A model of magnesium metabolism in young sheep. Magnesium absorption and excretion

BY A. B. ROBSON[1], A. C. FIELD[2], A. R. SYKES[2] AND A. E. McKINNON[1]

[1]*Centre for Computing & Biometrics, and* [2]*Animal & Veterinary Sciences Group, Lincoln University, Canterbury, New Zealand*

A model of Mg metabolism in sheep is proposed. It is based on standard Michaelis–Menten enzyme kinetics to describe the transport of Mg across the rumen wall and passive diffusion to describe the absorption of Mg in the hindgut. Factors known to have an effect on Mg metabolism in farm animals, namely the concentrations of K and Mg in the diet, and the physico-chemical conditions within the rumen as determined by the type of diet, are incorporated into the model. Consideration of the rumen as the only site of Mg absorption provided an inadequate mechanistic description of Mg metabolism in sheep. To ensure compatibility between predicted Mg absorption and recent independent data sets for Mg balances, it was necessary to include in the model aspects of Mg absorption that operate in the hindgut. The results from this model suggest that there is a need for a series of experiments to determine the important aspects of Mg transport in the hindgut of sheep. Mechanisms of homeostasis are discussed.

Magnesium: Mathematical modelling: Hypomagnesaemia: Grass tetany

Hypomagnesaemia and the clinical deficiency hypomagnesaemic tetany, have been the focus of many studies during several decades (Wilson *et al.* 1969; Wilcox & Hoff, 1974; Larvor, 1976). These have shown the importance of many confounding interactions with dietary and other environmental factors. Thus although there are several valuable reviews of Mg metabolism (British Veterinary Association, 1960; Martens & Rayssiguier, 1980) and whole symposia devoted to the subject (Fontenot *et al.* 1983) a real understanding of the quantitative importance of the several processes involved has not been achieved.

A hierarchically structured and nested model was built on existing knowledge to enable prediction of the dynamic changes of Mg metabolism within a single animal, taking into account interacting dietary factors, and to create an integrative platform for the experimental testing of various hypotheses.

THE MODEL

The form of the model, shown in Fig. 1, is based on compartments and fluxes between them (Godfrey, 1983). Flow of material occurs between compartments and these flows are controlled by various physico-chemical processes. The general notation used to represent compartments and fluxes, together with appropriate units, is given in Tables 1 and 2. Basically the physical parameters associated with each compartment are quantity, concentration, surface area and volume for those compartments in the liquid phase
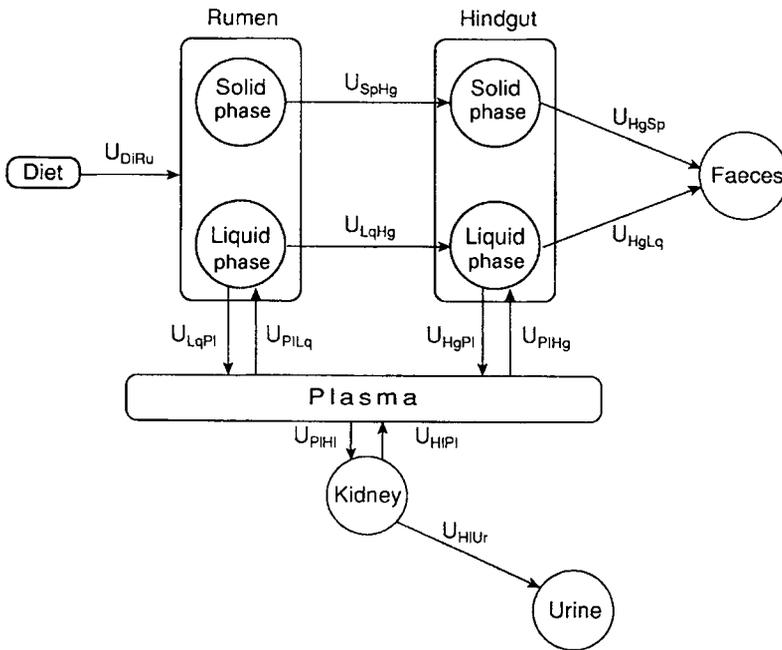
Fig. 1. Model of magnesium metabolism. For details of abbreviations, see Table 2.

Table 1. *Notation and units*

Notation*	Units	Description
$C_{i,j}$	mol/l	Concentration of substance i in compartment j
k_{jk}	–	General constant relating compartment j and compartment k
$K_{i,jk}$	mol/l	Michaelis–Menten affinity constant for transport of substance i between compartment j and compartment k
P_{jk}	mV	Potential difference between compartment j and compartment k
Q_j	mol	Quantity in compartment j
$r_{i,jk}$	/d	Fractional rate of passage of substance i from compartment j to compartment k
S_j	m^2	Surface area of compartment j
$U_{i,jk}$	mol/d	Flux of substance i from compartment j to compartment k
$V_{i,jk}$	mol/d	Michaelis–Menten saturation constant for transport of substance i between compartment j and compartment k
V_{bf}	litres/d	Volume blood flow
V_j	litres	Volume of compartment j
$\theta_{i,jk}$	–	Steepness parameter for transport of substance i between compartments j and k

* When i is omitted it is always magnesium. jk take values from Table 2.

while quantity alone is the parameter associated with those compartments in the solid phase.

In the model, the gut is represented by two major functional compartments. These can be closely allied to the reticulo-rumen, and a post-ruminal site denoted herein as the hindgut, and are depicted as the two pairs of compartments in the upper half of Fig. 1. In each compartment the amount of Mg is partitioned between a liquid phase and a solid

Table 2. *Labels for compartments*

Label	Description	Comment
Bw	Body weight	Not a compartment
Di	Diet	Not a compartment
Fa	Faeces	Not a compartment
Hg	Hindgut	
Hl	Henle loop (in kidney)	Not a compartment
Lq	Liquid phase compartment	
Pl	Plasma	
Ru	Total rumen compartment	
Sp	Solid phase compartment	
Ur	Urine	Not a compartment
Xm	Maximum (in some sense)	Not a compartment

phase form, with the liquid phase form being defined as that Mg which can be readily absorbed.

Plasma is the central compartment in the model. Further development of the model to include compartments representing cerebrospinal fluid and bone will be considered in a subsequent paper (A. B. Robson and A. R. Sykes, unpublished results). The net losses of Mg from the plasma that occur when female animals are either pregnant or lactating are not currently considered in this model.

General kinetics

The transport processes described herein use standard enzyme kinetics as applied to dynamic models (Gill *et al.* 1984, France *et al.* 1987). The transport of a substance i, at a concentration $C_{i,j}$, from the source compartment j to the sink compartment k is described by the flow rate $U_{i,jk}$ given by:

$$U_{i,jk} = \frac{V_{i,jk}}{\left[1 + \dfrac{K_{i,jk}}{C_{i,j}}\right]} \quad (1)$$

where $V_{i,jk}$ is the maximum velocity of transport and $K_{i,jk}$ is the Michaelis–Menten constant. This equation describes a process which proceeds linearly at low values of $C_{i,j}$ and then becomes saturated when $C_{i,j}$ exceeds $K_{i,jk}$.

In order to describe the partitioning of materials between the solid and liquid phases in the model, the sigmoidal response curves provided by Thornley (1976) and used in Gill *et al.* (1984) are used here. These sigmoidal curves are described by:

$$\frac{V_{i,jk}}{\left[1 + \left[\dfrac{K_{i,jk}}{C_{i,j}}\right]^{\theta_{i,jk}}\right]} \quad (2)$$

where $\theta_{i,jk}$ is a steepness parameter. The value of $\theta_{i,jk} = 1$ gives the ordinary Michaelis–Menten curve while successively higher values of $\theta_{i,jk}$ make the curve more and more

sigmoidal in a 'switch on' sense. When a 'switch off' form of the relationship is desired, the description of the curve becomes:

$$\frac{V_{i,jk}}{\left[1 + \left[\dfrac{C_{i,j}}{K_{i,jk}}\right]^{\theta_{i,jk}}\right]} \tag{3}$$

with a similar effect in that increasing values of $\theta_{i,jk}$ in this relationship enhance the steepness of the 'switch off' characteristic. A maximum likelihood program (Ross, 1980) was used to fit these curves to data in the literature.

Magnesium flows down the alimentary tract change as absorptive and excretive processes operate in the rumen, small intestine and large intestine (Grace & MacRae, 1972; Ben-Ghedalia *et al.* 1975; Tomas & Potter, 1976*a,b*). Grace (1983) summarized available data to show that the Mg fluxes in the small and large intestines were of comparable magnitude but opposite sign and that the major net loss from the alimentary tract occurred in the rumen.

The following sections describe the nature of the processes known to be operating in the rumen and the hindgut. Homeostasis of Mg within the body appears to be achieved by processes in the kidney (Quamme & Dirks, 1983). All of these absorptive and excretory processes are assembled as a series of equations which represents a mathematical model of Mg metabolism.

RUMEN COMPARTMENT

Partitioning of magnesium within the rumen

Grace *et al.* (1988) defined the liquid phase form of Mg in rumen digesta as that which existed in the supernatant fraction of a sample centrifuged at greater than $20\,000\,g$, and had a molecular mass of approximately 200 Da. The solid-phase form was considered to be that bound and unavailable directly for absorption. A chemical equilibrium between these phases was shown by Smith & Horn (1976) and Johnson *et al.* (1987) to be pH dependent. A 'switch off' type of curve (equation 4) was applied to describe the relationship between the proportion of ultrafilterable Mg and the pH of the rumen fluid, using the data of Smith & Horn (1976). This curve is defined by the equation:

$$Q_{Lq} = (0.861 \times Q_{Ru}/(1 + (pH/7.44)^{10.1})) + 0.055. \tag{4}$$

Transport between the rumen and plasma

There is bidirectional flow of Mg between the rumen and the plasma as shown in Fig. 1. The rates and control mechanisms of these flows have been estimated by the isotope studies of Martens *et al.* (1987). These are considered separately in the following sections.

Transport from the rumen to plasma. From the rumen fluid Mg is transported into the plasma by an active process (Martens *et al.* 1978) that is represented here by a simple Michaelis–Menten equation as follows:

$$U_{LqPl} = V_{Xm}/(1 + K_{LqPl}/C_{Lq}), \tag{5}$$

where

$$V_{Xm} = S_{Ru} \times V_{LqPl} \times (1.0 - 0.01251 \times P_{LqPl}). \tag{6}$$

Equation (6) contains the normal maximum saturated absorptive capacity for the rumen wall on a per area basis (V_{LqPl}) along with an inhibition factor included to account for the decrease in uptake that occurs as a result of the potential difference P_{LqPl} between the rumen fluid and the blood (Martens & Blume, 1986; Martens *et al.* 1987). The reduction in V_{Xm} that occurs as a consequence of this inhibition factor is of the same form as the inhibition factors used by Gill *et al.* (1984). They used a factor of the form:

$$(1 + i/J)^{-1},$$

which, since it is of the form:

$$(1 + x)^{-1},$$

can for x small be approximated as:

$$(1 - x),$$

the form used here. The justification for the values of the parameters used in equation (6) is given in the Appendix.

The value of V_{LqPl} was obtained from Martens *et al.* (1978), where unidirectional fluxes were measured at 2 mM-K concentration giving a value of 208 nmol/cm^2 per h which translates to a value of:

$$V_{LqPl} = 0\cdot05 \text{ mol/m}^2 \text{ per d.} \tag{7}$$

The value of K_{LqPl} of 2·43 mM was also obtained from Martens *et al.* (1978). The value of S_{Ru} (surface area of the rumen) was taken to be 0·332 m^2 which is the surface area of an equivalent sphere of volume 18 litres.

Transport from the plasma to rumen. The efflux of Mg from the blood into the rumen was assumed to be driven by the gradient of potential difference across the rumen wall. Values for this flow are given in the Appendix and from these, the reverse flow can be calculated as:

$$U_{PlLq} = 24\cdot0 \times 10^{-5} \times S_{Ru} \times (8\cdot0 + 0\cdot1434 \times P_{LqPl}) \tag{8}$$

where the constant term arises as a unit conversion factor from nmol/cm^2 per h to mol/m^2 per d and the rumen area is as described earlier. The coefficient of 0·1434 arises directly from $(11\cdot6 - 8\cdot0)/25\cdot1$ assuming that the effect of potential difference is also linear (Appendix).

Source of potential difference across the rumen wall. The source of the potential difference across the rumen wall in sheep was established by Scott (1966) who derived an expression which becomes:

$$P_{LqPl} = 43 \times \log_{10}(C_{K,Lq}) - 29\text{mV}, \tag{9}$$

where $C_{K,Lq}$ is the rumen concentration of K in the liquid phase in mmol/l. The results of the studies of Martens & Blume (1986) are in agreement with this. They found that the transmural potential difference (y) increased linearly with the logarithm of the K concentration (x):

$$y = 37\cdot1x - 28\cdot9\text{mV}.$$

Rumen outflow

The flow of digesta out of the rumen in both the liquid and solid phases was obtained from the experiments of Faichney (1986) who reported a liquid-phase fractional outflow rate of 2·5/d for a chopped hay diet and 1·5/d for a ground and pelleted diet made from the same material. The solid-phase fractional outflow rates were reported as 1·0/d and 0·5/d, respectively (Faichney, 1986).

The amount of Mg flowing from the rumen in the solid phase is thus:

$$U_{SpHg} = Q_{Sp} \times r_{SpHg}, \tag{10}$$

where Q_{Sp} is given by:

$$Q_{Sp} = Q_{Ru} - Q_{Lq} \tag{11}$$

and Q_{Lq} is given by equation (4).

The loss of Mg from the rumen in the liquid phase is given by the quantity of Mg in the liquid phase, multiplied by the fractional outflow rate of the liquid phase. Thus:

$$U_{LqHg} = Q_{Lq} \times r_{LqHg}. \tag{12}$$

Thus, the rumen Mg compartment is affected by the inputs of diet (U_{DiRu}) and backflow from the plasma (U_{PlLq}), along with the outflow to the plasma (U_{LqPl}) and outflows to the small intestine in both liquid phase (U_{LqHg}) and solid phase (U_{SpHg}). This gives, by conservation of mass:

$$\frac{dQ_{Ru}}{dt} = U_{DiRu} + U_{PlLq} - U_{SpHg} - U_{LqHg} - U_{LqPl}. \tag{13}$$

PLASMA COMPARTMENT

The quantity of Mg in the plasma compartment is increased by the contribution absorbed from the rumen (U_{LqPl}), the amount resorbed by the loop of Henle in the kidney (U_{HlPl}), and any Mg absorbed in the hindgut (U_{HgPl}). Outputs of Mg from the plasma compartment are the loss back to the rumen (U_{PlLq}), the output to the kidney (U_{PlHl}), and the net loss to the post-ruminal gastrointestinal tract (U_{PlHg}). A description of each of these flows follows.

U_{LqPl} and U_{PlLq} are defined by equations (5) and (8) respectively.

U_{PlHl} is given by:

$$U_{PlHl} = k_{PlHl} \times V_{bf} \times C_{Pl}, \tag{14}$$

where k_{PlHl} is the product of the glomerular filtration rate, taken to be a factor of 0·8 representing the essentially unbound state of Mg in plasma (Blincoe & Woodin, 1984; Woodin & Blincoe, 1985) and the proportion of blood flow to the kidneys for filtration which is assumed to have a value of 0·25 (Finco, 1989).

The cardiac output, V_{bf} is assumed to be given by:

$$V_{bf} = 54.7 \times Q_{Bw}, \tag{15}$$

from Pethick *et al.* (1981) where Q_{Bw} is the body weight.

The concentration of Mg in the plasma is given by:

$$C_{Pl} = Q_{Pl}/V_{Pl}, \tag{16}$$

where the V_{Pl} is assumed from Baldwin & Black (1979, Table 1) to be defined by:

$$V_{Pl} = 4\cdot41 \times 10^{-2} \times Q_{Bw}{}^{1\cdot07}. \tag{17}$$

Mg is absorbed strongly by the kidney by several processes, not all of which are clearly understood (Quamme & Dirks, 1983). The kidney does, however, act as the major regulator of the concentration of Mg in blood and a description of this process was given by Kemp *et al.* (1960). A Michaelis–Menten relationship of the following form is assumed:

$$C_{Pl} = V_{HlUr}/(1 + K_{HlUr}/U_{HlUr}),$$

which describes the concentration of Mg in plasma as a function of urinary output. The values of V_{HlUr} and K_{HlUr} were obtained by fitting the data of Kemp *et al.* (1960) using maximum likelihood program (Ross, 1980). This equation is rearranged to give:

$$U_{HlUr} = K_{HlUr} \times (V_{HlUr}/C_{Pl} - 1), \tag{18}$$

and thus U_{HlPl}, the input from the kidney that is effectively absorbed, is given by:

$$U_{HlPl} = U_{PlHl} - U_{HlUr}, \tag{19}$$

by conservation of mass.

There are no estimates of the bidirectional fluxes of Mg in those parts of the alimentary tract outside the rumen. The net endogenous loss of Mg from the plasma into the gastrointestinal tract was estimated as 3 mg/kg body weight per d (Agricultural Research Council, 1980) and we have adopted that value. This net endogenous loss represents the sum of all absorptive and secretive processes operating in the gastrointestinal tract, other than the major processes operating in the rumen and hindgut. This gives:

$$U_{PlHg} = 1\cdot25 \times 10^{-4} \times Q_{Bw}, \tag{20}$$

where the constant arises as a change of units to mol/d.

The flux of Mg from the hindgut to the body, U_{HgPl}, is described by equation (26).

All inputs and outputs for the plasma compartment sum to give:

$$\frac{dQ_{Pl}}{dt} = U_{LqPl} + U_{HlPl} + U_{HgPl} - U_{PlHl} - U_{PlHg} - U_{PlLq}. \tag{21}$$

HINDGUT COMPARTMENT

The third compartment of the model represents the Mg absorption observed to occur in the hindgut (Ben-Ghedalia *et al.* 1975; Reynolds *et al.* 1984; Dalley & Sykes, 1989). In the context of this model the hindgut is defined as the combination of two sections of the alimentary tract distal to the pylorus. In the proximal section, from the pylorus to the terminal ileum, a change in pH occurs with little absorption of Mg, whereas from the terminal ileum to the rectum, considerable absorption of Mg occurs.

The quantity of Mg in this third compartment is represented by Q_{Hg}. As before, there are two components within this third compartment representing the liquid and solid phases of Mg.

Partitioning of magnesium within the hindgut

Ben-Ghedalia *et al.* (1975) observed in sheep a decrease in the solubility of Mg in digesta from approximately 80 % at the pylorus (pH 3) to approximately 7 % at the terminal ileum (pH 8). Similar observations were made in cattle by van't Klooster (1967). Using these combined data a partitioning between soluble and insoluble fractions was obtained that was of the same form as that used in equation (4) to represent partitioning of Mg in the rumen. The equation used here is:

$$Q_{HgLq} = 0.8 \times Q_{Hg}/(1 + (pH/7.0)^{10.0}),\tag{22}$$

and Q_{HgSp} by difference is:

$$Q_{HgSp} = Q_{Hg} - Q_{HgLp}.\tag{23}$$

The pH of the hindgut used in equation (22) was taken as 7.96 from the work of Ben-Ghedalia *et al.* (1975).

Transport out of hindgut into faeces

Dixon & Nolan (1982) reported digesta flows of liquid and solid phases in multiple sections of the lower hindgut. Their values of unity for the fractional outflow rates for the liquid r_{HgLq1}, and solid r_{HgSp1}, phases, are used here. Thus the amounts of Mg flowing from the solid and liquid phase portions of the hindgut compartment are given by:

$$U_{HgSp} = r_{HgSp1} \times Q_{HgSp},\tag{24}$$

$$U_{HgLq} = r_{HgLq1} \times Q_{HgLq}\tag{25}$$

Absorption of magnesium from the hindgut

Field & Munro (1977) observed that urinary Mg excretion increased when Mg was infused into post-ruminal (omasal, abomasal or duodenal) sections of the tract. The nature of this change was linear with increasing Mg infusion and there appeared to be no effect of site of infusion. Tomas & Potter (1976*b*) also observed no difference between the effect of infusion of Mg into either the duodenum or the terminal ileum on the amount of Mg absorbed distal to the pylorus.

Field & Munro (1977) therefore assumed that '... the constant but low efficiency of absorption of infused Mg could denote passive diffusion or an active but unsaturated transport mechanism'. Ben-Ghedalia *et al.* (1975) also noted that 'As the formation of faecal pellets is connected with an intense absorption of water as the digesta passes along the colon, it is clear that this process also concentrates solutes and this would facilitate the absorption (passive) of Mg and possibly other elements from the colon'.

Based on this experimental evidence passive diffusion was chosen as the description of the mechanism of absorption from the hindgut. Consequently, the flow of Mg from the hindgut into the plasma is given by:

$$U_{HgPl} = k_{HgPl} \times (C_{HgLq} - C_{Pl}),\tag{26}$$

where C_{HgLq} and C_{Pl} are the concentrations of Mg in the liquid phase fraction of the hindgut and plasma respectively. The value of k_{HgPl} is unknown and was initially set at unity. Adjustment of this parameter is described on pp. 986–987.

The concentration of Mg in the liquid phase partition of the hindgut, C_{HgLq}, is determined from:

$$C_{HgLq} = Q_{HgLq}/V_{Hg}, \tag{27}$$

where:

$$V_{Hg} = 0.05 \times Q_{Bw.} \tag{28}$$

The constant of 0.05 in this equation relating volume of hindgut to body weight was taken from Dixon & Nolan (1982), who found that this parameter was not affected by diet.

Once in the hindgut all contributory components are subject to the partitioning caused by pH, the characteristic of which is given by equation (22). The output from the hindgut compartment to faeces is given by the combined flows of the liquid and solid phase outflows, thus:

$$U_{Fa} = U_{HgSp} + U_{HgLq}. \tag{29}$$

Equations (22) to (29) describe the component parts and fluxes relating to the hindgut compartment. All inputs and outputs for this compartment now sum to give:

$$\frac{dQ_{Hg}}{dt} = U_{SpHg} + U_{LqHg} + U_{PlHg} - U_{Fa} - U_{HgPl}. \tag{30}$$

Summary of model

The model is defined by equations (4) through to (30). It has three state variables: Q_{Ru}, Q_{Pl} and Q_{Hg}. There are three corresponding first-order, non-linear differential equations which can be integrated numerically for given initial conditions and parameter values. The program to solve the model was written in the simulation language ACSL (Mitchell & Gauthier, 1987) and run on a VAX computer. A fourth order Runge–Kutta procedure was used for the numerical integration. The initial values and parameter values used are shown in Table 3. The characteristics of the rumen contents used in these simulations are shown in Table 5.

RESULTS AND DISCUSSION

The model based on the physiological experiments described earlier was used initially to predict the maximum net absorption of Mg from the reticulo-rumen for sheep based on the data in the literature which have been summarized by Grace (1983). Second, the model was adjusted in light of these comparisons and the modified model was validated using unpublished data from Moredun Research Institute, Edinburgh (R. A. Dingwall and A. C. Field, unpublished results). The latter experiment was chosen since their sheep were continuously fed on different diets which resulted in different pH values in ruminal digesta and the dietary intakes of K and Mg were independently varied. Finally the absorption of Mg from the hindgut was incorporated into the model's predictions.

The simulation runs used a hypothetical 25 kg sheep consuming 800 g DM/d with Mg intakes ranging between 0.2 g/d and 8.0 g/d and K concentrations in the rumen ranging between 25 mM and 125 mM. These conditions represent a superset of the range of values

Table 3. *Initial conditions and parameter values*

	Value	Units	Comment
$C_{K,Lq}$	25·0	mmol/l	Rumen potassium concentration
C_{PI0}	1·0	mmol/l	Initial plasma magnesium concentration
C_{Lq0}	2·5	mmol/l	Initial rumen magnesium concentration
V_{HlUr}	1·146	mmol/l	Kidney V_{max}
K_{HlUr}	4·166	mmol/d	Kidney K_m
V_{LqPI}	0·05	mol/m^2 per d	Rumen wall V_{max}
K_{LqPI}	2·43	mmol/l	Rumen wall K_m
V_{Lq}	5·0	litres	Rumen liquid volume
S_{Ru}	0·332	m^2	Rumen surface area
Q_{Lq0}	0·3	g	Initial quantity in rumen liquid
Q_{Ru0}	0·42	g	Initial quantity in rumen total
Q_{Hg0}	2·0	g	Initial quantity in hindgut total
k_{HgPI}	0·0		(See pp. 986–987)

for the ruminal K concentration (34·2 mM and 97·6 mM) and Mg intakes between 1·9 and 2·1 g/d used in the experiments of Tomas & Potter (1976c).

Initial simulation runs predicted a maximum value of only 0·15 g/d for the net absorption of Mg from the rumen ($U_{LqPI} - U_{PILq}$) under steady-state conditions. This compared with actual values of absorption of between 0·3 and 0·6 g/d observed at dietary Mg intakes between 1·6 and 2·0 g/d (Grace & MacRae, 1972; Grace *et al.* 1974; Tomas & Potter, 1976b; Field & Munro, 1977) which suggested that the parameter describing the maximum possible amount of Mg absorbed from the rumen (V_{Xm}) was too small. This parameter is defined by equation (6) which relates V_{Xm} to the rumen surface area, S_{Ru}, the maximum absorptive capacity on a per area basis, V_{LqPI}, and the potential difference across the rumen wall. The surface area used here (0·332 m^2) comes from considering the serosal surface of the rumen to be that of a sphere of equivalent volume (18 litres). The potential difference calculated to exist across the rumen wall using the constants defined in Table 3 and equation (9) is 31·1 mV, a value which compares well with the observed values of Tomas & Potter (1976c). However, when this value for the potential difference is substituted in equation (6) and a value of 0·05 mol/m^2 per d is used for V_{LqPI}, a value of V_{xm}, the maximum possible amount of Mg absorbed from the rumen, of 0·01 mol/d is obtained. This value of V_{xm} represents a maximum possible unidirectional uptake from the rumen of 0·24 g/d. The value of 0·15 g/d mentioned earlier arises when the complete equations (5) and (8) are used, which takes into account the physiological value for the concentration of Mg in the rumen fluid and the flux of Mg from the blood into the rumen. The adjustment of the parameter, V_{LqPI}, describing the maximum possible uptake across the rumen wall is considered in the next section.

Adjustment of rumen wall saturation constant (V_{LqPI})

The value for the rumen wall saturation constant (V_{LqPI}) was taken from the *in vitro* experiments of Martens *et al.* (1978). The sensitivities of the model to this parameter were evaluated by simulating the range of dietary concentrations for Mg described earlier, as well as altering the values for V_{LqPI}. Results from this simulation are shown in Table 4.

A comparison of the results in Table 4 with the summary of observed values given by Grace (1983) suggested the need to increase V_{LqPI} fourfold from 0·05 mol/m^2 per d to

Table 4. *Effect of changes in* V_{LqPl} *(mol/m^2 per d) on faecal and urinary excretion (g/d) and plasma concentration (mmol/l) of magnesium for animals whose diets contain between 1·6 and 2·0 g magnesium/d**

Dietary intake	V_{LqPl}	Faeces	Urine	Plasma
1·6	0·05	1·52	0·08	0·51
	0·10	1·35	0·25	0·82
	0·15	1·20	0·40	0·92
	0·20	1·06	0·54	0·97
	0·25	0·93	0·67	1·00
	0·30	0·82	0·78	1·02
	0·35	0·73	0·87	1·03
	0·40	0·65	0·95	1·04
	0·45	0·59	1·01	1·04
1·7	0·05	1·52	0·08	0·52
	0·10	1·45	0·25	0·82
	0·15	1·29	0·41	0·92
	0·20	1·14	0·56	0·97
	0·25	1·01	0·69	1·00
	0·30	0·90	0·80	1·02
	0·35	0·79	0·91	1·03
	0·40	0·71	0·99	1·04
	0·45	0·64	1·06	1·07
1·8	0·05	1·71	0·08	0·53
	0·10	1·54	0·26	0·83
	0·15	1·38	0·42	0·93
	0·20	1·23	0·57	0·98
	0·25	1·09	0·71	1·00
	0·30	0·97	0·83	1·02
	0·35	0·86	0·94	1·04
	0·40	0·77	1·03	1·05
	0·45	0·69	1·11	1·05
2·0	0·05	1·91	0·09	0·54
	0·10	1·73	0·27	0·84
	0·15	1·56	0·44	0·93
	0·20	1·40	0·60	0·98
	0·25	1·25	0·75	1·01
	0·30	1·12	0·88	1·03
	0·35	1·00	1·00	1·04
	0·40	0·89	1·11	1·05
	0·45	0·80	1·20	1·06

* Simulated with rumen pH 5·9, r_{LqHg} 1·5 and r_{SpHg} 0·5 (diet A from Table 5).

0·2 mol/m^2 per d to bring the fluxes of Mg from the rumen to the plasma into the observed range of 0·3 to 0·6 g/d. This represents a change in the maximum quantity of Mg that can be absorbed from an 18 litre capacity rumen with a zero potential difference across the rumen wall, from 0·4 g/d to 1·6 g/d. Such a change is justified in that the movement of Mg across the rumen wall is known to be an active transport process which requires functioning Na^+-K^+ ATPases (Martens *et al.* 1978). The reduction in the functioning of these enzymes from an *in vivo* to an *in vitro* situation has been observed by Ferreira *et al.* (1966) as a rapid fall in the potential difference across the rumen wall within a few minutes

of death with a potential difference of approximately 7–10 mV present up until 90 min after death.

Martens *et al.* (1978) also reported a change in transport characteristics between the first and second hours post mortem. Their value of V_{LqPl} of 208 nmol/cm^2 per h was calculated over the entire 2 h observation period. They observed that the potential difference across the isolated rumen epithelium of 8·2 mV measured during the first hour fell to 5·1 mV in the second hour. This first hour value represents approximately 25–30 % of the potential difference observed in later work (Martens & Blume, 1986; Martens *et al.* 1987).

Other workers have observed at least 0·4 g Mg/d being absorbed from the rumen alone. McLean *et al.* (1984) measured up to 0·6 g/d being absorbed proximal to the pylorus in a balance trial and Martens & Blume (1986) observed 0·71 mmol/h (0·4 g/d) being absorbed from a rumen using the washed rumen technique where the observed potential difference was 26·1 mV.

Further justification for the adjustment upwards of V_{LqPl} arises when the work of Martens (1983) is considered. The temporarily isolated rumen technique was used to estimate the saturation kinetics of Mg efflux across the rumen wall in calves. The parameters obtained were a V_{max} of 120·3 μmol/min (0·173 mol/d) and a K_m of 11·43 mM. These animals were 10–12 months old, ranged in weight from 170–240 kg, and had rumen contents of approximately 25 kg. If the rumen volume is assumed to be 75 litres, then this equates to a sphere of equivalent surface area of 0·860 m^2, which gives a V_{LqPl} of 0·20 mol/m^2 per d. For these reasons the value of V_{LqPl} was set at 0·2 mol/m^2 per d.

Once the predicted values of Mg absorbed from the rumen were in the experimental range, the value of k_{HgPl}, the constant relating the absorption of Mg from the hindgut to the plasma, was adjusted to give the realistic values for the amount of Mg absorbed at this site that were reported by Grace (1983).

Model challenge

The characteristics of the rumen contents observed in the experiments of R. A. Dingwall and A. C. Field (Moredun Institute, Edinburgh) are shown in Table 5. Results from the model, omitting any potential absorption from the hindgut, obtained by setting k_{HgPl} to zero in equation (26), and the respective dietary intakes used by Dingwall and colleagues are shown in Table 6. As can be seen there is an acceptable level of agreement except at the higher levels of intake where a lower than observed urinary excretion and a higher than observed faecal excretion indicates that insufficient absorption is predicted. Given that the values of V_{LqPl} had been altered as described earlier, the sensitivity of these urinary and faecal excretion values to changes in V_{LqPl} were ascertained. Results of this analysis are shown in Table 7. Variations in V_{LqPl} over a fourfold range away from the value of

Table 5. *Characteristics of rumen contents used in simulations*

Diet	Nature of diet	pH	Rumen K (mmol/l)	r_{LqHg} (/d)	r_{SpHg} (/d)
A	Pelleted	5·9	27	1·5	0·5
B	Chopped hay	6·45	45	2·5	1·0

Table 6. *Results of the present model* (lower value) compared with experimental data from R. A. Dingwall and A. C. Field (unpublished results; upper value)*

Nature of diet†	Mg intake (g/d)	Mg excretion (g/d)		Plasma Mg (mmol/l)
		Faeces	Urine	
Diet A, cereal (rumen pH 5·9 (SE 0·04))	0·88	0·54	0·36	0·82
		0·52	0·36	0·90
	2·71	1·58	1·16	1·07
		2·04	0·67	1·00
	4·51	3·01	1·53	1·07
		3·76	0·74	1·01
	6·11	3·72	1·95	1·22
		5·32	0·77	1·01
Diet B, hay (rumen pH 6·45 (SE 0·04))	1·44	1·05	0·28	0·99
		1·12	0·32	0·87
	3·44	2·71	0·66	0·92
		2·95	0·48	0·95
	5·32	4·53	0·80	1·12
		4·78	0·54	0·97
	7·22	6·14	1·24	1·15
		6·65	0·57	0·97

* Omitting any absorption from the hindgut ($k_{HgPl} = 0$).
† For details of diets and rumen content characteristics, see Table 5.

$0.2 \, mol/m^2$ per d originally chosen were not effective in overcoming this discrepancy between observed and model excretion values at all dietary levels of Mg.

The results of the model including absorption from the hindgut, with k_{HgPl} set at 2·5, are represented in Table 8 in the same format as previously, and indicate much closer agreement between the model and the unpublished results of Dingwall and Field. That absorption occurs in the hindgut has been observed by several other workers (Tomas & Potter, 1976b; McLean *et al.* 1984) whose data are not shown by Grace (1983).

In terms of the model discussed here it is possible to hypothesize that the nature of the hindgut absorption acts as a homeostatic mechanism. Under conditions of little or no absorption of Mg occurring in the reticulo-rumen the concentration of Mg in the colonic digesta can be expected to increase, thus allowing increased absorption of Mg at that site as described by equation (26). Conversely, as more dietary Mg is absorbed in the forestomachs the concentration of Mg in colonic digesta is reduced and the quantity of Mg absorbed in the hindgut decreases. This hypothesis suggested by these model results is consistent with the experimental observations of McLean *et al.* (1984) and Dalley *et al.* (1997). In fact, an inspection of the model results presented in Table 8 of the current paper compare favourably with the experimental results of Table 2 of Dalley *et al.* (1997). ·

Conclusions

The work described herein evaluated a three-compartment model which took into account the major factors, from physiological experiments, that influence Mg absorption from the digestive tract in ruminants and quantitatively described their effects. A function to describe hindgut absorption was necessary in order to explain satisfactorily current

Table 7. *Model results, omitting absorption from the hindgut, as a function of changes in V_{LqPl} compared with unpublished results from R. A. Dingwall and A. C. Field*

Nature of diet†	Mg intake (g/d)	V_{LqPl}	Mg excretion (g/d)		Plasma Mg (mmol/l)
			Faeces	Urine	
Diet A, cereal	0·88	0·05	0·83	0·05	0·37
(rumen pH 5·9 (SE 0·04))		0·10	0·71	0·17	0·73
		0·15	0·60	0·28	0·84
		0·20*	0·52	0·36	0·90
		0·25	0·45	0·43	0·93
		0·30	0·40	0·48	0·95
		0·35	0·36	0·52	0·96
		0·40	0·32	0·56	0·97
		0·45	0·30	0·58	0·98
	2·71	0·05	2·60	0·10	0·57
		0·10	2·41	0·29	0·86
		0·15	2·22	0·48	0·95
		0·20*	2·04	0·67	1·00
		0·25	1·87	0·84	1·02
		0·30	1·70	1·00	1·04
		0·35	1·55	1·16	1·06
		0·40	1·41	1·30	1·06
		0·45	1·28	1·43	1·07
	4·51	0·05	4·38	0·11	0·61
		0·10	4·17	0·33	0·88
		0·15	3·96	0·53	0·97
		0·20*	3·76	0·74	1·01
		0·25	3·56	0·94	1·04
		0·30	3·36	1·14	1·05
		0·35	3·16	1·34	1·07
		0·40	2·97	1·53	1·08
		0·45	2·79	1·71	1·08
	6·11	0·05	5·97	0·11	0·62
		0·10	5·75	0·38	0·88
		0·15	5·53	0·55	0·97
		0·20*	5·32	0·77	1·01
		0·25	5·11	0·98	1·04
		0·30	4·90	1·20	1·06
		0·35	4·69	1·40	1·07
		0·40	4·48	1·61	1·08
		0·45	4·28	1·82	1·09
Diet B, hay	1·44	0·05	1·42	0·02	0·15
(rumen pH 6·45 (SE 0·04))		0·10	1·31	0·12	0·64
		0·15	1·21	0·23	0·79
		0·20*	1·12	0·32	0·87
		0·25	1·04	0·40	0·92
		0·30	0·96	0·48	0·95
		0·35	0·89	0·55	0·97
		0·40	0·82	0·62	0·99
		0·45	0·77	0·67	1·00

(continued)

Table 7. (*Continued*)

Nature of diet†	Mg intake (g/d)	V_{LqPl}	Mg excretion (g/d)		Plasma Mg (mmol/l)
			Faeces	Urine	
Diet B, hay	3·44	0·05	3·39	0·05	0·38
(rumen pH 6·45 (SE 0·04))		0·10	3·24	0·20	0·76
		0·15	3·10	0·34	0·89
		0·20*	2·95	0·48	0·95
		0·25	2·82	0·62	0·99
		0·30	2·68	0·76	1·01
		0·35	2·55	0·89	1·03
		0·40	2·42	1·02	1·04
		0·45	2·30	1·14	1·05
	5·32	0·05	5·26	0·06	0·43
		0·10	5·10	0·22	0·79
		0·15	4·94	0·38	0·91
		0·20*	4·78	0·54	0·97
		0·25	4·62	0·70	1·00
		0·30	4·47	0·85	1·03
		0·35	4·32	1·00	1·04
		0·40	4·17	1·15	1·06
		0·45	4·02	1·30	1·06
	7·22	0·05	7·15	0·07	0·46
		0·10	6·98	0·24	0·80
		0·15	6·82	0·40	0·92
		0·20*	6·65	0·57	0·97
		0·25	6·49	0·73	1·01
		0·30	6·32	0·90	1·03
		0·35	6·16	1·06	1·05
		0·40	6·00	1·22	1·06
		0·45	5·84	1·38	1·07

* Value used.
† For details of diets and rumen content characteristics, see Table 5.

Table 8. *Results of the present model* (lower value) compared with experimental data from R. A. Dingwall and A. C. Field (unpublished results; upper value)*

Nature of diet†	Mg intake (g/d)	Mg excretion (g/d)		Plasma Mg (mmol/l)
		Faeces	Urine	
Diet A, cereal (rumen pH 5·9 (SE 0·04))	0·88	0·54	0·36	0·82
		0·44	0·44	0·94
	2·71	1·58	1·16	1·07
		1·55	1·14	1·05
	4·51	3·01	1·53	1·07
		2·79	1·64	1·08
	6·11	3·72	1·95	1·22
		3·92	2·06	1·09
Diet B, hay (rumen pH 6·45 (SE 0·04))	1·44	1·05	0·28	0·99
		1·03	0·41	0·92
	3·44	2·71	0·66	0·92
		2·68	0·76	1·01
	5·32	4·53	0·80	1·12
		4·31	1·00	1·04
	7·22	6·14	1·24	1·15
		5·98	1·23	1·06

* Including absorption from the hindgut ($k_{HgPl} = 2·5$).
† For details of diets and rumen content characteristics, see Table 5.

experimental observations from balance trials. Constants of proportionality used in the three-compartment model must still be verified by further experimentation, particularly those associated with absorption of Mg in the hindgut.

REFERENCES

Agricultural Research Council (1980). *The Nutrient Requirements of Ruminant Livestock.* Slough: Commonwealth Agricultural Bureaux.

Baldwin, R. L. & Black, J. L. (1979). *Simulation of the Effects of Nutritional and Physiological Status on the Growth of Mammalian Tissues: Description and Evaluation of a Computer Program. Animal Research Laboratories Technical Paper* no. 6. Melbourne: Commonwealth Scientific and Industrial Research Organization.

Ben-Ghedalia, D., Tagari, H., Zamwel, S. & Bondi, A. (1975). Solubility and net exchange of calcium, magnesium and phosphorus in digesta flowing along the gut of the sheep. *British Journal of Nutrition* 33, 87–94.

Blincoe, C. & Woodin, T. S. (1984). Chemical state of magnesium in plasma. *Journal of Inorganic Biochemistry* 21, 209–214.

British Veterinary Association (1960). *Conference on Hypomagnesaemia.* London: British Veterinary Association.

Dalley, D. E., Isherwood, P., Sykes, A. R. & Robson, A. B. (1997). Effect of intraruminal infusion of potassium on the site of magnesium absorption within the digestive tract in sheep. *Journal of Agricultural Science, Cambridge* 129, 99–105.

Dalley, D. E. & Sykes, A. R. (1989). Magnesium absorption from the large intestine in sheep. *Proceedings of the New Zealand Society of Animal Production* 49, 229–232.

Dixon, R. & Nolan, J. (1982). Studies on the large intestine of sheep. 1. Fermentation and absorption in sections of the large intestine. *British Journal of Nutrition* 47, 289–300.

Faichney, G. J. (1986). The kinetics of particulate matter in the rumen. In *Control of Digestion and Metabolism in Ruminants*, pp. 173–195 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice-Hall.

Ferreira, H. G., Harrison, F. A., Keynes, R. D. & Nauss, A. H. (1966). Observations on the potential across the rumen of sheep. *Journal of Physiology* 187, 615–630.

Field, A. C. & Munro, C. S. (1977). The effect of site and quantity on the extent of absorption of Mg infused into the gastro-intestinal tract of sheep. *Journal of Agricultural Science, Cambridge* 89, 365–371.

Finco, D. R. (1989). Kidney function. In *Clinical Biochemistry of Domestic Animals*, 4th ed., pp. 496–542 [J. J. Kaneko, editor]. San Diego, CA: Academic Press, Inc.

Fontenot, J. P., Bunce, G. E., Webb, K. E. & Allen, V. G. (editors) (1983). *Proceedings of the John Lee Pratt International Symposium on the Role of Magnesium in Animal Nutrition.* Blacksburg, VA: Virginia Polytechnic Institute and State University.

France, J., Gill, M., Thornley, J. H. M. & England, P. (1987). A model of nutrient utilization and body composition in beef cattle. *Animal Production* 44, 371–385.

Gill, M., Thornley J. H. M., Black, J. L., Oldham, J. D. & Beever, D. E. (1984). Simulation of the metabolism of absorbed energy-yielding nutrients in young sheep. *British Journal of Nutrition* 52, 621–649.

Godfrey, K. (1983). *Compartmental Models and their Application.* London: Academic Press.

Grace, N. D. (1983). The site of absorption of magnesium in ruminants. *In Proceedings of John Lee Pratt International Symposium on the Role of Magnesium in Animal Nutrition*, pp. 107–120 [J. P. Fontenot, G. E. Bunce, K. E. Webb and V. G. Allen, editors]. Blacksburg, VA: Virginia Polytechnic Institute and State University.

Grace, N. D., Caple, I. W. & Care, A. D. (1988). Studies in sheep on the absorption of magnesium from a low molecular weight fraction of the reticulo-rumen contents. *British Journal of Nutrition* 59, 93–108.

Grace, N. D. & MacRae, J. C. (1972). Influence of feeding regimen and protein supplementation on the sites of net absorption of magnesium in sheep. *British Journal of Nutrition* 27, 51–55.

Grace, N. D., Ulyatt, M. J. & MacRae, J. C. (1974). Quantitative digestion of fresh herbage by sheep. *Journal of Agricultural Science, Cambridge* 82, 321–330.

Johnson, C. L., Helliwell, S. H. & Aubrey Jones, D. A. (1987). Magnesium metabolism in the rumens of lactating dairy cows fed on spring grass. *Quarterly Journal of Experimental Physiology* 73, 23–31.

Kemp, A., Deijs, W. B., Hemkes, O. J. & van Es, A. J. H. (1960). Basic source of magnesium metabolism III. Intake and utilization of magnesium from herbage by lactating cows. In *Conference on Hypomagnesaemia*, pp. 23–32. London: British Veterinary Association.

Larvor, P. (1976). [28]Mg kinetics in ewes fed normal or tetany prone grass. *Cornell Veterinarian* 66, 413–429.

McLean, A. F., Buchan, W. & Scott, D. (1984). Magnesium absorption in mature ewes infused intraruminally with magnesium chloride. *British Journal of Nutrition* 52, 523–527.

Martens, H. (1983). Saturation kinetics of magnesium efflux across the rumen wall in heifers. *British Journal of Nutrition* **49**, 153–158.

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. *Quarterly Journal of Experimental Physiology* **71**, 409–415.

Martens, H., Gabel, G. & Strozyk, H. (1987). The effect of potassium and the transmural potential difference on magnesium transport across an isolated preparation of sheep rumen epithelium. *Quarterly Journal of Experimental Physiology* **72**, 181–188.

Martens, H., Harmeyer, J. & Michael, H. (1978). Magnesium transport by isolated rumen epithelium of sheep. *Research in Veterinary Science* **24**, 161–168.

Martens, H. & Rayssiguier, Y. (1980). Magnesium metabolism and hypomagnesaemia. In *Proceedings of the 5th International Symposium on Ruminant Physiology*, pp. 447–466 [Y. Ruckebush and P. Thivend, editors]. Lancaster: MTP Press Ltd.

Mitchell, E. E. L. & Gauthier, J. (1987). *Advanced Continuous Simulation Language (ACSL)*. Concord, MA: Mitchell & Gauthier Associates.

Pethick, D. W., Lindsay, D. B., Barker, P. J. & Northrop, A. J. (1981). Acetate supply and utilization by the tissues of sheep *in vivo*. *British Journal of Nutrition* **46**, 97–110.

Quamme, G. A. & Dirks, J. H. (1983). Renal magnesium transport. In *Review of Physiology, Biochemistry and Pharmacology*, vol. 97, pp. 69–110 [K. Ullrich, editor]. Berlin: Springer-Verlag.

Reynolds, C. K., Bell, M. C. & Sims, M. H. (1984). Changes in plasma, red blood cell and cerebrospinal fluid mineral concentrations in calves during magnesium depletion followed by repletion with rectally infused magnesium chloride. *Journal of Nutrition* **114**, 1334–1341.

Ross, G. J. S. (1980). *MLP Maximum Likelihood Program.* Lawes Agricultural Trust, Rothamsted Experimental Station.

Scott, D. (1966). The effects of sodium depletion and potassium supplementation upon electrical potentials in the rumen of the sheep. *Quarterly Journal of Experimental Physiology* **51**, 60–69.

Smith R. H. & Horn, J. P. (1976). Absorption of magnesium, labelled with magnesium-28, from the stomach of the young steer. In *International Symposium on Nuclear Techniques in Animal Production and Health as Related to the Soil/Plant System*, pp. 253–260. Vienna: International Atomic Energy Agency.

Thornley, J. H. M. (1976). *Mathematical Methods in Plant Physiology.* London: Academic Press.

Tomas, F. M. & Potter, B. J. (1976a). The site of magnesium absorption from the ruminant stomach. *British Journal of Nutrition* **36**, 37–45.

Tomas, F. M. & Potter, B. J. (1976b). Interaction between sites of magnesium absorption in the digestive tract of sheep. *Australian Journal of Agricultural Research* **27**, 437–446.

Tomas, F. M. & Potter, B. J. (1976c). The effect and site of action of potassium upon magnesium absorption in sheep. *Australian Journal of Agricultural Research* **27**, 873–880.

van't Klooster, A. Th. (1967). De toestand van calcium, magnesium en enkele andere mineralen in darminhoud en mest van herkauwers in verband met hun resorptie (The status of calcium, magnesium and some other minerals in intestinal contents and manure of ruminants in relation to their resorption). *Mededelingen Landbouwhogeschool Wageningen* **67**, 1–135.

Wilcox, G. E. & Hoff, J. E. (1974). Grass tetany: an hypothesis concerning its relationship with ammonium nutrition of spring grasses. *Journal of Dairy Science* **57**, 1085–1089.

Wilson, G. F., Reid, C. S. W., Molloy, L. F., Metson, A. J. & Butler, G. W. (1969). Grass tetany. I. Influence of starch and peanut oil supplements on the plasma magnesium, calcium and phosphorus levels in grazing dairy cows. *New Zealand Journal of Agricultural Research* **12**, 467–488.

Woodin, T. S. & Blincoe, C. (1985). Distribution of plasma magnesium in various vertebrates. *Comparative Biochemistry and Physiology* **81A**, 507–509.

APPENDIX

Martens *et al.* (1987) established in an *in vitro* experiment, with small sheets of rumen epithelium less than 1 h old, that there is an effect on the unidirectional flows in both directions caused by the potential difference across the rumen wall. The source of this potential difference, due largely but not necessarily entirely to the K concentration *in vivo*, is not important. The observed reductions in both forward and reverse flows from their work are reproduced in the Table.

The coefficient of 0·01251 in equation (6) was obtained as follows. From the Table it can be seen that the flux at zero potential difference is 76·1 nmol/cm^2 per h and the flux at

Table. *Fluxes (nmol/cm^2 per h) of Mg^{2+} across isolated rumen epithelium.*

(Modified from Martens *et al.* 1987)

Group	Potential difference (mV)	Forward flux	Reverse flux
K$^+$ (70 mM)	25·1	52·2	11·6
K$^+$ (70 mM)	0·0	76·1	8·0
Control (5 mM)	26·1	47·4	12·2
Control (5 mM)	0·0	72·1	6·5

25·1 mV is 52·2 nmol/cm^2 per h. Assuming that the inhibition by the potential difference of the unidirectional flux is a linear effect, the relationship can be described by:

$$V_{Xm}/(S_{Ru} \times V_{LqPl}) \; \alpha \; 76{\cdot}1 - ((76{\cdot}1 - 52{\cdot}2)/25{\cdot}1) \times P_{LqPl} \qquad (A1)$$

$$\alpha \; 76{\cdot}1 - 0{\cdot}952 \times P_{LqPl}. \qquad (A2)$$

This represents the flux in absolute terms and to convert it to relative terms it is necessary to divide by 76·1 to obtain the proportionate reduction in maximum flux caused by the effect of potential difference. The equation now becomes:

$$V_{Xm}/(S_{Ru} \times V_{LqPl}) = 1{\cdot}0 - 0{\cdot}01251 \times P_{LqPl}. \qquad (A3)$$