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Magnesium homeostasis in cattle: absorption and excretion

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Abstract

Magnesium (Mg^{2+}) is an essential mineral without known specific regulatory mechanisms. In ruminants, plasma Mg^{2+} concentration depends primarily on the balance between Mg^{2+} absorption and Mg^{2+} excretion. The primary site of Mg^{2+} absorption is the rumen, where Mg^{2+} is apically absorbed by both potential-dependent and potential-independent uptake mechanisms, reflecting involvement of ion channels and electroneutral transporters, respectively. Transport is energised in a secondary active manner by a basolateral Na^+/Mg^{2+} exchanger. Ruminal transport of Mg^{2+} is significantly influenced by a variety of factors such as high K^+ concentration, sudden increases of ammonia, pH, and the concentration of SCFA. Impaired Mg^{2+} absorption in the rumen is not compensated for by increased transport in the small or large intestine. While renal excretion can be adjusted to compensate precisely for any surplus in Mg^{2+} uptake, a shortage in dietary Mg^{2+} cannot be compensated for either via skeletal mobilisation of Mg^{2+} or via up-regulation of ruminal absorption. In such situations, hypomagnesaemia will lead to decrease of a Mg^{2+} in the cerebrospinal fluid and clinical manifestations of tetany. Improved knowledge concerning the factors governing Mg^{2+} homeostasis will allow reliable recommendations for an adequate Mg^{2+} intake and for the avoidance of possible disturbances. Future research should clarify the molecular identity of the suggested Mg^{2+} transport proteins and the regulatory mechanisms controlling renal Mg excretion as parameters influencing Mg^{2+} homeostasis.

Key words: **Rumen: Epithelial transport: Tetany: Cows: Kidneys**

Introduction

Magnesium (Mg^{2+}) is an essential mineral[(1)] and its binding is of central importance for enzymic reactions after combining with the enzyme or substrate. The cytosolic concentration of the free, ionised Mg^{2+} ion is about 1 mmol/l, but the total intracellular concentration is much higher since numerous anions such as phosphate groups in nucleic acids or ATP^{4-} bind Mg^{2+}[(2)]. Furthermore, Mg^{2+} acts as a modulator of synaptic transmission in the central nervous system (CNS)[(3,4)], at the motoric endplate[(5)], in immunological pathways[(6)] and in timekeeping[(7)]. Importantly, Mg^{2+} is involved in the gating of ion channels[(8)]. Many transient receptor potential (TRP) channels are regulated by Mg^{2+} in a voltage-dependent manner[(9)] and are involved in the transport of cations across the ruminal epithelium[(10–12)].

The modulation of channel function in the CNS by Mg^{2+} is probably the reason for neurological symptoms such as ataxia, recumbency, convulsions, and finally tetanic muscle spasms in hypomagnesaemia and has been well known in cattle for some 80 years as grass tetany[(13,14)].

The present review attempts to outline the principles of Mg^{2+} homeostasis with particular emphasis on the site and mechanism of Mg^{2+} absorption, renal excretion and possible imbalances such as tetany.

Magnesium homeostasis

Plasma magnesium

Despite the absence of a (known) specific regulatory system, Mg^{2+} in plasma is kept within the range of 0·9–1·2 mmol/l, provided that influx (via absorption) into the extracellular space (ECS) including plasma is larger than the efflux (requirement and excretion). In humans, six genomic regions have been implicated in the maintenance of plasma Mg^{2+} concentration[(15)], and similar gene loci may explain the heritability of plasma Mg^{2+} concentration in dairy cows (0·20–0·43)[(16)].

Plasma Mg^{2+} is known to be influenced in a non-specific manner by catecholamines[(17)], insulin[(18)] and parathyroid hormone (PTH)[(19)]. In addition, epidermal growth factor has recently been shown to have magnesiotropic effects via TRPM6 channels, which regulate renal and intestinal Mg^{2+} absorption[(20)].

Distribution of magnesium

Some 60–70 % of total body Mg^{2+} is bound in the skeleton. A further 30 % is found in the intracellular space (ICS) but only 1–5 % of intracellular Mg^{2+} is in the ionised form[(21)]. The Mg^{2+} in the ECS only reflects about 1 % of total body Mg^{2+}.

Abbreviations: 1,25(OH)2D3, 1,25-dihydroxyvitamin D3; BW, body weight; CNNM, cyclin and CBS domain divalent metal cation transport mediator; CNS, central nervous system; CSF, cerebrospinal fluid; DCT, distal convoluted tubule; ECS, extracellular space; ICS, intracellular space; PD, potential difference; PTH, parathyroid hormone; TAL, thick ascending limb of Henle; TRP, transient receptor potential.

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Between 20 and 40 % of plasma Mg^{2+} is bound to albumin and globulin and some 10 % complexes with small anions such as citrate, phosphate and bicarbonate, so that 50–70 % are ionised.

According to a classical estimate[22], the total content of Mg^{2+} within the body of calves can be calculated from:

$$Mg\,(g) = (0{\cdot}655 \times BW\ (kg)) - 3{\cdot}5, \quad (1)$$

where BW is body weight in kg.

Provided that a similar relationship holds for adult ruminants, the total body Mg^{2+} of a cow with a BW of 700 kg should be roughly 455 g, of which approximately 320 g would be skeletal, about 130 g intracellular, while only about 4–5 g would be found in the total ECS.

Regulation of magnesium homeostasis

The range of plasma Mg^{2+} primarily depends on the influx of Mg^{2+} from the gastrointestinal tract into the ECS (a) and on the efflux from the ECS into milk (b), into the ICS including soft tissue and bones during growth and the fetus during pregnancy (c), and into the intestine as endogenous secretion (d). Mg^{2+} not required for b–d is excreted into urine (e). Plasma Mg^{2+} concentration thus depends on the daily Mg^{2+} balance and is given by:

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

where a is Mg^{2+} absorption (g/d) (influx), b is Mg^{2+} efflux in milk (g/d), c is Mg^{2+} uptake (efflux) into the ICS (g/d), d is intestinal Mg^{2+} secretion (efflux) (g/d), and e is renal Mg^{2+} excretion (efflux) (g/d).

A scheme of Mg^{2+} metabolism for a cow with a BW of 700 kg and a milk production of 40 kg/d is given in Fig. 1.

In an adult and not growing, non-pregnant cow, net uptake of Mg^{2+} into the ICS and bone at adequate Mg intake is marginal, so that equation (2) can be simplified to:

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

where a is Mg^{2+} absorption (g/d) (influx), b is Mg^{2+} efflux in milk (g/d), d is endogenous Mg^{2+} secretion (efflux) (g/d), and e is renal Mg^{2+} excretion (efflux) (g/d).

Because Mg^{2+} absorption (a) rarely equals Mg^{2+} efflux, additional mechanisms are necessary for the adjustment, which is very efficiently controlled by the kidneys at Mg^{2+} surplus. However, mobilisation from the skeleton or the cytosol is very limited[21]. This suggests that Mg^{2+} influx was very rarely limited during evolution. Obviously, Mg^{2+} intake and absorption (a) were generally above requirement (b + c + d), so that an efficient renal excretion of the surplus was sufficient for the regulation of Mg^{2+} homeostasis (e). Moreover, Mg^{2+} is not very toxic, and hence transient hypermagnesaemia (rapid influx > efflux) is well tolerated[23]. Therefore, absorption from the gastrointestinal tract is the key factor determining plasma Mg^{2+} levels, which can only be kept constant when the daily requirement is replaced by an adequate absorption. A better comprehension of the gastrointestinal absorption (influx) and its large variation[24] appears to be a key factor for understanding Mg^{2+} homeostasis.

Mg^{2+} absorption from the ruminant gastrointestinal tract

Site of magnesium absorption

Early studies *in vivo* suggested the distal part of the small intestine as the site of Mg^{2+} absorption[25] and Storry[26] stated that 'there is no evidence to assume that ... a mechanism exists

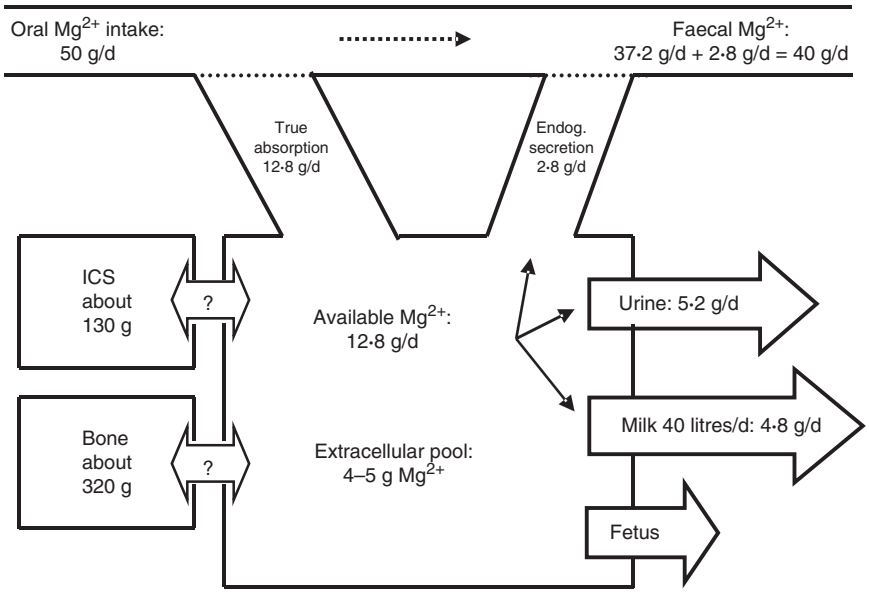

Fig. 1. Scheme of Mg^{2+} metabolism in a non-pregnant dairy cow of 700 kg body weight (BW). The daily Mg^{2+} intake is 50 g, with true Mg^{2+} absorption being 12·8 g/d (25·6 %). The true absorption is reduced by an endogenous (Endog.) secretion of 2·8 g/d (4 mg/kg), which accounts for an apparent absorption or Mg^{2+} digestion of 10 g/d (20 %). An amount of 14·8 g Mg^{2+}/d is used for 40 kg milk secretion (120 mg/l) and the surplus (5·2 g/d) is excreted via the kidneys into 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, as in sheep[26]. The unidirectional flow of Mg^{2+} into and out of the intracellular space (ICS) and bone is not known and net flux into the ICS or bone is zero at constant BW. In pregnant cows in late gestation a flux of 0·2 g Mg^{2+}/d towards the fetus has to be included[207].

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for the transport of calcium and magnesium across the rumen epithelium'. In contrast, Harrison *et al.*[27] observed Mg^{2+} absorption proximal to the duodenum and Marongiu from the rumen fluid[28]. In a later study, Rogers & van't Klooster[29] measured mineral flow rates along the gut and demonstrated absorption before the duodenum. Martens[30] summarised data from sheep and cows. The rate of absorption before the duodenum increased linearly with Mg^{2+} intake[31]. A net secretion was observed into the small intestine; the same amount was absorbed in the large intestine.

Regarding the location of Mg^{2+} absorption before the duodenum, uptake from the omasum but not from the abomasum was demonstrated[32,33]. However, Martens & Rayssiguier[34] showed that, *in vitro*, the transport capacity of the rumen epithelium was large and predominant.

Physiological significance of the rumen. An important finding was that Mg^{2+} absorption from the forestomachs was essential for maintaining normal plasma Mg^{2+} and a precondition for Mg^{2+} homeostasis[35]. Reduced Mg^{2+} absorption from the forestomach was not compensated for by absorption in the intestine[36].

Absorption from the large intestine. Mg^{2+} absorption was shown to switch from the hindgut to the developed rumen after dietary transition from milk to solid feed in calves[37] and lambs[38]. Mg^{2+} absorption from the hindgut is maintained in adult animals and can be used for the treatment of acute hypomagnesaemia[39].

Mechanism of ruminal Mg^{2+} transport

Scott[40] analysed the passive driving forces across the rumen epithelium and concluded that the chemical gradient of Mg^{2+} for passive movement from the rumen to plasma was opposed by the stronger electrical gradient (blood side positive 30–60 mV), which thus prevented passive paracellular uptake of the Mg^{2+} ion from the rumen to blood. Accordingly, Mg^{2+} transport across the rumen epithelium has to be energised.

The exclusion of passive paracellular diffusion suggests active, transcellular transport, which was deduced both from *in vitro* and *in vivo* experiments[41–43] showing: (a) net transport of Mg^{2+} from the rumen to blood; (b) saturation kinetics; (c) significantly lower transport at lower temperature; and (d) reduced transport by inhibition of Na^+/K^+-ATPase (ouabain or dinitrophenol). Furthermore, it was shown that 'bulk flow' could not explain ruminal Mg^{2+} transport[44], as had been proposed for rats[45].

The movement of Mg^{2+} across the multilayered epithelium includes: (a) uptake across the apical membrane; (b) the passage of Mg^{2+} across the various epithelial layers of the multilayered epithelium; and (c) release across the basolateral membrane. In cases where the passive gradients are sufficient, transepithelial transfer may further involve (d) possible paracellular passive movement.

Epithelial mechanisms. For many decades, characterisation of the transcellular transport pathway suffered from a lack of knowledge about Mg^{2+} transport. The existence of Mg^{2+} ion channels was still widely considered an unproven hypothesis. However, transport mechanisms for other ions (for example, Na^+ or Ca^{2+}) had been clearly established in other epithelia, such as in rabbit ileum[46]. Simply put, transport of ions across epithelia is either influenced by the transepithelial potential difference (PD_t) or not. By varying the electrical driving force for a specific ion (ξ)[46], and plotting the measured flux rate over ξ, it is possible to differentiate between the PD-independent flux (given by the *y*-intercept in the plot) and the PD-dependent flux (given by the slope of the plotted curve).

Potential-dependent Mg^{2+} uptake: The suggestion that Mg^{2+} transport occurred with the passage of a charge was deduced from a reciprocal relationship between an increase in ruminal PD_t and a decrease in ruminal Mg^{2+} transport[36,47]. PD_t can be calculated from the apical potential difference (PD_a) and the basolateral potential difference (PD_b): $PD_t = PD_a - PD_b$. In this relationship, the sign convention is such that the apical (ruminal) side is set to ground level and an increase in the passage of cations from the apical to the serosal (blood) side will lead to a more positive PD_t and a less negative PD_a, thus reducing the driving force for apical Mg^{2+} uptake. Mucosal to serosal Mg^{2+} transport rates (J_{ms} Mg^{2+}) revealed a linear correlation between ξ (PD_t) and J_{ms} Mg^{2+} within –25 and + 25 mV[48]. The obtained slope confirmed the suggestion of PD-dependent J_{ms} Mg^{2+} transport with uptake as an ion (for example, channel-mediated), but also exhibited an intercept of the *y*-axis, which represents a PD-independent component (for example, via co-transport).

A PD-dependent uptake mechanism for Mg^{2+} in the apical membrane is supported by data from microelectrode experiments. Leonhard-Marek & Martens[48] measured a PD_a under open circuit conditions of –67·3 mV (cytosol negative). An increase in the mucosal K^+ concentration depolarised PD_a and increased PD_t. These experiments suggested that the apical membrane is permeable to K^+, with non-selective cation channels from the TRP family such as TRPV3 and TRPA1 likely candidates[10].

In further flux measurements, Mg^{2+} transport was reduced not only by elevation of the PD_t but also by the apical K^+ concentration[47,48]. Depolarisation of PD_a by K^+ is the most likely explanation for the reduced mucosal to serosal flux of Mg^{2+} (J_{ms}) at high concentrations of K^+ (80 mmol/l) and argues for the uptake of Mg^{2+} by a PD-dependent mechanism. Since the ionised intracellular Mg^{2+} concentration (0·54 mmol/l)[49] is lower than the concentration of Mg^{2+} in the rumen, the uptake of Mg^{2+} is driven by the electrochemical gradient across the apical membrane.

The ruminal Mg^{2+} channel: The significant correlation between changes of PD_a and Mg^{2+} transport led to the suggestion of an apical Mg^{2+} channel[47], long before such channels were cloned. Channel-mediated transport of Mg^{2+} is now well established[50]. Thus, hypomagnesaemia in man is now known to be caused by the mutation of a channel of the TRP gene family, TRPM6[51]. TRPM6 plays a key role in the intestinal and renal absorption of Mg^{2+} in mice[50]. Expression of mRNA encoding for this protein by the rumen epithelium suggests a similar role in the ruminal absorption of Mg^{2+}[10].

A further member of this channel family, TRPM7, has been demonstrated in ruminal epithelial cells as mRNA[10,52] and

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protein[52] and is thought to play a role in intracellular Mg^{2+} homeostasis[53]. Since experiments in TRPM7-deficient mice by Ryazanova *et al.*[54] demonstrate disturbed intestinal Mg^{2+} absorption, an additional role in epithelial transport has been proposed. It has been suggested that both candidate genes are of functional importance for epithelial transport since both TRPM6 and TRPM7 subunits may be required to form a functional Mg^{2+} channel[55]. MagT1 is a further candidate gene for the PD-dependent uptake pathway in ruminal epithelial cells[52,56].

Potential difference-independent (electroneutral) Mg^{2+} uptake: In addition to the channel-mediated pathway, a second, PD-independent Mg^{2+} uptake pathway mediates Mg^{2+} transport[48]. The charge of Mg^{2+} is compensated by co-transport with anions or counter-transport of cations. Interestingly, the intake of high levels of readily fermentable carbohydrates[57] increased Mg^{2+} digestion. Furthermore, SCFA or CO_2 enhanced ruminal Mg^{2+} absorption *in vivo*[58] and stimulated J_{ms} Mg^{2+} *in vitro*[59]. Since both fermentation products acidify the epithelium, $Mg^{2+}/2H^+$ exchange has been proposed to represent this transport mechanism[59,60].

However, Schweigel & Martens[61] found no experimental evidence for directly coupled $Mg^{2+}/2H^+$ exchange in isolated ruminal epithelial cells of sheep and suggested a co-transport of Mg^{2+} with an anion such as HCO_3^- or Cl^-. Furthermore, the conductance of ruminal TRP channels for monovalent cations is activated by exposure to SCFA, possibly related to swelling of the cells[62,63]. This opens the possibility that the stimulation of Mg^{2+} by SCFA and CO_2 may not exclusively represent PD-independent Mg^{2+} transport but also involves stimulation of PD-dependent mechanisms. Finally, the activity of the ruminal vacuolar H^+-ATPase modulates Mg^{2+} transport[61], possibly by increasing PD_a and thus enhancing the uptake of Mg^{2+}. Such a mechanism would represent functional albeit not fixed Mg^{2+}/H^+ exchange. Currently, neither the stoichiometry nor the molecular identity of the PD-independent Mg^{2+} transporter is known.

Physiological consequences of two uptake mechanisms: Given that the rumen is the essential site of Mg^{2+} absorption under various feeding conditions, it has been proposed that both mechanisms work in parallel by 'job sharing' with an efficient uptake at all Mg^{2+} concentrations. At low ruminal Mg^{2+} concentrations, the PD-dependent and K^+-sensitive mechanism might mediate Mg^{2+} transport with high affinity and low capacity. This became apparent in experiments by Ram *et al.*[64] and Care *et al.*[42]. High ruminal K^+ intake reduced Mg^{2+} absorption to a higher extent at low ruminal Mg^{2+} concentration. Consequently, a possible negative effect of K^+ intake will be pronounced at high ruminal K^+ (> 50 mmol/l) and low ruminal Mg^{2+} (<2 mmol/l) concentration (see below).

Vice versa, the PD-independent and K^+-insensitive mechanism has a high capacity and low affinity and will thus primarily mediate transport at high Mg^{2+} (> 3 mmol/l) concentrations. This uptake mechanism relies exclusively on the chemical gradients of the involved ions and will rise with increasing Mg^{2+} concentration (Table 1).

Mg^{2+} transport within the epithelium: The rumen epithelium is a squamous multilayered epithelium forming a functional syncytium comparable with the classical model of frog skin[65]. Connections between cells of the various layers are formed by proteins such as connexin 43[66].

Basolateral extrusion: Mg^{2+} extrusion is related to the uptake of Na^+. Reduction of serosal Na^+ reduced J_{ms} Mg^{2+}[67] and in ruminal epithelial cells, the release or uptake of Mg^{2+} was dependent on the direction of the Na^+ gradient[68]. Furthermore, application of imipramine, an inhibitor of Na^+/Mg^{2+} exchange, reduced Mg^{2+} transport[12,68]. The characterisation of Na^+/Mg^{2+} exchange in HEK (human embryonic kidney) cells has revealed that the human gene *SLC41A1* (solute carrier family 41 member 1) encodes for this Mg^{2+}-transporting protein[69,70]. The Na^+/Mg^{2+} exchanger is indirectly energised by Na^+/K^+-ATPase[43]. Although evidence for the extrusion of Mg^{2+} from giant squid axons via Na^+/Mg^{2+} exchange had previously been obtained[71], ruminants were arguably the first mammalian species in which evidence for a (secondary) active epithelial Mg^{2+} transport could be obtained in an essential site of Mg^{2+} absorption (Fig. 2).

Passive paracellular Mg^{2+} transport: The flux of Mg^{2+} from the serosal to the mucosal side (J_{sm} Mg^{2+}) is entirely passive[48] with a permeability in the range of some 1×10^{-6} cm/s. This low passive flow rate limits passive transport and is unimportant under *in vivo* conditions.

Saturation of Mg^{2+} transport

Mg^{2+} transport saturates *in vitro*[41] and in studies *in vivo*[42,72,73] and probably includes the combined transport capacities of both uptake mechanisms. However, this saturation has never been observed in conventional balance studies. Weiss[74] and Schonewille *et al.*[24] analysed Mg^{2+} intake and digestion in cows and found a linear correlation between Mg^{2+} intake and Mg^{2+} digestion, respectively. The observed saturation under experimental conditions simulated, but very likely did not represent, the real *in vivo* conditions[42,72,73]. Mg^{2+} was almost certainly ionised in these model studies and available for transport[42,72,73]. It is to be assumed that in the normal rumen

Table 1. Characteristics of magnesium transport across the rumen epithelium

	Luminal Mg^{2+} uptake			
Ions	Driving force	Properties	Nomenclature	Basolateral Mg^{2+} extrusion
Mg^{2+}	Electrical gradient (PD_a)	High affinity Low capacity	PD-dependent K^+-sensitive	Na^+/Mg^{2+} exchanger
Mg^{2+} + anions (?)	Chemical gradient	Low affinity High capacity	PD-independent K^+-insensitive	Na^+/Mg^{2+} exchanger

PD_a, apical potential difference.

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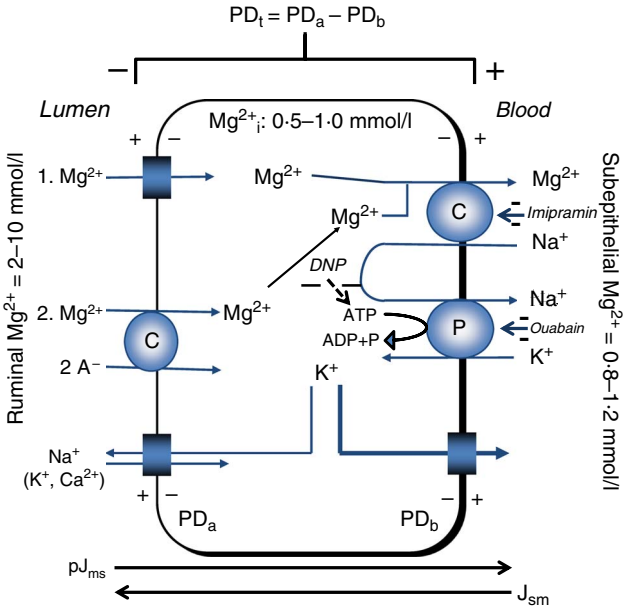

Fig. 2. Representation of transepithelial ruminal Mg^{2+} transport. The multi-layered epithelium is simplified to one compartment. Passive Mg^{2+} uptake is driven (1) mainly by the apical potential difference (PD_a) or (2) by the chemical gradient of the involved free ions. The PD-dependent uptake (1) is thought to involve homo- or heteromeric assemblies of the transient receptor potential channel proteins TRPM6 and TRPM7. The molecular identity of PD-independent (2) uptake is unknown. Basolateral extrusion occurs via Na^+/Mg^{2+} exchange via solute carrier family 41 member 1 (SLC41A1). The negative effects of inhibitors (–) on various steps of Mg^{2+} transport are printed in *italics*. pJ_{ms} and J_{sm} represent the passive flow through the paracellular pathway. The cylindrical scheme represents a channel. PD_t, transepithelial potential difference; PD_b, basolateral potential difference; Mg^{2+}_i, intracellular ionised (free) Mg^{2+}; DNP, 2,4-dinitrophenol; A^-, anion; C, carrier; P, pump (Na^+/K^+-ATPase). Example for PD_t (+15 mV) = PD_a (–45 mV) – PD_b (–60 mV). Depolarisation of PD_a by an increase of ruminal K^+ increases PD_t.

fluid, Mg^{2+} is only partially ionised. Indeed, chelating Mg^{2+} by EDTA severely depresses Mg^{2+} transport[75].

Modulation of ruminal Mg^{2+} transport

In one of his first publications, Sjollema[14] reported the composition of tetany-prone grass with high concentrations of K^+ and N, low concentrations of Na^+, while levels of Mg^{2+} were moderate but not low. Hence, this disease 'does not arise by inadequate intake of Mg^{2+} alone'[76]. Hypomagnesaemic tetany also occurred after changing the diet despite equal Mg^{2+} content[77], and a decrease in plasma Mg^{2+} was observed even with an increase in Mg^{2+} intake[78]. Today, there can be no doubt that various dietary factors interfere with Mg^{2+} transport.

The classical implications of K^+

High K^+ intake significantly reduced Mg^{2+} digestion, plasma Mg^{2+} concentration and, consequently, urinary excretion in sheep[79] and cows[80]. The reduced Mg^{2+} digestion was caused by a decrease in Mg^{2+} absorption and not by an increase in endogenous Mg^{2+} loss[81].

Site of K^+ effect. A higher K^+ intake reduced Mg^{2+} absorption from the forestomachs. This reduction was not compensated for in the small or the large intestine[36]. Furthermore, K^+ infusion into the abomasum or ileum did not affect Mg^{2+} absorption[82].

The effect of K^+ and Mg^{2+} concentrations. There is considerable evidence showing that the effect of K^+ depends on both ruminal K^+ and Mg^{2+} concentration.

Role of K^+ intake: Inhibition of Mg^{2+} absorption is pronounced between 1 and 3 % K^+ in DM and is attenuated at higher K^+ concentrations[83]. In agreement with this conclusion, Schonewille *et al.*[84] did not find a correlation between Mg^{2+} digestion and high K^+ content of the diet within the range of 2·9 to 4·4 % of DM. Notably, Martens *et al.*[58] observed that the absorption of Mg^{2+} from the temporarily isolated rumen of heifers dramatically decreased between 25 and 75 mmol K^+/l in the artificial rumen fluid, but not between 75 and 100 or 120 mmol K^+/l. This agrees with the logarithmic relationship between mucosal K^+ concentration and PD_a[48].

Role of Mg^{2+} intake: The proposed model of 'job sharing' (Table 1) of the two uptake mechanisms suggests that the effect of K^+ also depends on the Mg^{2+} concentration. The reduction of Mg^{2+} absorption by K^+ must be higher if Mg^{2+} is mainly transported via the K^+-sensitive, PD-dependent mechanism. Ram *et al.*[64] fed sheep increasing amounts Mg^{2+} at two levels of K^+ intake. Mg^{2+} absorption was reduced by 54 % at low Mg^{2+} intake and by 27 % at high Mg^{2+} intake.

The increase in K^+ intake elevates ruminal K^+[64] and reciprocally decreases Na^+ concentration[85]. Neither the rumen volume nor the passage rate was changed by K^+ intake, excluding dilution of ruminal Mg^{2+} concentration or enhanced outflow[64].

Meta-analysis of Mg^{2+} digestion: reduction by K^+

The quantity of the effect of K^+ on Mg^{2+} was analysed in a meta-analysis by Weiss[74] in cows, yielding the following relationship:

$$\text{Digestible Mg}^{2+} = 4{\cdot}5\ (\text{SEM}\ 4{\cdot}0)\text{g/d} + 0{\cdot}24\ (\text{SEM}\ 0{\cdot}07) \times \text{Mg}^{2+}\ \text{intake} - 4{\cdot}4\ \text{g/d}\ (\text{SEM}\ 2{\cdot}2) \times \text{K}^+, \quad (4)$$

where digestible Mg^{2+} and Mg^{2+} intake are given in g/d, and K^+ is given as % K^+ in DM (thirty-nine diets, 162 cows).

Schonewille *et al.*[24] performed a second meta-analysis with a different set of experiments and with a larger number of diets and cows, yielding:

$$\text{Mg}^{2+}\ (\text{true absorption}) = 3{\cdot}6\ \text{g/d}\ (\text{SEM}\ 0{\cdot}67) + 0{\cdot}2\ (\text{SEM}\ 0{\cdot}01) \times \text{Mg}^{2+}\ \text{intake} - 0{\cdot}08\ \text{g/d}\ (\text{SEM}\ 0{\cdot}014) \times \text{K}^+, \quad (5)$$

where Mg^{2+} true absorption and intake are given in g/d, and K^+ is given as g/kg in DM (sixty-eight diets, 323 cows).

True absorption can be transferred to apparent absorption (= digestible Mg^{2+}) by correction for endogenous Mg^{2+} secretion (700 kg BW × 4 mg/kg/d = 2·8 g/d)[86,87]:

$$\text{Mg}^{2+}\ (\text{apparent absorption}) = 3{\cdot}6\ \text{g/d} - 2{\cdot}8 + 0{\cdot}2 \times \text{Mg}^{2+}\ \text{intake} - 0{\cdot}08\ \text{g/d} \times \text{K}^+; \quad (6)$$

$$\text{Digestible Mg}^{2+} = 0{\cdot}8\ \text{g/d} + 0{\cdot}2 \times \text{Mg}^{2+}\ \text{intake} - 0{\cdot}08\ \text{g/d} \times \text{K}^+, \quad (7)$$

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Where Mg^{2+} (apparent absorption), digestible Mg^{2+} and intake are given in g/d, and K^+ is given in g/kg in DM.

At a K^+ concentration of 1% in the DM, the apparent Mg^{2+} digestion is slightly lower (20%) than in the calculation of Weiss[74] (24%). However, Mg^{2+} digestion is more depressed at low Mg^{2+} intake.

The linear decrease in digestible Mg^{2+} with rising ruminal K^+ (equations 4 and 7) is in contradiction to the discussed reduction of an effect of K^+ at a higher Mg^{2+} intake. The major reason is probably the experimental design. The experiments of Ram *et al.*[64] and Martens *et al.*[58] were performed under identical conditions. Equations 4 and 5 are the result of meta-analyses of many balance studies.

The role of Na^+

Insufficient Na^+ intake releases aldosterone and decreases Na^+ in both saliva and rumen fluid, while K^+ is increased[88–90]. Accordingly, Na^+ deficiency in sheep caused a decrease of Na^+ in saliva and rumen fluid, an increase of K^+ in both liquids, and an enhanced PD_t, while Mg^{2+} absorption from the rumen decreased (see Table 2)[90]. All of these changes were abolished by repletion of Na^+. Furthermore, intravenous infusion of aldosterone in sheep caused an increase in K^+ and a decrease in the Na^+ concentration in the rumen. Concomitantly, ruminal Mg^{2+} concentration rose, while plasma Mg^{2+} declined[91]. Since aldosterone alone does not change Mg^{2+} absorption[92], these effects are best explained by the aldosterone-induced elevation of the ruminal K^+ concentration.

Notably, K^+ concentration in saliva can reach some 100 mmol/l in Na^+-deficient animals. Assuming a salivary flow rate of 200 litres/d, this leads to a total influx of some 780 g K^+/d and presents a significant risk for reduced Mg^{2+} absorption. The condition is easily overlooked, because overt clinical signs of Na^+ deficiency are usually missing and because the large Na^+ pool in the rumen can be mobilised to cover deficiency for a long time[93]. Furthermore, as the K^+ concentration in the rumen fluid increases, the absorption of Na^+ is enhanced[85], which may help to compensate for Na^+ deficiency.

Young spring grass frequently contains extremely low concentrations of Na^+[94] and was suggested as a risk factor as early as 1966 by Metson *et al.*[95]: 'If low sodium is confirmed as yet another stress factor in the development of hypomagnesaemia, most of the present analyses [of grass] would undoubtedly qualify as tetany prone'. This suggestion is in agreement with the observation of Butler[96] about a negative relationship between the low Na^+ content of grass and the incidence of tetany. Vice versa, grass tetany caused by Na^+ deficiency can be prevented by supplementation with NaCl[97].

Table 2. Na^+ deficiency and high K^+ intake change the same rumen parameters and have identical effects on Mg^{2+} absorption

	Rumen			
	K^+	Na^+	PD_t	Mg^{2+} absorption
High K^+ intake	↑	↓	↑	↓
Na^+ deficiency	↑	↓	↑	↓

PD_t, transepithelial potential difference; ↑, increase; ↓, decrease.

Protein and ammonia

Tetany-prone young grass in spring exhibits a high concentration of crude protein[14], that causes an increase of up to 70 mmol/l ruminal ammonia[98] and is associated with grass tetany[99]. (The term ammonia is used without discrimination between NH_3 and NH_4^+. Chemical symbols are used when a specification is required.) Relationships between ammonia and Mg^{2+} absorption have been tested with contradictory results: both inhibition of Mg^{2+} absorption and no effect on Mg^{2+} digestion at high ruminal ammonia, depending on the experimental conditions. A decrease in Mg^{2+} absorption was observed at a sudden increase in ruminal NH_4^+ concentration. Intraruminal application of large amounts of ammonium acetate in cows caused a decrease both in plasma Mg^{2+} concentration and urinary Mg^{2+} excretion[76]. When working with sheep[34] or young heifers[58], respectively, Mg^{2+} absorption from the temporarily isolated rumen was severely reduced by increasing NH_4^+ concentrations which agrees with studies of the rumen pouch[42].

However, alterations in Mg^{2+} metabolism were not observed in chronic experiments with a delay in sampling after raising ruminal NH_4^+ concentrations[100,101]. These observations led to the hypothesis that an acute increase in ruminal NH_4^+ reduces Mg^{2+} absorption, but that when ruminal NH_4^+ remains elevated for a period of days, an adaptational response normalises Mg^{2+} absorption. Gäbel & Martens[101] tested this hypothesis *in vivo*. Acute addition of artificial rumen fluid with 40 mmol NH_4^+/l into the isolated sheep rumen significantly reduced Mg^{2+} absorption. In balance experiments, ruminal NH_4^+ was rapidly increased from 4·81 (SD 0·18) to 47·9 (SD 3·1) mmol/l within 1 d. Mg^{2+} excretion in urine transiently decreased from 385 to 255 mg/d over 2 d, but on the 3rd day, urinary Mg^{2+} increased and returned to control values, despite high ruminal NH_4^+ (36·1 (SD 4·8) mmol/l). Obviously, a sudden change in N intake and NH_4^+ concentration impairs Mg^{2+} absorption, but adaptation occurred within 3 d.

The reason(s) for the temporary reduction of Mg^{2+} absorption by NH_4^+ have not been studied. Ammonia is transported across the rumen epithelium both as NH_3 and NH_4^+, depending on the pH[102]. At a (physiological) pH of < 7·0, NH_4^+ is predominantly transported across cation channels in the apical membrane[10,102], decreasing PD_a[103] and increasing PD_t[101]. Since a variable fraction of the NH_4^+ that is taken up is extruded in the form of NH_3, protons are released and decrease the cytosolic pH[10,103], which stimulates apical Na^+/H^+ exchange and Na^+ transport[102]. These intraepithelial alterations of PD_a and intracellular pH (pH_i) offer some suggestions. Firstly, PD_a changes by some 10 mV when NH_4^+ is elevated to 40 mmol/l[103] which may inhibit channel-mediated uptake in analogy to what has been discussed for K^+. However, interactions between pH_i and Mg^{2+} transport are a further possibility. Thus, the enhanced uptake of Na^+ due to stimulation of Na^+/H^+ exchange[102] should elevate cytosolic Na^+ (Na^+_i), which can be expected to interfere with basolateral extrusion of Mg^{2+} via Na^+/Mg^{2+} exchange. The possible mechanisms of adaptation are still unclear.

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Ruminal pH

Only unbound Mg^{2+} in solution is available for transport across the ruminal epithelium and, accordingly, chelating Mg^{2+} by EDTA strongly reduces Mg^{2+} transport[75]. The range of free Mg^{2+} in the ruminal fluid varies from 34 to 77 % of the total amount[104,105] and depends on various factors[78,105,106]. One major factor determining the digestion of Mg^{2+} is the particle size of MgO[106]. Furthermore, free Mg^{2+} concentration in the rumen depends on pH[105]. The curvilinear relationship between rumen pH and Mg^{2+} solubility exhibits a steep slope between pH 5 and 7, which varies with diet[105,107,108] (Fig. 3). Most likely, increasing pH leads to the deprotonation of anionic binding sites in the ingested matter which are then available for binding of Mg^{2+}. An enhancing effect of low ruminal pH on Mg^{2+} digestion was suggested early on by Wilcox & Hoff[109], and is probably related to an increase in unbound Mg^{2+}. Horn & Smith[107] found a close and negative relationship between rumen pH and Mg^{2+} absorption before the duodenum. The obvious effect of pH on ionised Mg^{2+} is very likely the major reason for the influence of the diet on Mg^{2+} absorption, particularly with regard to carbohydrates. A causal relationship cannot be deduced from these studies, but the pH determines Mg^{2+} solubility with consequences for transport.

There is also reason to believe that Mg^{2+}-transporting proteins may be affected directly by changes in pH. Thus, patch clamp studies demonstrate that the conductance of monovalent cations is enhanced by a low pH in cells overexpressing TRPM6 and TRPM7[110]. At present it is unclear if Mg^{2+} conductance is similarly affected. Conversely, an acidic pH has been shown to decrease the expression of TRPM6 and other Mg^{2+}-transporting proteins[111], which probably contributes to the renal Mg^{2+} wasting that is observed in metabolic acidosis in man[21,112]. Interestingly, chronic usage of proton pump inhibitors impairs gastrointestinal Mg^{2+} absorption[113]. The possible role of ruminal pH in the aetiology of grass tetany is not clear, because both higher[107] or lower pH has been reported[114]. However, the close inverse correlation between ruminal pH and Mg^{2+} absorption before the duodenum[107] suggests that a high ruminal pH interferes with Mg^{2+} digestion, particularly at low DM intake in cold weather (H Meyer, personal communication) or as a consequence of pre-existing subclinical hypomagnesaemia with plasma Mg^{2+} concentration $\leq 0{\cdot}8$ mmol/l and no visible clinical signs such as ataxia or muscle spasms.

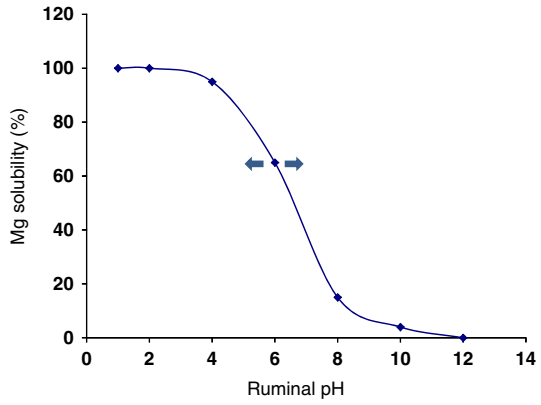

Fig. 3. Scheme of Mg^{2+} solubility in rumen fluid (redrawn from Dalley *et al.*[105]). The slope of Mg^{2+} solubility between pH 5 and 7 is influenced by the diet (←, →).

Mg^{2+} absorption and readily fermentable carbohydrates

A low level of fermentable carbohydrates in tetany-prone grass has been suggested to decrease Mg^{2+} availability[95]. Vice versa, drenching of grazing dairy cattle with a starch solution increased plasma Mg^{2+} concentration[115] and digestion of Mg^{2+}[57] although Mg^{2+} absorption was not consistently improved[116]. In ruminal fluid, the addition of fermentable carbohydrates causes: (a) an increase in the concentration of SCFA[117], (b) a decrease in pH[117], which (c) enhances Mg^{2+} solubility[105], (d) a decrease in NH_4^+ concentration, and (e) an increase of the number and size of rumen papilla[118], with the latter increasing the area for Mg^{2+} absorption[119]. Hence, Mg^{2+} digestion was enhanced in sheep by lactose[120].

The exact mechanism of the stimulation of Mg^{2+} transport by SCFA or HCO_3^-/CO_2 is not clear[60]. Notably, addition of fermentable carbohydrates to the diet with production of SCFA enhanced Mg^{2+} absorption from the caecum of rats[121] and, in mice, inulin increased Mg^{2+} absorption and expression of TRPM6 and TRPM7 in the hindgut[122]. In studies with goats, Schonewille *et al.*[123] have demonstrated that the depressive effect of K^+ can be compensated for by the addition of fermentable carbohydrates. Various reasons for this are conceivable. Influx of protonated SCFA with subsequent dissociation can be expected to lead to cell swelling, which, in turn, enhances monovalent currents both in cells hyperexpressing TRPM7 channels[124] and in native ruminal epithelial cells[62,63]. However, the most likely hypothesis is that the PD-independent pathway is stimulated by SCFA. Replacement of SCFA by gluconate significantly reduced the J_{ms} flux of Mg^{2+} and reduced uptake into cells[59,61]. This reduction in Mg^{2+} transport does not reflect binding by gluconate, because gluconate only weakly binds Mg^{2+}[125], and does not affect the epithelial transport of Mg^{2+}[126].

Mg^{2+} intake and digestion

The meta-analyses of Weiss[74] and Schonewille *et al.*[24] demonstrated a linear correlation between Mg^{2+} intake and digestible Mg^{2+}, suggesting a constant rate of Mg^{2+} absorption with no adaptation. However, McAleese *et al.*[127] orally dosed $^{28}Mg^{2+}$ in sheep and observed a higher $^{28}Mg^{2+}$ absorption at deficient Mg^{2+} intake. In line with these findings are the results of Schweigel *et al.*[52,56]: incubation of isolated rumen epithelial cells in a low- or high-Mg^{2+} medium caused a corresponding increase or decrease of in- and efflux mechanisms of Mg^{2+}. Although the expression of TRPM7 was only slightly altered, both the expression of the Na^+/Mg^{2+} exchanger[52,56,128], corresponding to SLC41[70], and the Mg^{2+} channel MagT1 increased significantly at low Mg^{2+} incubation and vice versa[52], supporting the assumption of the adaptation of Mg^{2+} transport at low Mg^{2+}.

Allsop & Rook[129] suggested that Mg^{2+} absorption is suppressed after increasing plasma Mg^{2+} concentration by intravenous infusion and concluded that 'the most probable major

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site of action is therefore on the uptake of Mg from the reticulorumen'. Martens & Stössel[23] tested this hypothesis and measured Mg^{2+} absorption from the isolated rumen in sheep. Mg^{2+} (net) transport was not influenced by increased plasma Mg^{2+} concentration or after 5 weeks of hypomagnesaemia, which is in contrast to the suggestion of McAleese *et al.*[127] and can probably be explained by the method used: sheep were orally dosed with equal amounts of $^{28}Mg^{2+}$, and the appearance of the isotope in blood was taken as Mg^{2+} absorption. However, it is highly likely that the ratio between the radioactive isotope ($^{28}Mg^{2+}$) and the total concentration of Mg^{2+} was much higher in Mg^{2+}-deficient sheep than in controls. Accordingly, a higher absorption of $^{28}Mg^{2+}$ into blood could be expected even without a change of the total rate of Mg^{2+} – a possibility that was not considered by the authors since absorption from the rumen was not known at that time.

Endogenous Mg^{2+} secretion

Storry[26] made an estimation of Mg^{2+} secretion in various secretions of sheep (saliva, gastric juice, bile, etc.) and estimated a daily secretion of 192 mg/d in a 40 kg sheep or 4·8 mg/kg (live weight), with similar secretion rates of 3·4 and 5·04 mg/kg found by Care[130]. A significant part of the endogenous Mg^{2+} loss is related to high flow rates of saliva. In sheep, Dua & Care[131] estimated a secretion of about 40 % of the Mg^{2+} amount in the ECS or 2–3 mg/kg (live weight). This involuntary endogenous loss of Mg^{2+} is not constant. In sheep on an artificial, low-Mg^{2+} diet, secretion dropped to 0·4–1·4 mg/kg[129], which is probably related to the linear correlation between plasma Mg^{2+}concentration and endogenous secretion of Mg^{2+} into the gut in general[132], and into the small intestine[31] or the bile in particular[130].

Schonewille & Beynen[87] summarised data for the endogenous Mg^{2+} secretion by dairy cows (within a range from 1·5 to 6·0 mg/kg) and proposed 4 mg/kg, a value that is also used by the Gesellschaft für Ernährungsphysiologie (German Society for Nutritional Physiology)[86].

Animal breeds and Mg^{2+} absorption

The digestion of Mg^{2+} in cows[133] and ruminal Mg^{2+} transport are influenced by animal breed[126]. Greene *et al.*[133] have shown that Mg^{2+} absorption is greater in Brahman than in Jersey, Holstein or Hereford cows. Leonhard-Marek *et al.*[126] measured the net Mg^{2+} transport *in vitro* across isolated rumen epithelium of four breeds of sheep (Merino, Schwarzkopf, Skudde and Heidschnucke). Skudde transported significantly less Mg^{2+} under short-circuit conditions. The wide variation of Mg^{2+} digestion seen in different studies might have a genetic background and may contribute to heritability of Mg^{2+} in plasma[16]. The significance of a genetic variation of Mg^{2+} transport proteins has been shown in man, where mutation of TRPM6 channels reduced transcellular Mg^{2+} transport in the intestine and kidney[50].

Vitamin D and Mg^{2+} homeostasis

PTH and vitamin D_3 are the principal regulators of Ca^{2+} metabolism. Interactions between PTH, calcitriol and Mg^{2+} in cows are well established[19,134,135], but the results are, in some cases, contradictory[136,137]. Calcitriol increased plasma Ca^{2+} and Mg^{2+} concentrations in hypomagnesaemic sheep[134] and Ca^{2+} concentration in cows, but decreased Mg^{2+} concentration[135,138]. A calcitriol-dependent uptake of Mg^{2+} into soft tissue has been suggested[135,139]. Calcitriol did not change faecal excretion in cattle[135], although calcitriol increased Mg^{2+} absorption from the rumen in sheep[140].

The infusion of bovine PTH in cows caused an increase in 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), Ca^{2+} and Mg^{2+} in plasma and a decrease in Mg^{2+} in urine[19], indicating enhanced Mg^{2+} resorption in the kidney. Dua *et al.*[141] observed a trend for increased Mg^{2+} absorption from the reticulo-rumen of sheep after the onset of PTH or PTH-related protein infusions.

While interactions between the PTH and $1,25(OH)_2D_3$ axis and Mg^{2+} metabolism can thus be observed, the physiological significance of this interaction is not clear. The effect of $1,25(OH)_2D_3$ on epithelial Ca^{2+} transport is classical and related to increased expression and activity of TRPV5 and TRPV6 channels[50]. These channels are non-selective cation channels with a high selectivity for Ca^{2+} over monovalent cations. However, a certain, albeit low, permeability to Mg^{2+} is to be expected. In summary, the possible stimulation of Mg^{2+} transport by $1,25(OH)_2D_3$ or effects of PTH should be considered as a side-effect of Ca^{2+} homeostasis.

Ionophores and Mg^{2+} digestion

Ionophores like monensin and lasalocid significantly increase Mg^{2+} digestion[142]. Both ionophores lowered ruminal K^+ concentrations in steers, suggesting a diminution of the reduction of K^+ on Mg^{2+} transport.

Sequestration of magnesium

A new environment, temperature changes or prolonged transport of animals may lead to a shift in the distribution of Mg^{2+} from the ECS into the ICS[143]. The stress hormone adrenaline has well-documented effects. Rayssiguier[17] intravenously infused adrenaline in sheep and observed a rapid decline in plasma Mg^{2+} concentration. This decrease was blocked by the β-receptor inhibitor propranolol. Adrenaline or theophylline stimulates lipolysis and increases NEFA, as a possible cause of sequestration[144]. Prevention of both lipolysis and increase in NEFA by application of sodium nicotinate abolished changes in plasma Mg^{2+} concentration in theophylline-treated sheep[145]. Furthermore, β-agonists such as adrenaline activate the Mg^{2+} channel TRPM7, stimulating uptake of Mg^{2+} into the cytosol[146]. Since TRPM7 is expressed throughout the body, a sequestration of Mg^{2+} into the cytosolic compartment is to be expected. The pathogenesis of transport tetany probably involves this adrenaline-dependent type of hypomagnesaemia[147]. It may also play a role as a secondary factor in classical grass tetany, in particular after the onset of the first clinical signs and may function as a trigger for tetanic muscle spasms.

Urinary Mg^{2+} excretion

Adjusted renal handling (influx ≠ efflux) is a precondition for the regulation Mg^{2+} homeostasis (Fig. 1) and includes two steps: filtration and re-absorption[21].

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Mg^{2+} filtration

Plasma Mg^{2+} varies from 0·9 to 1·2 mmol/l. Some 60–80 % or 0·48–0·96 mmol/l of plasma Mg^{2+} is ultrafiltrable so that, on a daily basis, roughly 29–59 g Mg^{2+}/d will appear in the glomerular filtrate of a cow of 650 kg BW (calculated with glomerular filtration rate (GFR) data of Murayama *et al.*[148]). Possible effects of GFR on Mg^{2+} filtration are not known.

Re-absorption proximal tubule. In the proximal tubule 20–30 % of the filtered Mg^{2+} is reabsorbed[21]. The fractional reabsorption rate in this part of the nephron is remarkably constant and probably occurs passively in an unregulated manner.

Re-absorption ascending limb of Henle. Most Mg^{2+} (60–70 %) is reclaimed in the thick ascending limb of Henle (TAL)[21]. The paracellular and passive transport in the TAL is mainly driven by PD_t (lumen positive). Energised by the basolateral Na^+/K^+-ATPase, this potential is generated by the apical uptake of Na^+, K^+ and Cl^- via NKCC2 with subsequent recycling of K^+ via renal outer medullary K (ROMK) channels and basolateral extrusion via ClC-Kb. Mg^{2+} absorption is mediated by the tight junctional channel protein, claudin-16 (paracellin-1), which interacts with claudin-19 to form a cation-selective channel[149,150]. Reduction of the passive driving force by blocking NKCC2 with furosemide[151] increases magnesuria in sheep[145]. Mg^{2+} transport in the TAL is stimulated by PTH[152] in rabbits and, accordingly, a reduced urinary excretion of Mg^{2+} has been found after PTH infusion *in vivo* in cows[19].

The passive transport across this pathway is regulated by Mg^{2+} availability. Hypomagnesaemia in mice increases both claudin-16 protein and mRNA abundance, while Mg^{2+}-loaded animals down-regulated claudin-16[153]. The expression of claudin-16 is inhibited by calcitriol[154] and further influenced by a variety of hormones such as glucagon, insulin, calcitonin, vasopressin or isoproterenol[155], which makes it difficult to evaluate these effects *in vivo*. Furthermore, Ca^{2+} transport via claudin-16 is reduced by Mg^{2+}[156]: 'A competitive transport of Mg^{2+} and Ca^{2+} via the common paracellular route in TAL could explain the coupling between Mg^{2+} and Ca^{2+} excretion'[157] (see below).

The remarkable roles of NKCC2, ROMK, CIC-Kb, and claudins 16 and 19 in Mg^{2+} homeostasis clearly emerge from genetic studies in human subjects[21]. Thus, Mg^{2+} homeostasis is severely impaired by a mutation of the claudin-16 gene[158]. Patients with this autosomal recessive disorder suffer from hypomagnesaemia, hypermagnesuria and hypercalciuria. In Japanese black cattle homozygous deletion (not mutation) of the claudin-16 gene has been reported[159,160], with reduced renal Mg^{2+} clearance and reabsorption[161].

Re-absorption distal tubule. Approximately 5–10 % of the filtered Mg is reabsorbed in the distal convoluted tubule (DCT) via active transport. Luminal Mg^{2+} uptake is mediated by TRPM6, driven by PD_a[21]. Renal TRPM6 is regulated by epidermal growth factor, which has been considered to be the first autocrine/paracrine magnesiotropic hormone[20]. Mg^{2+} deficit increases TRPM6 mRNA and protein expression in mice[162,163]. Neither PTH nor $1,25(OH)_2D_3$ stimulated TRPM6 expression in the kidney[162]. Interestingly, TRPM6 expression is influenced by the acid–base status of the animal. Metabolic acidosis decreases renal TRPM6 expression and thus increases Mg^{2+} excretion, whereas metabolic alkalosis led to the opposite effects[111]. The tight control of Mg^{2+} transport by TRPM6 has led to the conclusion that TRPM6 functions as a gatekeeper of Mg^{2+}. The efflux mechanism across the basolateral membrane is still uncertain, but may involve Na^+/Mg^{2+} exchange as in the rumen[12,128] or the intestine (cyclin and CBS domain divalent metal cation transport mediator 4; CNNM4)[164].

The adaptation of Mg^{2+} transport in the TAL and DCT has raised questions regarding the signalling cascade. Particularly intriguing is the rapid adaptation of Mg^{2+} excretion by the reabsorption of almost all filtered Mg^{2+} under low dietary Mg^{2+} intake, so that plasma Mg^{2+} concentration is almost perfectly maintained. Because mutation of the Ca-sensing receptor (CaSR) causes disturbances of Mg^{2+} homeostasis in man[165], the CaSR is emerging as an important player in the regulation of reabsorption of both Ca^{2+} and Mg^{2+} via luminal and basolateral sensing mechanisms[21,157]. More recently, Stuiver *et al.*[166] identified a protein (CNNM2), the mutation of which causes a disturbance in Mg^{2+} homeostasis. CNNM2 is located in the basolateral membrane of the TAL and DCT, and is up-regulated under Mg^{2+} deficiency. CNNM2 'might contribute to a Mg^{2+} sensing mechanism rather than transporting Mg^{2+} itself' and should thus considered to be a Mg^{2+} homeostatic factor[167].

Urinary Mg^{2+} excretion

The adaptation of renal Mg^{2+} transport activity in cows to various levels of intake has been illustrated by Schonewille[168] and Holtenius *et al.*[169]. Urinary excretion of Mg^{2+} rises in a quasi-exponential manner with plasma Mg^{2+} concentration. However, urinary Mg^{2+} drops rapidly with falling plasma Mg^{2+}, but levels off at 0·61–0·73 mmol/l, after which Mg^{2+} almost ceases to be excreted in urine[170]. Accordingly, a dairy cow with a plasma Mg^{2+} concentration < 0·8 mmol/l has to be considered at risk of hypomagnesaemia. This range of Mg^{2+} concentration appears to be a threshold. In a recent meta-analysis of Mg^{2+} metabolism in man, a concentration of ≥ 0·87 mmol/l leads to substantial urinary Mg^{2+} excretion[171].

Urinary Mg^{2+} excretion is a more sensitive indicator of Mg^{2+} availability than the plasma concentration. Rook & Balch[172] observed a much more pronounced decline of Mg^{2+} in urine than in plasma following a change in diet. The tight control of Mg^{2+} transport activity, particularly in the DCT[21] but also in the TAL[153], explains these classical observations.

The adjustment of renal Mg^{2+} excretion to changes in dietary intake with altered Mg^{2+} absorption (influx) not only ensures the maintenance of Mg^{2+} homeostasis in most feeding situations (Fig. 1), but also provides the practitioner with a diagnostic tool. According to the data of Kemp[173], Mg^{2+} influx can be considered to be sufficient at urinary $Mg^{2+} > 4·4$ mmol/l, while a range of 0·87–4·4 mmol/l might indicate a risk of Mg^{2+} shortage. Urinary $Mg^{2+} < 1$ mmol/l is probably a reliable indicator of insufficient intake/absorption.

Interaction of magnesium and calcium

Mutual interactions of transport between Ca^{2+} and Mg^{2+} have been observed[174]. Hypercalcaemia caused a large increase in

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urinary Mg^{2+} excretion. Vice versa and again in rats, infusion of Mg^{2+} caused an increase of urinary Ca^{2+} associated with a reduction in Ca^{2+} uptake via TRPV5[175]. A mutual interaction of Ca^{2+} and Mg^{2+} has also been found in cows, with negative interactions observed both on the level of the kidney[176] and the rumen[42,177].

Magnesium in milk

The Mg^{2+} concentration in milk is much higher than in plasma and exhibits a high heritability (0·60) in cows[178]. The higher Mg^{2+} concentration in milk requires active transport from plasma to milk. Nothing is known about this mechanism, which is most probably genetically determined and subject to modulation or regulation, leading to the wide variation in milk Mg^{2+} concentration. Cerbulis & Farrell[179] analysed Mg^{2+} in the milk of different breeds with a range of 99–120 mg/l, with one cow at 268 mg/l. The average concentration of Mg^{2+} in the milk of all animals was 112 mg/l, close to the recommendation of Schonewille & Beynen[87] of 120 mg/l. Assuming a milk yield of 30–40 litres/d, a cow will lose some 3–5 g Mg^{2+}/d, which approaches the total amount of Mg^{2+} in the ECF (see Fig. 1). It is important to realise that Mg^{2+} efflux via milk is continued probably with some (genetic) variation even in hypomagnesaemic cows[180] so that excretion of Mg^{2+} in milk exacerbates Mg^{2+} deficiency.

Goff & Horst[181] suggest that the concentration of Mg^{2+} in colostrum is 100 mg/l, although higher values of 238–322 mg/l were found by Shappel *et al.*[182] on the day of parturition in heifers and cows, with a rapid and exponential decline postpartum within 2 to 3 d to the normal level of 120 mg/l. The total amount in colostrum on the day of parturition amounted to 1·57–4·97 g/d. The rapid change of Mg^{2+} in milk after parturition probably explains the much higher concentration of Mg^{2+} in early colostrum[183]. Kehoe *et al.*[183] reported 733 mg/kg (range 230–1399 mg/kg) in the colostrum of fifty-five fully milked out cows from different herds within 4 h of calving. Assuming a volume of 5 litres yields a rough estimate of 3·6 g Mg^{2+} excretion in colostrum results, which underlines the significant Mg^{2+} demand at parturition.

Magnesium and tetany

Plasma Mg^{2+} and tetany

Sjollema[13,14] first demonstrated the relationship between the clinical symptoms of grass tetany and hypomagnesaemia. However, the Mg^{2+} concentration in the plasma of afflicted animals exhibits some variation (Table 3), and the severity of the nervous disturbances is not closely related to the plasma Mg^{2+} concentration[184]. Possibly, the speed of plasma Mg^{2+} decline promotes the onset of clinical manifestations[185].

Table 3. Status of Mg^{2+} metabolism and plasma Mg^{2+} concentration

	Blood Mg^{2+}	
Mg^{2+} status	mmol/l	mg/100 ml
1. Normal Mg^{2+}	0·9–1·2	2·19–2·92
2. Uncertainty	0·8–0·9	1·95–2·19
3. Subclinical hypomagnesaemia	0·7–0·8	1·70–1·95
4. Symptomatic hypomagnesaemia	< 0·7	< 1·70

At values below 0·9 mmol/l, both an adequate supply of Mg^{2+} or impending clinical hypomagnesaemia are possibilities, so that a safe assessment of Mg^{2+} status should involve a determination of urinary Mg^{2+} excretion. Even then, difficulties in judging Mg^{2+} status can be clearly seen in a study involving non-pregnant lactating cows with normal Mg^{2+} intake (29–32·5 g/d) and plasma Mg^{2+} concentration of 0·75–1·1 mmol/l[186]. After intravenous infusion of Mg^{2+} and despite a slight increase in plasma Mg^{2+} in four of the nine animals, the fractional renal Mg^{2+} excretion decreased, indicating Mg^{2+} retention after the Mg^{2+} load and pointing towards a possible Mg^{2+} deficit. Despite these uncertainties, low plasma Mg^{2+} concentrations almost invariably precede the onset of neurological symptoms with impaired function of the CNS.

Clinical hypomagnesaemia. Classical hypomagnesaemic tetany was originally observed a few days after cows had been let out to graze in spring[22]. At first sight, it appears surprising that the relatively large Mg^{2+} pools in the ICS (130 g) or bones (about 320 g) of cattle cannot acutely be mobilised to maintain physiological plasma Mg^{2+}[22], although a small mobilisation of 0·5 g/d has been reported in cows[170], comparable with observations in human subjects[21]. Mobilisation of Mg^{2+} from bone is unlikely, because the ratio between Ca^{2+} and Mg^{2+} in bone is 42 to 1, and substantial withdrawal from bone would disrupt Ca^{2+} homeostasis[187]. Furthermore, both PTH secretion and sensitivity of bone to PTH are decreased under conditions of hypomagnesaemia or alkalosis[188]. Cytosolic Mg^{2+} is only partly available for redistribution too; only 1–5 % is available in the ionised form with the rest bound primarily to ATP or sequestered in microsomes and mitochondria[21]. Accordingly, a massive efflux of Mg^{2+} from the cytosol into the ECS might interfere with cellular energy metabolism and cellular enzyme function.

Impaired function of the central nervous system. Hypomagnesaemic tetany is observed frequently as plasma Mg^{2+} drops below 0·7 mmol/l[189] and was originally suggested to be caused by impaired synaptic transmission at the motoric endplate[190]. This hypothesis was not confirmed by Todd & Horvath[191]. The possible involvement of the CNS was first discussed by Chutkow & Meyers[192] at low Mg^{2+} concentrations in the cerebrospinal fluid (CSF) of Mg^{2+}-deficient rats. The hypothesis of a decreased Mg^{2+} concentration in the CSF as a reason for clinical signs was tested by Meyer & Scholz[193] in Mg^{2+}-deficient sheep by measuring the Mg^{2+} concentration in plasma and CSF. They found that while the Mg^{2+} concentration in the CSF is kept constant over a wide range of plasma Mg^{2+} concentrations, it begins to decrease at plasma levels < 0·5 mmol/l so that at < 0·25 mmol/l, Mg^{2+} in CSF decreases almost linearly with the concentration in plasma. Allsop & Pauli[194] further tested the discussed causal correlation between Mg^{2+} in CSF and clinical signs. Mg^{2+} concentrations of < 0·25 mmol/l in the solution of CSF perfusion produced episodes of tetany that were abolished by higher Mg^{2+} concentrations. Because these effects were not accompanied by changes in blood parameters,

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the clinical symptoms were considered to be caused by the non-controlled activation of muscles by processes within the CNS. However, little is known about the regulation of Mg^{2+} in the CSF. After rectal infusion of $MgCl_2$, Reynolds *et al.*[195] observed that the Mg^{2+} concentration in the CSF remained constant in calves with normal plasma Mg^{2+}, while in calves with subnormal plasma Mg^{2+} (< 0·75 mmol/l), an increase in Mg^{2+} was observed in the CSF with a delay up to 120 min. These results suggest carrier-mediated transport into the CSF and might explain why a rapid decline of plasma Mg^{2+} causes a fall of Mg^{2+} in the CSF, whereas a slow decrease allows for sufficient Mg^{2+} transport into the CSF. This conclusion agrees with the observation of Allcroft & Burns[185] who suggested that the speed at which plasma Mg^{2+} level decreases is critical for triggering clinical symptoms.

There are a number of reasons why a drop of Mg^{2+} in the CSF might trigger hyperexcitability. Mg^{2+} is a physiological antagonist of Ca^{2+}-induced transmitter release at synapses[196], and low Mg^{2+} in the CSF might facilitate Ca^{2+}-dependent transmitter release and the excitation of CNS neurons that, amongst others, activate muscles. The activity of the glutamatergic NMDA receptor (*N*-methyl-D-aspartate) in the CNS is inhibited by external Mg^{2+} in a PD-dependent manner and at low Mg^{2+} in the CSF, more receptors are activated, which should result in hyperexcitability[3,197]. Furthermore, the activity of the inhibitory γ-aminobutyric acid (GABA) receptor is enhanced by Mg^{2+}. Conversely, the inhibitory effects of GABA are reduced when Mg^{2+} falls, facilitating neuronal activation[4]. Hence, a decrease of Mg^{2+} in the CSF induces hyperexcitability of excitatory neurons (NMDA) while reducing activity of inhibitory neurons (GABA).

The effect of Ca^{2+} concentration in the CSF on the onset of clinical symptoms is still controversial. Reynolds *et al.*[195] and Allsop & Pauli[194] observed diminished Mg^{2+} and Ca^{2+} concentrations in the CSF. However, plasma Ca^{2+} concentration did not correlate with clinical symptoms in sheep[189].

Subclinical hypomagnesaemia

It is important to note that the appearance of clinically relevant neurological symptoms is not obligatory, even when plasma levels of Mg^{2+} are low. Hypomagnesaemia of about 0·5 mmol/l was induced in sheep by feeding a low-Mg^{2+} diet for 5 weeks without appearance of any neurological symptoms[23]. It is very likely that Mg^{2+} concentration in the CSF is maintained when the induction of hypomagnesaemia with a low-Mg^{2+} diet is gradual (see above). It should be noted that even in the absence of clear neurological symptoms, animals may suffer from various non-neurological manifestations of hypomagnesaemia.

Interactions between hypomagnesaemia and the regulation of Ca^{2+} metabolism were observed early on. Thus, Allen *et al.*[198] showed a correlation between subclinical hypomagnesaemia and the occurrence of milk fever with plasma Mg concentrations of <0·8 mmol/l. Subclinical hypomagnesaemia has a negative effect on the release of PTH[136,199,200], the functioning of PTH on the target organ[201,202] and the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ (calcitriol)[203]. Moreover, in organ cultures of fetal rat bone, the release of Ca by supplementing $1,25(OH)_2D_3$ or PTH was reduced at low (<0·8 mmol/l) Mg concentration[204]. Furthermore, regulation of Ca homeostasis was found to deteriorate with induction of secondary hypocalcaemia in calves with hypomagnesaemia[200]. These results correspond very well with *in vivo* observations of Sansom *et al.*[205], who found that the mobilisation of Ca from bone was lowered significantly in cows with hypomagnesaemia. A subsequent study by van de Braak *et al.*[206] confirmed these findings.

Conclusions and perspectives

A correlation between the clinical symptoms of 'grass staggers' or 'grass tetany' and hypomagnesaemia more than 80 years ago initiated myriads of studies about Mg^{2+} metabolism in ruminants. These studies led to a stepwise improvement in understanding the pathogenesis: (a) hypomagnesaemia was not caused by a Mg^{2+}-deficient diet, but by reduced availability from the diet; (b) the site and mechanisms of Mg^{2+} absorption were described; and (c) the factors that influence Mg^{2+} transport and digestion were characterised. Despite a considerable increase in knowledge about the pathogenesis and prevention of hypomagnesaemic tetany, many open questions remain. Further work is necessary to identify if channels other than TRPM6, TRPM7 or MagT1 contribute to PD-dependent uptake of Mg^{2+} in the rumen. In particular, the role of various other TRP channels expressed by the rumen has to be clarified[10]. The PD-independent uptake mechanism is still not well characterised and its molecular identity is unknown[60,61]. Furthermore, the antagonism between Mg^{2+} and Ca^{2+} in the gut and the kidney deserves attention. In particular, renal excretion *in vivo* is of major interest, because renal Mg^{2+} transport is regulated according to the Mg^{2+} requirement. A better understanding of this mechanism could lead to improved diagnosis of the Mg^{2+} status of cattle.

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