Research 22, 127-
Vemana S, Pandey S, Larsson H (2008) Intracellular Mg^{2+} is a voltage-dependent pore blocker of HCN channels. Am J Physiol Cell Physiol 295, C557-C565.
Verdaris J, Evans J (1976) Diet calcium and pH versus mineral balances in Holstein cows 84 days pre- to 2 days postpartum. J Dairy Sci 59, 1271-1277.
Wada M, Tamura A, Takahashi N, Tsukita S (2013) Loss of claudins 2 and 15 from mice causes defects in paracellular Na flow and nutrient transport in gut and leads to death and malnutrition. Gastroenterology 144, 369-380.
Wang C, Beede D (1992) Effect of diet magnesium on acid-base status and calcium metabolism of dry cows fed acidogenic salts. J Dairy Sci 75, 829-836.
Ward, Parker (1999). Field evidence of metabolic stress in dairy cows? Occasional Publication No. 24 - Br Soc Anim Sci 24, 21-26.
Waterman D, Swenson T, Tucker W, Hemken R (1991) Role of magnesium in the dietary cation/anion balance equation for ruminants. J Dairy Sci 74, 1866-1873.

Weiss W (2004) Macromineral digestion by lactating cows: factors affecting Mg digestibility of magnesium. J Dairy Sci 87, 2167-2171.
Wilcox G, Hoff J (1974) Grass Tetany: An hypothesis concerning its relationship with ammonium nutrition of spring grasses. J Dairy Sci 57, 1085-1089.
Wilson R (1963) An attempt to induce hypomagnesaemia in whethers feeding high level of urea. Vet Rec 75, 698-699.
Wilson G, Reid C, Molloy C, Metson A, Butler G (1969) Influence of starch and peanut oil on plasma magnesium in grazing dairy cows. N Z J Agric Res 12, 467-488.
Wylie M, Fontenot J, Greene L (1985) Absorption of magnesium and other macrominerals in sheep infused with potassium in different parts of the digestive traxt. J Anim Sci 61, 1219-1229.
Xin Z, Tucker W, Hemken R (1989) Effect of reactivity rate and particle size of magnesium oxide on magnesium availability, acid-base balance, mineral metabolism, and milking performance of dairy cows. J Dairy Sci 72, 462-470.
Yamazaki D, Funato Y, Miura J, Sato S, Toyosawa S, Furutani K, Kurachi Y, Omori Y, Furukawa T, Tsuda T, Kuwabata S, Mizukami S, Kikuchi K, Miki H (2013) Basolateral Mg^{2+} extrusion via CNNM4 mediates transcellular Mg^{2+} transport across epithelia: A mouse model. PLOS Genetics 9, e1003983
Yano F, Horiuchi K, Matsui T, Yano H, Kawashima R (1984) Effect of 1α (OH)D_3 injection on Ca and Mg absorption in thyroparathyroidectomized sheep. Canad J Anim Sci, 64, 223-224.
Zhou H, Clapham D (2009) Mammalian MgT1 and TUSC3 are required for cellular magnesium uptake and embryonic development. PNAS 106, 15750-15755.

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Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.
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