A model of magnesium metabolism in young sheep: transactions between plasma, cerebrospinal fluid and bone

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An extension of the previously proposed model of Mg metabolism (Robson et al. 1997) has been developed to consider the transactions of Mg that are associated with cerebrospinal fluid (CSF) and bone. The representation of the CSF as a single Mg compartment with uptake from the plasma described by Michaelis–Menten kinetics gives very good agreement with published experiments. Analysis of the available information on resorption of Mg from adult bone indicates that this process makes a negligible contribution to Mg homeostasis and can be omitted from the model.

Magnesium: Mathematical modelling: Hypomagnesaemia: Grass tetany: Simulation

Hypomagnesaemic tetany occurs in ruminant animals when the Mg concentration of the cerebrospinal fluid (CSF) falls below approximately 0·66 mmol/l (Meyer & Scholz, 1972). Normally the concentration of Mg in CSF is higher than that of plasma (Meyer & Scholz, 1972; Beal & Bligh, 1977). Bito (1969) and Oppelt et al. (1963) have demonstrated that an active transport mechanism is involved in transporting Mg from blood to CSF, and Oppelt et al. (1963) further showed that diffusion and bulk filtration are responsible for the removal (of Mg) from the CSF.

Pauli & Allsop (1974) found that the onset of hypomagnesaemic tetany in two groups of four dairy cows, both grazing spring pasture, was more closely associated with low CSF Mg levels than with low plasma Mg levels. In the affected animals (group A) the CSF levels were 0·52 mmol Mg/l with plasma levels of 0·22 mmol Mg/l, whereas the unaffected animals (group B) had CSF and plasma levels of 0·77 and 0·16 mmol Mg/l respectively. Allsop & Pauli (1975a) showed that the CSF concentration in grazing dairy cows was significantly lower in clinically affected (0·51 mmol Mg/l) than in non-tetanic animals (0·73 mmol/l), despite the plasma Mg concentrations being similar (0·20 mmol Mg/l v. 0·15 mmol Mg/l respectively).

In a later experiment in sheep, Allsop & Pauli (1975b) were able to induce episodes of tetany by perfusion of the ventriculolumbar space with synthetic CSF containing <0·25 mmol Mg/l, and could abolish these tetanic episodes by perfusion with fluid of normal Mg concentration (1·00 mmol/l).

From these field and experimental observations, it is apparent that the clinical symptoms of hypomagnesaemic tetany are caused by a reduction in the CSF Mg concentration and it is thus important to understand the processes that contribute to low CSF Mg levels.

Bone has a Ca:Mg ratio of about 50:1 (Sykes & Russell, 2000) and so is a potential source of Mg for contributing to whole-animal Mg homeostasis, particularly in young animals. Field (1960) estimated the quantity of Mg available from bone in sheep to be 160–200 mg. This is equivalent to the estimated endogenous faecal loss for 1 d for an animal of 60 kg body weight (Agricultural Research Council, 1980). In cattle, Storry & Rook (1963) estimated the Mg available from bone to be about 0·5 g/d, which is small in comparison to the 2·4 g Mg required per d for the production of 20 litres milk.

The present paper reviews the mechanisms describing the inflow and outflow of Mg between plasma and CSF and develops a mechanistic description of them for inclusion in our earlier model of Mg metabolism in sheep (Robson et al. 1997). We also investigate the possible contribution that the Mg reservoirs in bone could make to Mg homeostasis.

Methodology

Relationship between plasma and cerebrospinal fluid magnesium

The relationship between the concentration of Mg in the CSF (y, mg/100 ml) and the Mg concentration in plasma...
Tables 1 and 2 of Robson and clearance rates of CSF. The notation used follows developments that describe the volume, production were used to extend this model to include a compartment mass balance equation:

\[
\frac{dQ_{Cs}}{dt} = U_{PicS} - U_{CpiS},
\]

where \( Q_{Cs} \) is the amount of Mg in the CSF, \( U_{PicS} \) is the rate of flow of Mg from plasma to the CSF and \( U_{CpiS} \) is the rate of flow of Mg from the CSF to plasma.

The detailed expressions and parameters for \( U_{PicS} \) and \( U_{CpiS} \) are derived in the following sections.

Cerebrospinal fluid volume

The relationship between brain mass (\( M_B \), g) and body weight (\( W \), kg) has been established for many mammals by Brody (1945) as:

\[
M_B = 9.96W^{0.697}.
\]

Other workers (see Peters, 1983, Appendix IVe) have found similar allometric relationships. Given that the specific gravity of brain tissue is 1.040 (Cserr, 1971), brain volume can be readily estimated.

The brain volume of Jersey cows was measured by Cox (1977), who found that animals with a mean body weight of 318 kg had a brain volume of 445 ml. This compares well with a value of 531 ml computed using equation 3. Cox (1977) also obtained the average CSF volume by two different experimental methods and reported a value of 186 ml as the average of the two methods. Consequently, CSF volume (\( V_{Cs} \)) was estimated as 42 % of the brain volume and is given by:

\[
V_{Cs}(ml) = 0.42 \times \frac{9.96}{1.04} W^{0.697} = 4.02W^{0.697}.
\]

Cerebrospinal fluid production and clearance rates

Cserr (1971) gives the CSF production rate across many mammalian species as 0.5 % CSF volume per min. This can be expressed as 7.2 \( V_{Cs} \) per d. CSF is produced mainly by the choroid plexus and is cleared back into the blood by bulk flow through the arachnoid villi.

The CSF volume as computed earlier (212 ml for a 320 kg animal) is small compared with the plasma volume estimated at 19.2 litres by Cox (1977) using a value of 60 ml plasma/kg body weight (Panaretto, 1964). Therefore, the effect of clearance of Mg from the CSF on the plasma Mg concentration will be negligible. Since the combined volume of brain and CSF system is contained in the fixed volume of the skull and spinal column, it is assumed that the CSF clearance rate is equal to the CSF production rate.

Magnesium uptake and clearance in the cerebrospinal fluid

The rate of flow of Mg (mol/d) from the CSF to the plasma is given by the product of the CSF clearance rate and the concentration of Mg in that secretion. Therefore:

\[
U_{CpiS} = 7.2V_{Cs}C_{Cs}.
\]

Using

\[
C_{Cs} = \frac{Q_{Cs}}{V_{Cs}},
\]

we can write:

\[
U_{CpiS} = r_{MgCpiS}Q_{Cs},
\]

where \( r_{MgCpiS} = 7.2/d \).

Transport of solutes across the choroid plexus requires energy and is sensitive to the effects of dinitrophenol and ouabain (Cserr, 1971). This energy dependency and the transport rate temperature sensitivity (Davson, 1967) are the hallmarks of an active transport process commonly represented by Michaelis–Menten kinetics (Martens et al. 1978). The saturation relationship in equation 1 is consistent with such a process. Michaelis–Menten kinetics describe the rate of change \( (\dot{y}) \) of a substance in or from a system in terms of the active concentration \( (x) \) of that
substance in the system such that:

\[ v = \frac{V_{\text{max}}}{1 + \frac{K_m}{C_{Pl}}} \]  

(8)

By plotting \( v \) vs. \( x \) as abscissa against \( v \) as ordinate (the Eadie–Hofstee plot), \( K_m \) and \( V_{\text{max}} \) can readily be obtained (Martens, 1983). In our case, \( x \) corresponds to the concentration of Mg in plasma \( (C_{Pl}) \) and \( v \) corresponds to the rate of flow of Mg from plasma to the CSF per unit volume of CSF, so that:

\[ U_{PlCs} = vC_{Cs} = \frac{V_{\text{max}}C_{Cs}}{1 + \frac{K_m}{C_{Pl}}}. \]  

(9)

The \( V_{Cs} \) term in equation 9 scales the flow rate of Mg between plasma and the CSF according to the volume of the CSF.

If we assume that the results reported by Meyer & Scholz (1972) were for animals whose CSF Mg concentrations were at steady state, we can set equation 2 to zero so that:

\[ U_{PlCs} = \frac{V_{\text{max}}C_{Cs}}{1 + \frac{K_m}{C_{Pl}}} = r_{MgCsPl}C_{Cs}C_{Pl}. \]  

(10)

Using the values from Meyer & Scholz (1972, Table 3) (i.e. measured values for \( C_{Pl} \) and \( C_{Cs} \)) we created an Eadie–Hofstee plot (Martens, 1983) based on equation 10 and obtained estimates for \( V_{\text{max}} \) and \( K_m \) of 7.890 and 0.112 mmol Mg/l respectively.

Testing the model of cerebrospinal magnesium

Our plan was to extend the model defined in Robson et al. (1997) by the addition of equations 2, 4, 7 and 9 with the necessary alterations to the plasma mass-balance equation being made. Here, we intended to first test equations 2, 7 and 9 to determine if they were a reasonable representation of the Mg dynamics between plasma and CSF.

Both Allsop & Pauli (1985) and Meyer & Scholz (1972) have reported experiments in which the time course of plasma and CSF Mg concentrations were measured in animals on various dietary Mg regimens. Allsop & Pauli (1985) carried out their experiment on six lactating Friesian cows that were fed a low-Mg diet for 16d. Two of these animals were given an intravenous infusion of Mg after being on the Mg-deficient diet for 10d. Meyer & Scholz (1972) carried out their experiment on sheep that were also fed a low-Mg diet. Both experiments were simulated using the model developed as described earlier. The measured plasma Mg concentrations were used as the values of \( C_{Pl} \). These were linearly interpolated where necessary. The initial values of both \( C_{Pl} \) and \( C_{Cs} \) used in the simulations were taken as the time zero values reported by the authors.

As we are working with concentrations of Mg in plasma and in the CSF and if we assume that the volume of the CSF \( (V_{Cs}) \) is constant, we can rewrite equation 2 with concentration as the state variable as follows:

\[ \frac{dC_{Cs}}{dt} = \frac{V_{\text{max}}}{1 + \frac{K_m}{C_{Pl}}} - r_{MgCsPl}C_{Cs}. \]  

(11)

This is independent of CSF volume \( (V_{Cs}) \).

For the Meyer & Scholz (1972) experiment, the values of both plasma and CSF Mg concentrations were taken from the 'Young animals' column of their Table 3, which shows the mean values of measurements taken from a number of animals as a function of the 'Depletion time'. Where the depletion time is given as a range of days, the midpoint of the range was used as the time value.

To simulate the procedure reported by Allsop & Pauli (1985) in which the plasma and CSF Mg concentrations were measured for 16d following the introduction of a depletion diet, the values of plasma and CSF Mg concentrations were taken from their Fig. 1. To simulate the procedure where 10d on a depletion diet was followed by an intravenous infusion of Mg, the plasma and CSF Mg values were taken from their Fig. 2. We compare our CSF results with their measurements from the ventricular region.

Rate of magnesium removal from bone

The skeleton contains approximately 80% of whole-body Mg (Larvor, 1983). Because there is no known mechanism for direct resorption of Mg from bone, this potential reservoir will not contribute significant amounts of Mg to assist with whole-animal Mg homeostasis unless the rate of Ca mobilization, and consequently the rate of Mg mobilization, is high. The rate at which Ca is mobilized from the skeleton is discussed in the following section.

Role of magnesium in bone turnover

Moate et al. (1980) studied the relationship between dietary Ca absorption in normal and hypomagnesaemic sheep in an attempt to ascertain whether or not Ca absorption in the gastrointestinal tract was affected by the level of Mg in plasma. Their hypothesis was that if hypomagnesaemia induced mobilization of Ca, and consequently Mg from bone, then the Ca requirement of the animal would be lowered and this would be observed as a reduction in dietary Ca absorption. They were unable to observe a change in the percentage of administered 45Ca that was absorbed between Mg-depleted sheep (plasma Mg concentration 0.38 mmol/l) and control sheep (plasma Mg concentration 0.81 mmol/l). They concluded that there was no effect on the gastrointestinal absorption of Ca, and therefore change in bone turnover, as a consequence of Mg deficiency.

Contreras et al. (1982) investigated the effects of normomagnesaemia and hypomagnesaemia (< 0.85 mmol/l) on the rate of Ca mobilization in cattle using an intravenous infusion of Na EDTA. Hypomagnesaemia reduced the rate of Ca chelation that these authors concluded reflected reduced mobilization from bone.

In a similar experiment, Terashima et al. (1988) examined the effects of diets known to influence the plasma Mg status of animals on the rate of Ca mobilization from
bone of wether sheep. Four diets were used (g/kg): control diet (Mg 2.3; K 7.6); low-Mg diet (Mg 0.4; K 7.5); control plus high-K diet (Mg 1.9; K 49.0); low Mg high-K diet (Mg 0.4; K 46.8). Plasma levels of Mg on these diets were 1.05, 0.55, 0.74 and 0.61 mmol/l respectively. As in the experiments of Contreras et al. (1982) discussed earlier, Ca mobilization was determined by intravenous infusion of EDTA for 60 min. The authors observed that 4.98, 3.83, 3.23 and 3.58 mmol Ca were mobilized during the infusion period. From these observations they concluded that hypomagnesaemic sheep may be susceptible to hypocalcaemia due to depressed ability for Ca mobilization. These findings are supported by endocrine studies that have shown low concentrations of parathyroid hormone in sheep (Shiga et al. 1980) and low responsiveness of the isolated perfused tibia of dogs to parathyroid hormone (Rude et al. 1978) in Mg deficiency.

Gardner (1973) found the rate of mobilization of Mg from body reserves of ewes subjected to peritoneal dialysis was 17–80 mg Mg/d. Haemodialysis with a Mg-free medium, however, produced mobilization rates of 20–30 mg Mg/h (480–720 mg Mg/d), which was sufficient to maintain the plasma level of Mg. When Ca was removed from an animal by dialysis with a Ca-free medium, there was a significant transfer of Mg from the animal to the dialysate. Conversely, when dialysis was performed with a Mg-free medium, there was a significant transfer of Ca from the animal to the dialysis medium. From these results, Gardner (1973) concluded that the site of this Mg reservoir was bone.

Van de Braak et al. (1986a,b) established that only dairy cows offered a low-Ca intake in association with low total feed intake and adequate Mg intake tended to increase Ca mobilization from bone when infused with Na EDTA in the peripartum period. They further demonstrated (Van de Braak et al. 1987a,b) that the rate of Ca mobilization from bone induced by Na EDTA infusion was reduced by a low dietary Mg intake. A further complication, if the rate of Mg release from the skeleton is dependent on bone turnover, is their observation (Van de Braak et al. 1987a) that bone Ca resorption makes only a minor contribution to the total Ca supply at parturition in older cows.

The work reported here investigates the level of plasma Mg at which Ca resorption is affected and whether or not it is possible to consider bone turnover as giving access to a reservoir which may be utilized by the animal for Mg homeostasis.

### Results and discussion

**Model of magnesium in the cerebrospinal fluid**

The simulation results of the Meyer & Scholz (1972) experiment and the two procedures from Allsop & Pauli (1985) are shown in Fig. 1 and Figs. 2 and 3 respectively. Given that there has been no adjustment of model parameters, the agreement between the observed and simulated CSF Mg concentrations is very satisfactory. It is important to note that, because equation 11 is independent of CSF volume, these results do not provide verification of equation 4, which estimates CSF volume from animal body weight.
deficient in that it considers the source of Mg to the cerebrospinal system to be only of choroid plexus origin. Cox (1977), however, states that he was unable to observe differences between the lumbar and ventricular CSF Mg concentrations, despite the fact that with 28Mg-labelled material he was able to observe a temporal difference in the arrival of radioactivity at these different sites in the CSF system. Allsop & Pauli (1985) observed that when depleted cows were repleted with intravenous Mg, the ventricular CSF Mg concentration was restored to normal levels within 24 h, but it was a further 48–72 h before the lumbar CSF was restored to normal levels. This also suggests Mg transport down a diffusional concentration gradient in the cerebrospinal column or alternatively, a secondary source of Mg transport into the CSF operating in the lumbar region.

The transport of Mg across the rumen wall is affected by intra-ruminal K concentration (Martens & Blume, 1986). Pauli & Allsop (1974) observed an increase in plasma K concentration in animals suffering from grass tetany and it is possible that similar K-dependent transport mechanisms operate at the choroid plexus as occur at the rumen wall. CSF is known to be electrically slightly positive with respect to blood by a few mV (Bito, 1969; Csern, 1971, p. 292), so the tranchoroidal potential could be generated by the K gradient. An effect of elevated K levels on the transport of Mg across the choroid plexus has been observed by Parkinson & Leaver (1980) in an anaesthetized sheep; mild hyperkalaemia (plasma K+ > 5 mmol/l) reduced, within minutes, the concentration of CSF Mg from 0·93 to 0·85 mmol/l (Parkinson & Leaver, 1980). There is support for this finding in the in vitro studies of Allsop (1986), in which choroid plexus Mg concentration decreased in response to an increase in K concentration in a perfusion medium introduced into the brain via an internal carotid artery.

It can be postulated that a possible mechanism for the onset of tetany is a stress reaction involving the adrenal–gluocorticoid axis, which increases plasma K+ concentration and, by similar mechanisms as pertain at the rumen wall, triggers the lowering of Mg transport across the choroid plexus. There is direct evidence, for example, that aldosterone can effect a direct and immediate change in Mg metabolism in the rumen (Charlton & Armstrong, 1989), and clinical experience certainly implicates stressful environmental conditions as trigger factors for clinical disease.

Hypocalcaemia has often been observed in association with clinical symptoms following prolonged hypomagnesaemia (Hemingway & Ritchie, 1965; Allsop & Pauli 1975a,b). The findings of Allsop (1986), from in vivo studies of the isolated perfused choroid plexus of sheep, that the rate of transfer of Mg into choroid plexus fluid was not affected by decreasing Ca concentration in the perfusing fluid suggests that the hypocalcaemia is a consequence rather than a cause of hypomagnesaemia, as argued in the next section, and therefore no attempt was made to allow for it in the model.

**Magnesium–bone interactions**

Table 1 summarizes data from the literature and presents derived values for a mean Ca resorption rate (R') that has been corrected for differing metabolic live weights (live weight0.75). When the derived values for R' are plotted

![Fig. 3. Simulation of the procedure reported by Allsop & Pauli (1985): plasma and cerebrospinal fluid magnesium concentrations were measured in two cows on a magnesium-deficient diet. After 10 d the animals were given an intravenous infusion of magnesium.](https://www.cambridge.org/core/journals/biological-and-nutritional-chemistry/article/fig/3/Simulation-of-the-procedure-reported-by-Allsop-Pauli-1985-plasma-and-cerebrospinal-fluid-magnesium-concentrations-were-measured-in-two-cows-on-a-magnesium-deficient-diet-After-10-d-the-animals-were-given-an-intravenous-infusion-of-magnesium)

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W, live weight; R’, calcium resorption rate corrected for metabolic live weight.
against the serum Mg levels, as shown in Fig. 4, a quadratic response is evident \((P<0.1)\). This is interpreted to mean that when the serum levels of Mg are greater than the turning point \((\text{approx. 0.91 mmol/l})\), a reduction in serum Mg tends to increase the Ca mobilization from bone. Conversely, when the Mg serum levels are \(<0.91 \text{ mmol/l}\), there is a decrease in the rate at which Ca is mobilized from bone. These biphasic responses are consistent with the findings of Buckle et al. (1968) of reductions of parathyroid hormone in parathyroid venous plasma when the Mg concentration in the perfusion medium of the isolated gland was increased and the reverse trend when the Mg concentration in the perfusion fluid was reduced.

It thus appears that the mobilization of Ca from bone occurs only when the Mg levels are near normal and when the plasma Mg falls the potential for bone resorption is inhibited. Littledike et al. (1983) observed that the ability of the bone to supply Ca and Mg to the blood is progressively inhibited as the extracellular Mg concentrations decrease in Mg deficiency. Both Terashima et al. (1988) and Van de Braak et al. (1987b) noted a reduction in Ca mobilization from bone when the plasma Mg levels were lowered, while Littledike et al. (1983) observed that as hypomagnesaemia became severe, acute marked decreases in plasma hydroxypoline concentrations also occurred, a further indication that bone resorption was inhibited.

From this analysis, it is clear that in general bone acts as a small reservoir of Mg. However, with a maximum Ca resorption rate of approximately \(0.27 \text{ mmol/h per kg live weight}^{0.75}\) (the maximum value in Fig. 4), a 500 kg animal could mobilize about 28.5 mmol Ca from bone/h. This value can be used to calculate the flux of Mg into the extracellular fluid in association with bone Ca turnover, on the assumption that Ca and Mg are resorbed non-preferentially. This assumption may require some caution in the light of the findings of Neuman & Mulryan (1971) that in synthetic apetite crystals produced in vivo, Mg was located in readily exchangeable surface positions and may therefore be preferentially mobilized. On the other hand, there is overwhelming evidence from direct mineral analysis of the whole skeletons of sheep during changes of skeletal mineral content of up to 30% that the Ca:Mg ratio remains constant (Sykes & Field, 1972; Sykes et al. 1974) or may even decrease in Ca deficiency (Field et al. 1975); this led Field et al. (1975) to speculate that Mg may substitute for Ca in skeletal mineral during Ca deficiency.

Assuming that Ca and Mg are resorbed from bone non-preferentially, and with a 50:1 ratio of Ca:Mg, this means that a maximum of \(0.57 \text{ mmol Mg/h}\) can be mobilized from the skeleton. This amounts to \(0.33 \text{ g Mg/d}\) for cattle, comparable with an independent estimate of \(0.5 \text{ g/d}\) (Storry & Rook, 1963). For a 25 kg sheep the comparable value is \(0.035 \text{ g Mg/d}\). These values are very small compared with either the daily endogenous losses of cattle or sheep (1500 and \(0.075 \text{ g Mg/d}\) respectively) or to the normal daily urinary excretion of approximately \(3.0 \text{ g Mg/d}\) and \(0.5 \text{ g Mg/d}\) respectively.

Consequently, the quantitative contribution to Mg dynamics from bone resorption is not considered to be an important component in our present model.

Conclusions

The extension of the model proposed here is able to account well for the dynamics of CSF Mg concentration observed in hypomagnesaemic ruminant animals. The possible quantitative contribution of bone turnover as a mechanism for Mg homeostasis has been discounted.

Acknowledgement

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References


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