

Chapter 2

A ROLE FOR MAGNESIUM IN THE REGULATION OF RUMINAL SODIUM TRANSPORT

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ABSTRACT

The ruminal epithelium is a stratified squamous epithelium that has evolved to display functions essential for the unique ability of cows and sheep to ferment dietary components like carbohydrates and protein, and to selectively absorb nutrients and minerals for the production of milk. A characteristic property of this tissue is its pronounced ability to transport magnesium against an electrochemical gradient. Absorption of magnesium is reduced by dietary elevation of ruminal potassium, leading to hypomagnesaemia that can reach clinical significance. Studies of the intact tissue and of isolated cells suggest that cellular magnesium uptake is decreased by apical depolarization of the ruminal membrane, resulting in both a lower cytosolic concentration and transepithelial transport of the element.

Another characteristic feature of this unusual epithelium is the expression of a sodium-conducting channel with functional properties that are clearly distinct from the epithelial sodium channel (ENaC) found in most mammalian epithelia. Thus, it has not been possible to demonstrate direct regulation of ruminal sodium transport by aldosterone, and the effects of amiloride are clearly limited to an inhibition of the sodium proton exchanger (NHE3) expressed by this tissue. Studies at the level of the animal and the tissue suggest that sodium conductance is enhanced by depolarization of the apical membrane. Recent *in vitro* studies have demonstrated that ruminal epithelial cells express non-selective cation channels in the apical membrane that are regulated by changes in cytosolic magnesium. We propose that the reduction in ruminal magnesium uptake observed after ingestion of high potassium fodder may be related to a role for magnesium in a signaling cascade that leads to an increase in the permeability of this non-selective cation channel for sodium, thus enhancing absorption of this ion from the rumen and restoring ruminal osmolarity, while contributing to the retention of potassium.

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INTRODUCTION

Compared to the extensive research on various molecules involved in signal transduction, Mg^{2+} has not gotten much attention. One of the reasons for this may be that in man, hypomagnesaemia is a fairly rare condition and one that is easily treated by magnesium supplements when it does occur – as in pregnancy or lactation. Thus, the stimulus for research in the area has been limited. Conversely and due to the large amounts of magnesium lost in milk, hypomagnesaemia in cows and sheep continues to lead to deaths of afflicted animals or even part of entire herds [32, 47, 92, 104, 118]. As a result, there has been ongoing interest in the uptake of magnesium in this species.

In the following, the focus will be on the ruminant in general and on the very peculiar organ that gives this species its name. Thus, this topic may not appear to be of general interest. However, we feel that the story that is emerging from many decades of research on how the ruminant has adapted to a dramatically altered nutritional situation by utilizing the interactions of Mg^{2+} with uptake of other cations may have implications for other physiological situations.

THE RUMINANT SPECIES: ADAPTATION TO A ROUGHAGE DIET

The ruminant species and mankind evolved in parallel as survivors of a relatively short period of mass extinction during which the forests of the Oligocene perished and were replaced with vast grasslands [11]. During this era, the ancestral ruminant, a small and forest-dwelling species [60], perfected its ability to utilize poorly digestible fiber and adapted to uptake of grass and roughage for its nutritional requirements. This accomplishment has been essential for the survival of these animals, and probably also for that of the humanoid apes which left the dwindling forests and began to follow and hunt these herds of ruminants.

Central to the ability of ruminants to subsist on a diet of grass while excreting large amounts of milk is the development of an advanced digestive system with several specialized compartments (reticulum, rumen, and omasum) that precede the actual stomach (abomasum) and the lower digestive tract (Figure 1). The largest of these forestomachs, the rumen, fills almost half of the entire abdominal cavity of these animals with a volume reaching 60 to 100 l in cows [138]. Lined with squamous, stratified epithelium which is keratinized and non-glandular [48, 53], this pouch is probably of esophageal origin [98] and serves as both a water reservoir [138] and a fermentation chamber in which poorly digestible forage is broken down and reassembled into digestible nutrients by an abundant flora of microorganisms [8, 57, 58, 156]. Maintenance of a pH that should be slightly acidic (5.5 - 7.0) and an osmolarity that is marginally hypotonic before or slightly hypertonic after a meal (to blood) is central to this fermentation process. Ruminal homeostasis is ensured by a number of factors that include production of ample amounts of saliva, continuous mixing of saliva with ruminal content by ruminal contractions and rumination of roughage, and the ability of the rumen to serve as a barrier between blood and rumen content while absorbing certain minerals and organic compounds in a controlled manner.

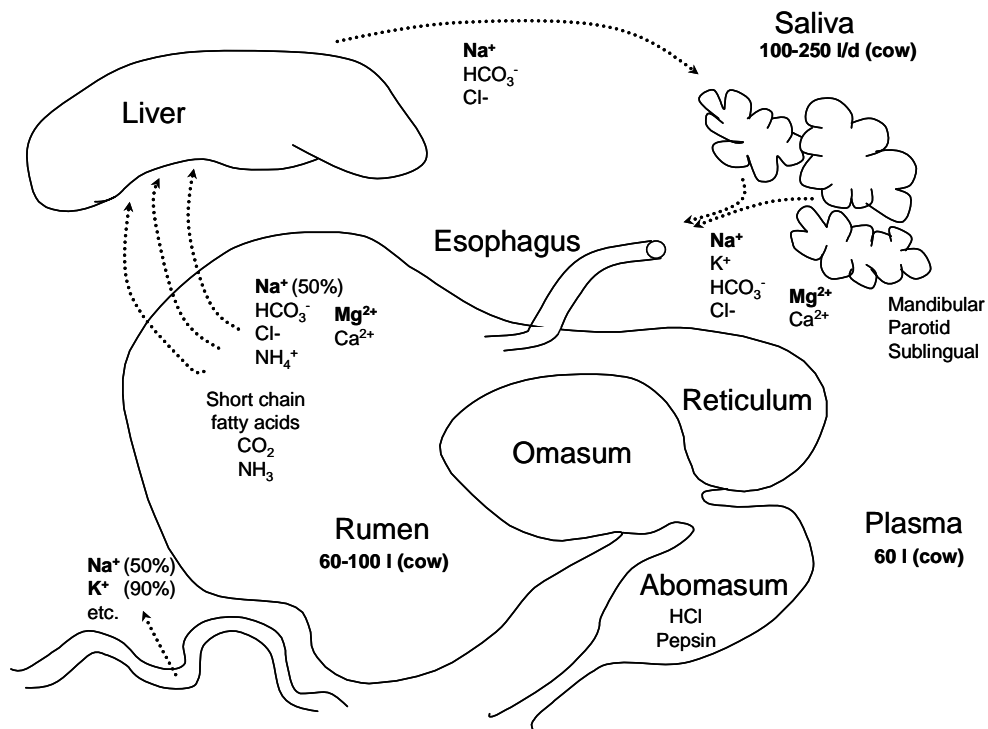


Fig 1: Schematic representation of the ruminant forestomach system: The glandular stomach (abomasum) is preceded by three compartments lined with stratified, squamous epithelium: the reticulum, the rumen, and the omasum. These forestomachs are involved in absorption of fermentation products and inorganic ions. Some 100 – 250 l/d of saliva are produced daily by the cow, representing 5 times the total plasma content of sodium, 50% of which is reabsorbed by the forestomachs. Over 30% of plasma Mg^{2+} content are excreted via saliva on a daily basis and are absorbed primarily in the rumen. Substantial amounts of both elements are secreted in milk (40 l/d).

SALIVA

A striking difference between ruminants and most monogastric species is the ability to produce an extremely large stream of well-buffered saliva that amounts to about 6-16 l/d in sheep [67] and 100 – 200 l/d in cattle [9, 15] with values of up to 250 l/d in lactating cows [23, 138] (Figure 1). This saliva is low in chloride (22 mmol/l) and contains large amounts of pH buffers such as HCO_3^- (120 mmol/l) and PO_4^- (30 mmol/l). After feeding, the flow rate but not the ionic composition of saliva increases, and can be so massive that the ADH and renin-angiotensin mechanism is activated [138]. Postprandial stimulation of salivary flow can be at least partially mimicked by the addition of volatile fatty acids to the rumen [15]. In contrast, in experiments in which ruminal pH was altered by addition of HCl, or ruminal osmolarity by addition of mannitol, no impact on the flow rate or composition of saliva could be observed in the sense of a feedback mechanism [15], although salivary secretion dropped when pH reached very low levels (<3.5) [15]. When strongly hypertonic solutions of sodium or potassium salts were infused into the rumen, or sodium salts or urea were infused into the blood, production of saliva decreased and the concentration of those substances increased in the saliva. Other treatments including physiological elevations of ruminal potassium content

had little effect on salivary composition [157]. Thus, evidence for sensors in the gut or rumen are scarce and salivary flow appears to be regulated by the composition of the portal blood [21, 138], possibly via hepatic sensors [119, 151].

A major fact that emerged from these studies is that if experimentally obtained saliva is not returned to the rumen, flow rate decreases to 50% of the initial value within two hours [15]. This decrease can be prevented by administration of NaHCO_3 containing solution to the rumen [14] and points towards the importance of ruminal reabsorption of these elements.

With the saliva, the animal secretes an amount of Na^+ that is 1.2-1.5 mol/d in sheep and 15 – 30 mol/d in cows [82] and is thus five times higher than the sodium content of the body, and 15 times higher than the amount of sodium consumed with the fodder [139]. Salivary secretion of HCO_3^- reaches levels that can lead to acidaemia when salivary flow is high, as after a meal [111, 112, 129, 139], stressing the importance of a rapid reabsorption of HCO_3^- .

Sodium content of saliva is strongly regulated by aldosterone, which is of considerable importance given the fact that in the natural diet of the ruminant, sodium is a scarce element. In sodium depleted sheep, salivary content of this mineral can fall dramatically from concentrations of about 180 mmol/l to as low as 40 mmol/l. Potassium excretion rises concomitantly from a normal level of 4 to 6 mmol/l to values of over 130 mmol/l [14]. Conversely, ruminal reabsorption of Na^+ is not regulated by aldosterone [85].

Crisis situations can occur in spring, when animals are quickly transferred from a diet of hay, which is comparably low in potassium, to a diet of fresh spring grass which is rich in potassium. In these situations, potassium levels in the rumen can rapidly peak to over 100 mmol/l, while sodium levels drop to lows of 30 mmol/l. To restore the balance for sodium, animals thus have to be able to absorb sodium actively against this very high background of potassium, while any absorption of potassium has to be tightly coupled to its excretion in the kidney. Given the enormous surface area and volume of the rumen, and the huge quantities of potassium rich roughage, the demands on this organ are thus clear: it has to prevent potassium from entering the blood stream too quickly, and it has to be highly able to transport sodium, bicarbonate, magnesium and calcium from the rumen to the plasma against an electrochemical gradient.

THE RUMEN

Histology and Organogenesis

Interestingly, the esophagus of mammals including humans has been shown to possess transporting properties in that it forms a tight barrier against the efflux of K^+ [68] and has considerable potential for the active transport of Na^+ [109]. The physiological function of this pronounced transport of electrolytes across the esophagus is unclear and probably merely serves maintenance of cell equilibrium. However, it is tempting to speculate that these transporting properties may have facilitated the adaptation of the esophageal wall to the requirements of forming a chamber for fermentation in the evolutionary process.

Of course, dramatic differences between the two types of tissue predominate. From an electrophysiological point of view, one pronounced difference is the fact that while participation of the rumen in the total absorption of potassium is small [49, 113], the tissue is

depolarized by high apical potassium [37, 38, 136]. This characteristic feature will be discussed in more detail further down.

The histology of ruminal epithelium has been studied in some detail [48] and shows a typical structure with stratum corneum, granulosum, spinosum and basale; tight junctions (claudin 1 and ZO1), gap junctions (connexin-43) and expression of increasing levels of the α subunit of the Na^+/K^+ -ATPase towards the basal membrane of the multilayered epithelium. The surface area is enlarged by leaf-like papillae of 10 to 15 mm length in the cow. Conversely, the abomasum is lined with a glandular epithelium which secretes hydrochloric acid and pepsin and is comparable to the gastric mucosa of animal with simple stomachs.

Studies of organogenesis in ruminant embryos show that the forestomach system develops from an extension of the primordial esophagus to the dorsal side of the primordial stomach, and is subsequently separated from the proper esophagus by constriction [98].

Ruminal Osmoregulation

For short periods of time following the ingestion of a meal, an osmotic gradient between blood and rumen fluid may develop that can exceed 200 mosmol/kg [139], with prefeed values usually being reached within about 3 – 4 hours [129]. The survival both of the microorganisms that digest the roughage, and of the cells lining the rumen depends on a rapid restoration of ruminal osmolarity to the normal level and a number of mechanisms have been described that may facilitate this process. While saliva plays a major role in elevating ruminal osmolarity after drinking [139], hypertonicity cannot be restored by influx of saliva in a reasonable amount of time since saliva is isotonic with blood.

Hyperosmolarity after ingestion of a meal is based in part on an increase in fermentation products that can cross the membrane via lipid diffusion (Figure 1). These include short chain fatty acids (SCFA) [6, 75, 87, 108, 120, 132], ammonia, [1, 16, 17, 41, 54, 87, 93] and CO_2 [6, 87, 97]. Besides lowering ruminal osmolarity and preventing the influx of water and ruminal distention, absorption of SCFA is of essential importance for the energy metabolism of ruminants [12, 13].

Conversely, ruminal hyperosmolarity following ingestion of non-ionic, non-diffusible substances or fermentation of easily digestible carbohydrates such as glucose or starch can lead to problems such as loss of appetite or even ruminal damage in unadapted animals [22, 105]. *In vitro* and *in vivo* experiments have established that in these cases, osmolarity is restored by an increase in water influx into the rumen [31, 42, 78, 122, 129, 158, 160].

Of the electrolytes, potassium shows the biggest rise in concentration [129]. Surprisingly and in marked contrast to the problems that can occur when osmolarity rises after ingestion of easily digestible carbohydrates, the rumen is highly able to deal with the osmotic challenge of large amounts of fresh, potassium rich grass. As potassium in the rumen rises to values that can reach 100 mmol/l, sodium concentration falls so that osmolarity within the rumen remains constant (at about 130 mmol/l) (Figure 2). The inverse regulation of both ions seems to be a constant factor under many different feeding regimes [24, 50, 59, 61, 80, 96, 113, 130, 131, 136, 149, 159, 160]. However, we are only beginning to understand the mechanism by which this regulatory response occurs.

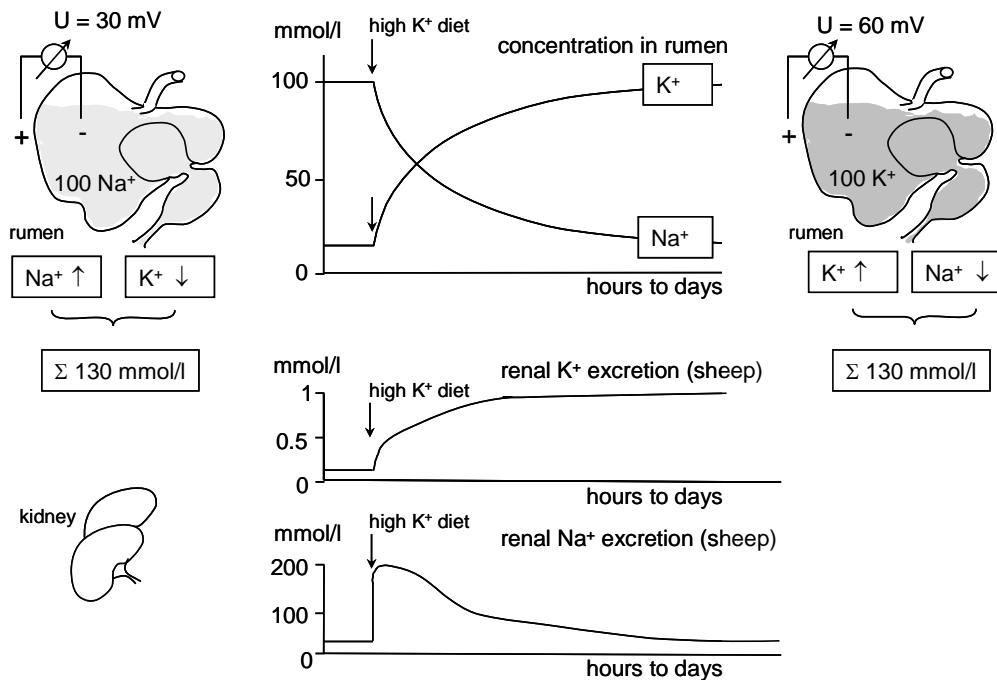


Fig 2: Ruminal osmoregulation: When sheep are transferred from a low potassium diet (i.e. hay) to a high potassium diet (i.e. grass), the concentration of potassium in the rumen continues to rise for a period of several hours to days. Concomitantly, ruminal concentration of sodium falls, so that the sum of both ions remains constant and ruminal osmolarity is restored. *In vivo* measurements show that the transepithelial potential increases with the concentration of potassium. Simultaneously, sodium excretion in urine peaks, while potassium excretion rises more slowly until maximum values are observed after about a week.

The Role of the Rumen in the Potassium Balance of Ruminants

Due to the large amounts of plant material that ruminants have to ingest to meet their energy requirements, daily intake of potassium can exceed 30 % of the total amount to be found in the body and is thus more than ten times higher than the dietary potassium intake of man (as related to body weight) [46, 69, 130]. As in man, about 90% of total potassium intake is absorbed [25, 46, 69, 112, 130, 149]. To prevent a rise to toxic concentrations in the plasma, potassium absorbed by the gut is rapidly redistributed to the cytosolic compartment of muscle, bone, liver and red blood cells, while the much slower renal corrections of disturbances in potassium balance require several hours [46, 70]. Thus, it should not come as a surprise that after a rapid transfer from a low potassium diet to one rich in potassium, the rumen is used as an additional storage space for this mineral [136] until urinary excretion of potassium has reached the level of intake. In the ruminant, this can take several hours or even days [149] (Figure 2).

Conversely, excretion of Na^+ sets in almost immediately after ingestion of a high potassium meal (1-3 hours) [25, 111, 112, 137, 149, 158, 160]. The sodium content of the diet does not appear to be related to this effect as natriuresis of sheep switched to a high

potassium, low sodium diet is larger than that of sheep switched to a medium potassium, medium sodium diet [30]. The kidney of the sheep may retain sodium even when there are ample amounts in the diet, and lose sodium when intake is severely reduced [160], thus reflecting the replacement of Na^+ by K^+ due to ruminal osmoregulation.

From what is known about renal potassium excretion [46], an increase in the delivery of sodium to the distal nephron should stimulate uptake via ENaC, leading to a more lumen negative potential, and enhanced potassium excretion. Therefore, the sodium absorbed from the rumen should help to stimulate renal potassium excretion before ruminal K^+ reaches the gut and plasma potassium levels begin to rise. The ability of the rumen to absorb sodium thus appears central both for potassium balance and ruminal osmoregulation after ingestion of large amounts of potassium rich fodder.

Transport of Sodium and Chloride across the Rumen

Considering that the ability of ruminants to extract scarce sodium from grass and concentrate it in milk has been known for a long time, studies of Na^+ transport across the ruminal epithelium came relatively late. However, and perhaps for this reason, the ruminal epithelium was among the first tissues in which active transport of ions was investigated [143] following Hans Ussing's discovery of active sodium transport in frog skin [152]. The reasons for this interest are obvious: the ruminal epithelium is a moderately tight tissue [109] and thus, very suitable for determining active transport. An additional advantage of this organ is that due to its enormous size, experiments can easily be performed on animals *in vivo* by surgical fistulation of the rumen. Ruminal content can be removed by hand from such fistulas and exchanged for other solutions. It appears that animals adapt to this procedure reasonably well and will continue to ingest hay, if provided, during manipulations of the rumen. The solutions applied should not be dramatically out of the (considerable) physiological range and should contain appropriate amounts of Na^+ , HCO_3^- , Ca^{2+} and Mg^{2+} if influx of saliva into the rumen is prevented in the technique of the isolated rumen.

The first study of ruminal transport was performed on goat rumen pouches *in situ* and left no doubt that chloride was being transported against a chemical gradient from rumen to blood [143]. In the same paper, and five years prior to the discovery of the Na^+/K^+ -ATPase [142], the authors also noted absorption of Na^+ against an electrochemical gradient, and the secretion of a small amount of K^+ into the ruminal pouch. Subsequently, Dobson [27-29] published results from *in vivo* experiments in the sheep demonstrating that sodium is actively transported across the tissue. In these studies, electrodes were used to measure the potential difference, PD_t , generated across the ruminal epithelium. A mean PD_t of 30 mV with the blood side positive was observed, and many subsequent studies have confirmed the hypothesis that this potential is generated by active transport of sodium across the ruminal epithelium, with chloride following along the electrochemical gradient generated by this transport [36-39, 53, 81, 83, 85, 86, 128, 131, 136, 160]. Half of the sodium that is secreted by saliva is thus absorbed by the rumen [27], corresponding to a recirculation of total body sodium 2-3 times a day across this tissue [138]. Thus, water and electrolyte balance in the ruminant cannot be understood without taking the ruminal-portal-salivary exchanges into account (Figure 1).

It has since been demonstrated that the major part of ruminal Na^+ transport takes place by apical uptake of Na^+ via NHE3 exchange [42, 82, 83, 145], blockable by amiloride (1 mmol/l) (Figure 3). Conversely, bumetanide and furosemide have no effect on Na^+ transport across the intact ruminal epithelium, and there is no other indication of Na^+ - K^+ - Cl^- cotransport in the rumen [83, 87].

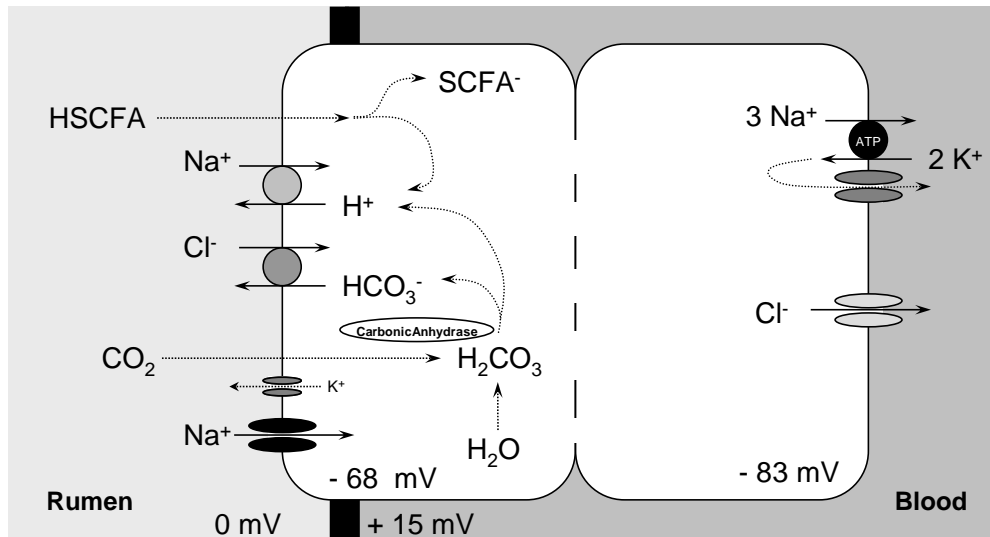


Fig 3: Current model of Na^+ transport across the ruminal epithelium: Na^+ transport from the rumen can occur both via sodium-proton exchange (NHE) coupled to chloride-bicarbonate exchange and via an electrogenic pathway. On the basolateral side, active transport of sodium occurs via the Na^+/K^+ -ATPase which generates a potential (blood side positive) that drives basolateral efflux of chloride through a channel. This, in turn, stimulates apical uptake of Cl^- via $\text{Cl}^-/\text{HCO}_3^-$ exchange and Na^+ via NHE. Carbon dioxide and short chain fatty acids (HSCFA) are thought to diffuse freely through the lipid membrane and stimulate electroneutral sodium transport. (It should be noted that the ruminal epithelium consists of more than two layers. Potential levels are for orientation and can vary considerably from tissue to tissue.)

Basolateral extrusion of Na^+ takes place via the Na^+/K^+ -ATPase mentioned above. Substantial evidence suggests that K^+ is mainly recycled via basolateral potassium channels, since apical potassium secretion is small [16, 39, 53, 73, 76]. The resulting negative cytosolic potential drives Cl^- out of the cell through a basolateral Cl^- channel [1, 74] and into the blood. This, in turn, may generate a chemical gradient for apical uptake of Cl^- via $\text{Cl}^-/\text{HCO}_3^-$ exchange [27, 82], utilizing HCO_3^- that is continuously produced from CO_2 diffusing into the cell across the apical membrane. Accordingly, Cl^- transport is sensitive to acetazolamide in the manner known from many other transporting epithelia [36]. It should be noted that electroneutral coupling of chloride with bicarbonate exchange ensures that the potential gradient built up by the Na^+/K^+ -ATPase does not break down. In line with this model, changes in chloride concentration do not lead to large changes of PD_i [52], although smaller effects of exchanging chloride for sulfate have been noted [37, 80]. However, any apical or paracellular conductance for chloride appears to be relatively small, or blocked by other ions under most circumstances.

Apical Na^+/H^+ exchange is stimulated both by CO_2 ($\Leftrightarrow \text{H}_2\text{CO}_3$) and by the large amounts of short chain fatty acids (SCFA) found in the rumen. Both diffuse through the apical membrane in their lipophilic form, and donate a proton for exchange with Na^+ after dissociation [40, 43, 45, 75, 133-135].

In contrast, the response of ruminal epithelium to the third major fermentation product of the rumen, ammonia, depends on ruminal pH [1, 2]. At an (unphysiologically high) pH of 7.4, addition of ammonia to the ruminal epithelium *in vitro* inhibits the uptake of Na^+ via NHE due to the influx of NH_3 with subsequent reconstitution to NH_4^+ [1] and an increase of cellular pH [97]. At the more acidic values to be found physiologically (5.5-7.0), the amount of NH_3 is reduced greatly due to protonation, and the dominant observation is a stimulation of Na^+ uptake due to uptake of NH_4^+ with subsequent cytosolic release of protons for exchange in NHE [1]. Thus, in the healthy rumen with physiological pH values, the major products of fermentation, short chain fatty acids, bicarbonate and ammonium, all stimulate sodium reabsorption as a requirement for the postprandial stimulation of salivary flow.

Electrogenic Na^+ Conductance in the Rumen

The epithelial sodium channel (ENaC) is the channel that is responsible for the conductance of sodium across most mammalian transporting epithelia in the lung, gut and kidney [71, 115]. Characteristic features are block by low doses of amiloride in the micromolar range, stimulation by aldosterone and lack of voltage dependence (although a slightly higher open probability after *hyperpolarization* of the cell has been observed [114]).

In contrast, amiloride given in a dose as high as 1 mmol/l does not significantly affect the PD_t and the short circuit current across the ruminal epithelium, although Na^+ transport via NHE is significantly reduced. In addition and contrary to expectations, extensive *in vivo* [85] and *in vitro* experiments (unpublished results) by our laboratory failed to show any regulation of ruminal sodium transport by aldosterone. Likewise, adrenalectomy did not affect the PD_t of the ruminal epithelium *in vivo* [131]. In micropuncture experiments on the intact epithelium, it was found that *depolarization* of the apical membrane decreases the fractional apical resistance [72, 122] and increases the conductance of the ruminal epithelium for sodium. Thus, the electrogenic Na^+ conductance of ruminal epithelium has none of the properties associated with the expression of epithelial sodium channels (ENaC).

In an attempt to find further characteristics of the electrogenic sodium conductance of forestomach epithelia, it was observed in Ussing chamber experiments that removal of mucosal Ca^{2+} , Mg^{2+} or both increased the PD_t and the short circuit current across the omasal [121] and ruminal epithelium [73]. At physiological concentrations of both divalent cations, the conductance saturates at a Na^+ concentration of about 30 mmol/l [116]. In light of the great physiological variability of ruminal Na^+ content (which varies from 25 mmol/l to above 100 mmol/l), this property is essential for maintaining sodium conductance at low levels of Na^+ , while preventing a massive Na^+ influx at higher concentrations which might damage cell homeostasis.

It should be noted that these experiments were performed in absence of an electrochemical gradient for sodium and can thus not be explained by opening of a paracellular shunt pathway due to removal of divalent cations. Since both short circuit current and potential difference rose almost immediately after removal of divalent cations, it can also be concluded that the Na^+/K^+ -ATPase is not the rate limiting step in sodium transport across the ruminal epithelium.

In microelectrode experiments on intact epithelia, removal of mucosal divalent cations led to a depolarization of the apical membrane [72], while paracellular conductance was not

affected. Maneuvers that have been shown to reduce cytosolic Mg^{2+} concentration in isolated cells such as the elevation of cAMP [77, 126], magnesium deprivation [73, 124], or reduction of serosal Na^+ [121], can also increase Na^+ conductance through forestomach epithelia.

In patch clamp experiments on isolated cells of the ruminal epithelium, we have observed similar effects: conductance for both Na^+ and K^+ increases when external divalents cations are removed. Interestingly, a further opening could be achieved by hyperpolarization, and it was found that the voltage dependence of this conductance was altered by the cytosolic level of Mg^{2+} [77, 147]. A residual sodium conductance can also be observed in physiological solutions with normal concentrations of external divalent cations (Figure 4 A and B). However, when the Mg^{2+} concentration in the pipette is set to values at the upper end of the range reported for intact ruminal epithelial cells [123, 124, 126], no significant conductance for Na^+ can be seen (Figure 4 C). Under these high Mg^{2+} conditions, sodium conductance could be stimulated by elevation of cytosolic cAMP, either by direct application to the cytosol via the patch pipette, or via PGE2 [146]. The latter effect may rest both on a reduction of cytosolic magnesium [126], and/or upon direct effects on the channel protein. It is noteworthy that an endogenous production of prostaglandins has been observed in intact ruminal epithelium [40].

Although the precise nature of the Na^+ conducting channel in ruminal epithelium has yet to be identified, our experiments suggest that electrogenic uptake of Na^+ by the sheep rumen is regulated by voltage dependent binding of external and internal magnesium to sites in the pore of a non-selective cation channel.

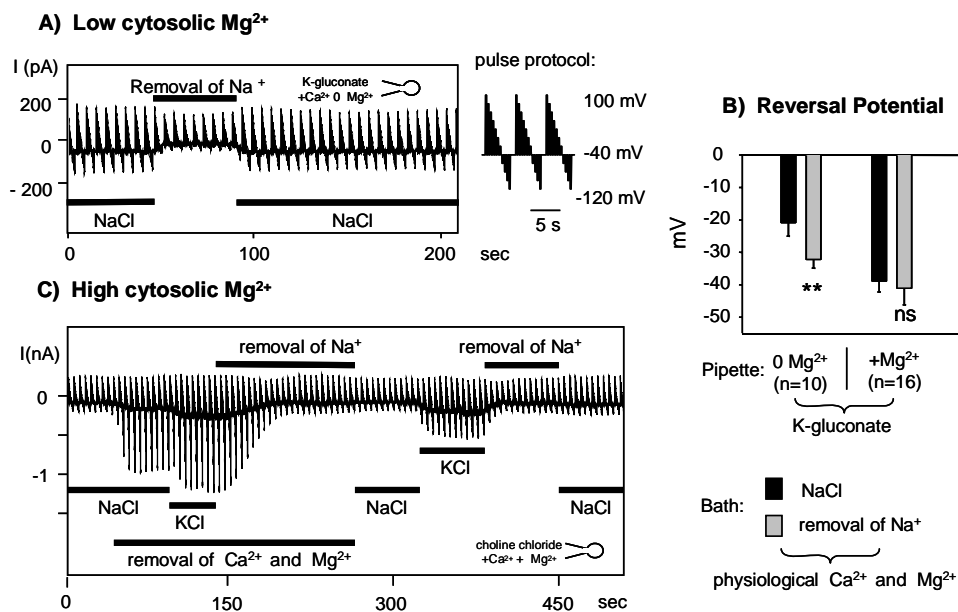


Fig 4: Sodium conductance of ruminal epithelial cells is blocked by external and internal Mg^{2+} : Original patch clamp recordings. Cells were filled with different pipette solutions, and currents in response to various voltages (pulse protocol) were measured. Removal of Na^+ from a bath solution with physiological concentrations of Ca^{2+} and Mg^{2+} resulted in a visible reduction of inward currents (A) and significant depolarization of the cells (B) when filled with a pipette solution that contained no Mg^{2+} . When Mg^{2+} was added to the cytosolic medium in a concentration of 0.9 mmol/l, effects of removing sodium from the bath solution were not significant. Removal of external Ca^{2+} , Mg^{2+} , or both increased both sodium and potassium conductance of the cells.

Mg²⁺ Gating of Ion Channels

Regulation of channel conductance by intracellular or extracellular magnesium is known from a large number of ion channels, such as the inwardly rectifying potassium channel, the NMDA receptor, a number of non-selective cation channels from the TRP family [3, 100, 154] or the epithelial sodium channel, ENaC [26].

While voltage gated channels respond to a change in membrane voltage by changes in the conformation of the channel protein, in magnesium gated ion channels, removal of Mg²⁺ in the solution eliminates the voltage dependence. Inwardly rectifying potassium channels have been studied extensively for many decades [10, 56, 117], and it appears that voltage dependence is related to binding of Mg²⁺ to a binding site on the intracellular side (Figure 5 A). When the interior of the cell is hyperpolarized versus the outside, the resulting potential gradient will tend to push positively charged ions into the cell and away from the cell membrane. If the binding of Mg²⁺ to the internal mouth of the channel is weak, the probability that Mg²⁺ will dissociate and move into the cell lumen rises, and thus, the number of channels that are open at any given moment will increase. A characteristic and distinctive property of the inward rectifier is that the conductance change is a function not of the absolute potential E , but of the potential difference $E - E(K^+)$ [56, 66] where $E(K^+)$ is the reversal potential for potassium. The reason for this is that as the external potassium concentration rises, influx of potassium ions will push the blocking Mg²⁺ ion out of internal mouth of the channel pore [55] and the channel will open at voltages where it would otherwise have been closed. Thus, paradoxically, a channel that normally only opens when it is hyperpolarized to low levels will also open when the external side is exposed to a high concentration of potassium. The relatively simple mechanism of Mg²⁺ block allows depolarization of the cell when extracellular potassium concentration rises and allows influx of potassium when the membrane voltage is below the Nernst potential for this ion, but prevents efflux of potassium out of the cell at higher voltage levels.

NMDA receptors of neurons are channels with a high permeability for calcium and have been studied extensively, facilitated undoubtedly in part by the low resting conductance of the neuron's cell membrane. These channels are opened by certain agonists, but conduct poorly in normal bathing medium with physiological concentrations of magnesium, even in the presence of an agonist [55]. However, when the cell is depolarized in the presence of an agonist, their conductance increases [4, 62]. In these channels, the binding sites for Mg²⁺ are thought to be inside the channel pore, within the region where transmembrane voltage drops from the external level to the level found on the internal side of the cell membrane. As above, it has been established that the affinity to Mg²⁺ changes as a function of membrane voltage (Figure 5 B). However, at depolarized membrane voltages, the probability of Mg²⁺ occupying these *external* sites is lower, the block is less pronounced, and the conductance of the NMDA receptor increases. Interestingly, this block appears to be modulated by binding of permeant monovalent cations to the external channel vestibule of NMDA receptors [110]. A further feature is an additional block with different binding affinity by *internal* Mg²⁺ [63]. It has been suggested that Mg²⁺ can permeate the channel at a low, voltage dependent rate and would thus impede the flow of other ions through the pore, with the number of channels blocked in this manner rising with the concentration of Mg²⁺. The different blocking properties of external or internal Mg²⁺ can be explained by channel models in which several ions move in single file through a long narrow pore with asymmetrical energy barriers [63], [56]. Note that

the electrical field within the channel pore and thus, the forces that bind and repel the blocking Mg^{2+} ion, depend on the other ions within the pore. It has been proposed that the combination of block by internal and external Mg^{2+} make the NMDA-activated channel a bidirectional rectifier with an optimal voltage range for conductance [63] (Figure 5 C).

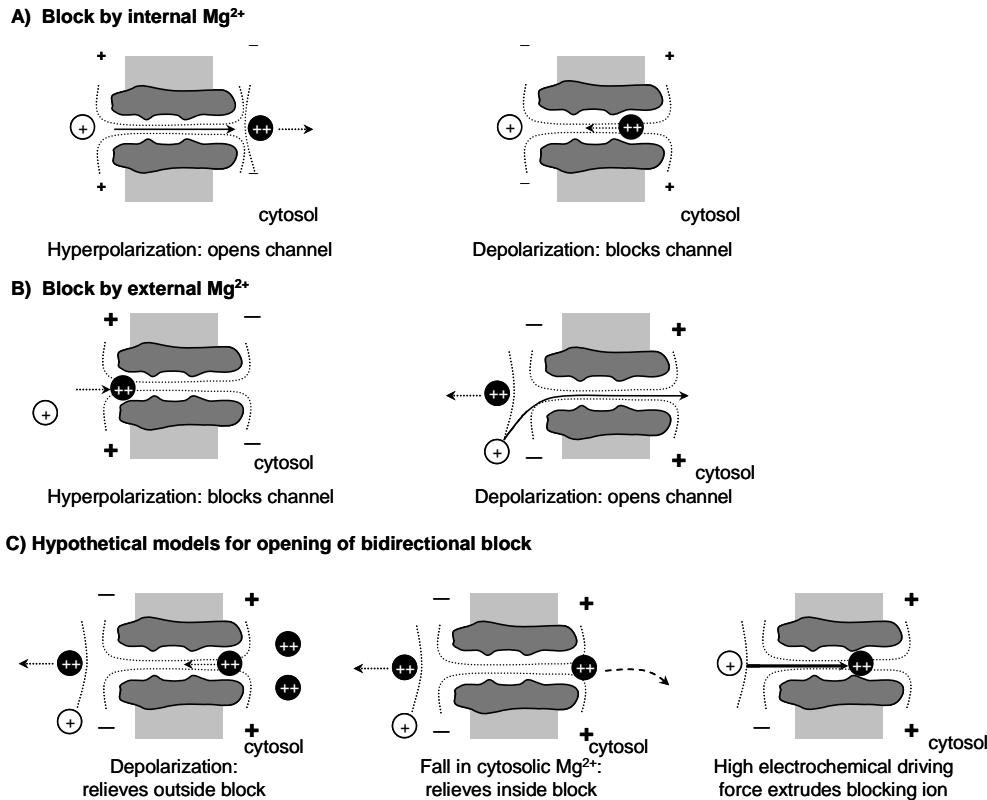


Fig 5: Voltage dependent block of ion channels by Mg^{2+} : some models (see text)

The epithelial calcium channels ECaC 1 (TRPV6) and ECaC 2 (TRPV5) are known for their extremely high Ca^{2+} selectivity ($P_{Ca}/P_{Na} > 100$) *in vitro*, and appear to be involved in Ca^{2+} reabsorption in kidney and intestine [106, 154]. When divalent cations are removed, they become permeable to monovalent cations and open at negative potentials with strong inward rectification. As with the inward rectifier, this rectification depends at least in part on the presence of intracellular Mg^{2+} . Block by external divalent cations is decreased when intracellular Mg^{2+} levels drop. As with the NMDA receptor, interaction with permeant cations has been observed. At high potentials, Mg^{2+} block is relieved, indicating that Mg^{2+} may be able to permeate the selectivity filter of the channel if the driving force is high enough [154].

In terms of conductance for sodium and block by divalent cations, the ruminal conductance for sodium has striking similarity to channels belonging to the TRP family [106]. However, in isolated cells of the ruminal epithelium, the blocking effects of divalent cations are much larger than any currents that might be attributed to a conductance for Ca^{2+} and Mg^{2+} , even if the electrochemical driving force is set to very high levels in patch clamp

experiments [148]. This is in line with the *in vitro* and *in vivo* observation that influx of Mg^{2+} into the cells and across the rumen saturates [85, 86, 123], although initial uptake rates are high and fast. Thus, while it cannot and should not be excluded that Mg^{2+} uptake by the rumen may involve channels from this group, these channels do not display the high rates of Mg^{2+} conductance reported for channels such as TRPM7 [95, 99].

Interestingly, low levels of messenger RNA of both TRPV5 and TRPV6 have been found in the rumen of sheep [162] where functionally, they may be involved in the transport of Ca^{2+} [120]. In the same study, a higher expression of both channels not just on the mRNA but also on the protein level was found in other parts of the gut of the sheep where absorption of Ca^{2+} is thought to play a minor role, and thus, the physiological role of these channels in the gastrointestinal tract needs to be clarified.

In the case of TRPV5, both high Ca^{2+} selectivity and sensitivity to extracellular divalent cations appear to depend on a single aspartate residue in the pore region between transmembrane domains 5 and 6 [101]. Point mutations can be expected to dramatically alter the conductance of ion channels, and the TRP family is no exception. Associated proteins may lead to further differences [153]. It should be noted that buffering of Ca^{2+} and Mg^{2+} in physiological solutions is by no means trivial [51, 79]. Since the cytosolic concentration of these ions has impacts on the selectivity of these channels, a prediction of their conductance *in vivo* from *in vitro* experiments has to be done with care. In most patch clamp studies [101] including our initial experiments with ruminal epithelial cells, [1, 101], the concentration of Mg^{2+} in the pipette solution is higher than the free concentration of the ion that is found in resting intact cells [123, 127], and the situation in the intact epithelium *in vivo* may be even more complex.

A recent study has shown that the esophageal epithelium of the rabbit possesses a sodium conductance with similar properties, and it has been suggested that in this tissue, ENaC subunits assemble to form a non-selective cation channel that is not blockable by amiloride [7]. Data from RT-PCR experiments in our laboratory suggest that alpha subunits of ENaC are expressed in ruminal epithelium (personal communication, Jatti Priesnitz). As mentioned, regulation of ENaC by intracellular Mg^{2+} has been demonstrated [26]. However, attempts to demonstrate other subunits have not been convincing so far, and while this is certainly an intriguing possibility, we do not feel that at this point that the involvement of other cation channels (i.e. TRP channels [100]) in ruminal sodium transport should be ruled out in light of the failure of aldosterone or amiloride to show any effects on electrogenic sodium conductance in the forestomach.

It may be argued that since the greater amount of sodium absorbed by the rumen is transported by the NHE as outlined above, any interest in a further mechanism is purely academic. However, the apical electrogenic entry of sodium determines the magnitude of the transepithelial potential (blood side positive), [27, 37-39, 82], and is thus an essential contributor to the total driving force for the basolateral efflux of chloride and other anions such as bicarbonate [27]. Basolateral efflux of chloride drives apical uptake of chloride, which, in turn, should stimulate Na^+ uptake via coupling of NHE with Cl^-/HCO_3^- exchange. More importantly, perhaps, stimulation of electrogenic sodium transport should have a negative impact on the electrochemical driving force for potassium efflux from the rumen. The central importance of electrogenic sodium transport for transruminal potential thus makes it a gate-keeper for the transport of ions across the rumen, and since the electrogenic Na^+

conductance of ruminal epithelium is regulated by changes in cytosolic magnesium, the uptake of this ion by the ruminal epithelium should be of some interest.

ABSORPTION OF MAGNESIUM

Interest in the uptake of magnesium by the ruminant is old and related to a surge of cases of an affliction known as “grass tetany” that led to widespread loss of cattle in the last century [90, 125]. After sudden transfer from normal winter rations to young spring grass, animals became afflicted with excitability, salivation, ataxia, recumbency, and tetanic muscle spasms [140, 141]. Despite normal magnesium content of the fodder, the animals were apparently unable to absorb this mineral and thus suffered from hypomagnesaemia. Sjollem’s hypothesis that the surge in frequency was related to the introduction of fertilizers containing potash has since been confirmed by a solid body of evidence which has been reviewed elsewhere [34, 90]. It should be noted that a number of other factors are known to contribute to malabsorption of magnesium that include sodium deficiency [118] and high protein content of the diet with subsequent high levels of ruminal ammonia [41].

Absorption of magnesium from the rumen has been studied extensively at the level of the animal, the tissue, and the cell. It has been established that the main site for absorption of magnesium in the ruminant is the forestomach [107]. When the rumen is bypassed, decreased plasma Mg^{2+} concentrations, decreased urinary Mg^{2+} excretion and increased fecal Mg^{2+} excretion is observed. This negative magnesium balance cannot be compensated for by post-ruminal infusion of Mg^{2+} [150].

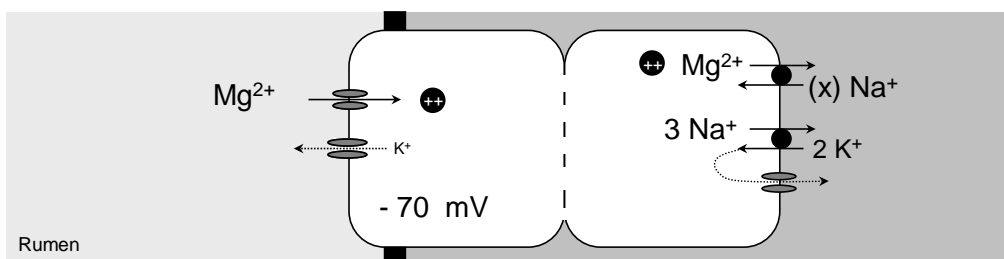
Given that the serosal side of the rumen is positive versus the mucosal side (30 mV), any paracellular transport will move Mg^{2+} from blood into the rumen [20]. Transport must thus be transcellular and active and was first demonstrated by Martens and Harmeyer in 1978 [86]. In the same study, it was shown that the transport is ouabain sensitive and saturable. The presence of basolateral Na^+/Mg^{2+} exchange coupled to Na^+/K^+ -ATPase has since been established [77, 126] as the mechanism underlying the active step of absorption (Figure 6 A).

In contrast, it has been shown that apical uptake depends on the electrochemical driving force for Mg^{2+} across the apical membrane (Figure 6 B). Depolarization of this membrane by potassium was found to reduce transport of Mg^{2+} across the rumen *in vivo* using various approaches that include the temporary isolation of the rumen from influx of saliva [19, 50, 80, 85, 87, 89, 94, 164]. Experiments across isolated ruminal epithelium in the Ussing chamber established that the potential difference, and not potassium concentration, were central to the reduction in the uptake of Mg^{2+} [76, 84]. Likewise, exposure of isolated cells of the ruminal epithelium in culture to high potassium solution resulted in a reduced uptake of Mg^{2+} [123, 124]. It has been shown that this maneuver depolarizes isolated cells of the ruminal epithelium via potassium channels [1]. In contrast, plasma magnesium concentration had no influence on the rate of Mg^{2+} absorption [91], and the effects of K^+ on Mg^{2+} absorption are not attributable to high levels of absorbed K^+ , but rather to the concentration of K^+ present in the rumen [113]. From these experiments, a model has emerged that resembles that developed for the distal convoluted tubule, where absorption occurs via an apical Mg^{2+} channel in conjunction with a basolateral Na^+/Mg^{2+} exchanger [65, 161]. In the context of this review, it is tempting to point out that in humans, a fall in plasma magnesium is known to lead to

kaliuresis [161] [64] [105], and that reduction in plasma Mg^{2+} levels should thus help with excretion of potassium.

It should be noted that there is evidence for a second transporting process that is not potential dependent and which facilitates uptake of magnesium into depolarized cells at higher ruminal potassium concentrations of this mineral [61, 76, 124]. In addition, a number of factors are known to stimulate absorption of magnesium from the rumen, including a rise in bicarbonate, short chain fatty acids, and a low but physiological pH of around 6.4 [90].

A) Low ruminal potassium



B) High ruminal potassium

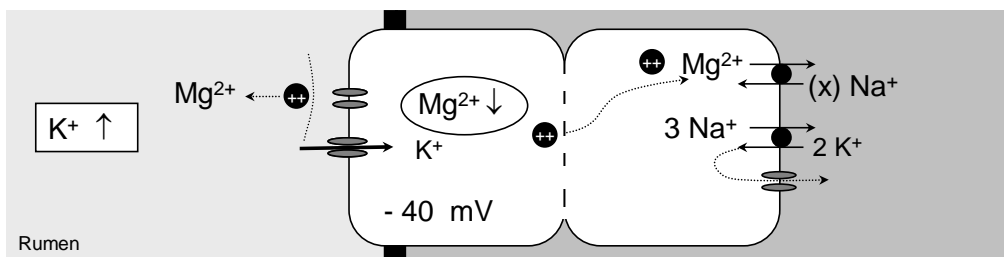


Fig 6: Reduction of Mg^{2+} uptake by elevation of ruminal potassium: Apical Mg^{2+} influx occurs down the electrochemical gradient, driven by the negative potential across the apical membrane (A). Basolateral efflux occurs in exchange for sodium. The stoichiometry has not been identified.

After elevation of ruminal potassium (B), the apical membrane is depolarized and the gradient for uptake of Mg^{2+} decreases. Cytosolic Mg^{2+} concentration drops, and thus, also basolateral extrusion. Note that the relative contribution of the potassium conductance to total membrane permeability may rise as ruminal potassium levels increase.

Interestingly, reductions in the absorption of Mg^{2+} were also observed after acute exposure to ammonia [18, 41, 54, 87, 89]. For reasons that are not clear at present, compensatory mechanisms set in after a few days [41] in this case, so that hypomagnesaemia is transient. It is possible to speculate about the role that the electrogenic vacuolar H-ATPase might play in this regulatory response [124] or about up-regulation of enzymes which are involved in ammonia metabolism in the rumen epithelium [102, 103].

The practical implications of these physiological background facts are clear and have led to a reduction in the frequency of the affliction. Adjusting cattle to a high potassium and high protein diet with production of ammonia must be done slowly. Mg^{2+} concentration in the rumen must be elevated by feeding Mg^{2+} salts to activate the non-potential dependent magnesium uptake mechanism. Fertilization techniques which greatly raise potassium intake should be avoided. In addition, care has to be taken that animals are not sodium depleted,

since this increases aldosterone levels, salivary excretion of potassium and thus, elevates ruminal levels of potassium [90] [118] with the detrimental effect on Mg^{2+} absorption from the forestomachs described above [88].

RUMINAL TRANSPORT OF POTASSIUM

Given the high concentration of potassium in the ruminant diet and that the volume of the rumen can exceed that of the plasma, it is clear that any absorption of potassium from this organ has to be slow. When total potassium absorption is related to feed intake and recirculation of saliva is not considered, the contribution of the rumen to total potassium absorption is marginal, and most studies regard the small intestine as the major site for the absorption of dietary potassium [69, 164].

However, when the rate of absorption is related to the concentration found in the rumen, a certain permeability of the ruminal wall for potassium becomes apparent. Secretion of potassium under experimental conditions with very low ruminal potassium concentration was first noted by Sperber and Hyden [143], and this finding has been confirmed many times [35, 59, 80, 130, 160]. The simplest explanation for this observation is to assume a leak of potassium into the rumen paracellularly from the blood side (4-5 mmol/l K^+), driven by the positive PD_t of about 30 mV (blood side positive). In this case, an increase in ruminal potassium should lead to an increase in the potential across the ruminal wall, and such a potential was, indeed, measured in feeding experiments [136]. Subsequent parallel *in vivo* and *in vitro* investigations [37, 38] confirmed that the potential rose strongly with the luminal concentration of potassium both in the completely isolated rumen in anaesthetized sheep, and in isolated ruminal epithelium in the Ussing chamber. Conversely, raising Na^+ from a basal level of 20 mmol/l had little (*in vivo*) or no (*in vitro*) effect [37, 38]. Later *in vivo* studies (under more physiological conditions with HCO_3^- and acetate instead of Cl^- as the major anions [18, 80]) confirmed the finding that K^+ concentration contributes more to overall transepithelial potential than concentrations of Na^+ exceeding 20 mmol/l. However, it should be noted that the increasing transepithelial potential with falling ruminal sodium concentration in these experiments suggests that any paracellular leak of sodium into the rumen has to be small. Thus, the simple model of a hypothetical "leak" pathway postulated above cannot be quite so simple after all and must, at the very least, be highly selective for potassium over sodium.

At first glance, these results seem to suggest that the efflux of potassium has to be considerable since the transruminal potential has to be continuously maintained by a corresponding flux of ions under *in vivo* non-equilibrium conditions. This is very clearly not the case. The ability of the rumen to concentrate potassium has already been outlined above (Figure 2). When sheep are transferred from a low potassium diet of hay and meals to a diet of high potassium grass from a heavily fertilized meadow, ruminal potassium concentrations can rise from levels of about 30 mmol/l to peaks of 100 mmol/l [136]. Concomitantly, the concentration of sodium falls from levels as high as 100 mmol/l to values of 25 mmol/l. Thus, while there can be no doubt about the coupling of potassium and transruminal potential [37, 38, 61, 76, 80, 84, 131, 155], and while a small passive flux of potassium down its electrochemical gradient has been demonstrated [59, 160], the major ion being absorbed under these conditions is sodium [59, 130, 144, 158, 160]. In line with this, a complete removal of

sodium (or application of ouabain) abolished the potential and the short circuit current across the ruminal epithelium [53], establishing Na^+ as the major ion responsible for the potential across the ruminal wall. An explanation for the failure to observe changes in transepithelial potential in response to the elevation of ruminal sodium above 20 mmol/l [37, 38] has since emerged: *in vitro*, electrogenic sodium conductance saturates at 30 mmol/l of Na^+ [116]. Thus, raising Na^+ concentration from 20 mmol/l to higher values should only have marginal effects.

These data do not support the originally proposed model of a major paracellular leak pathway for potassium and suggest a transcellular electrogenic uptake pathway highly selective for potassium over sodium, with potassium channels being ideal candidates. In line with this, a high concentration of potassium was found to depolarize the apical membrane of ruminal epithelium and decrease the fractional apical resistance [76]. *In vitro* studies showing that active, ouabain sensitive potassium secretion can take place under equilibrium conditions [39, 53, 76, 163], with evidence for the existence of additional basolateral potassium channels [76], support the model of channel mediated transcellular uptake.

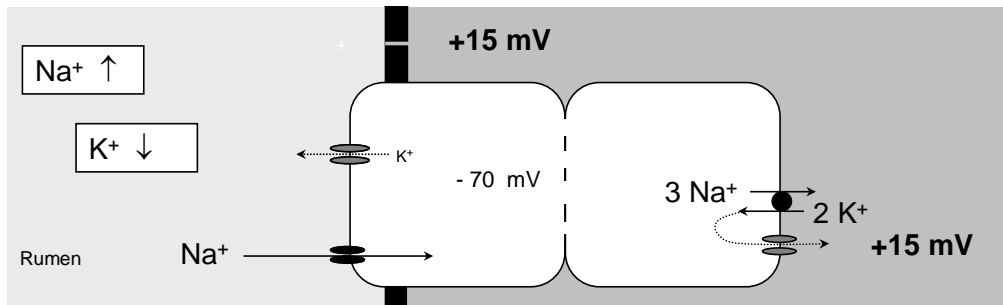
A similar notion of apical potassium channels evolved independently from *in vivo* experiments on sheep, demonstrating that ruminal uptake of ammonia cannot be explained exclusively by assumption of lipophilic diffusion of NH_3 [17]. *In vitro* experiments in the Ussing chamber followed, that postulated electrogenic uptake of NH_4^+ via a quinidine sensitive potassium channel [16]. This suggestion is supported by a recent study demonstrating stimulating effects of NH_4^+ on sodium transport, thus establishing that transport of NH_4^+ has to be transcellular [1]. Using the patch clamp technique, it was possible to directly demonstrate corresponding quinidine sensitive potassium channels with a conductance for both potassium and NH_4^+ in isolated ruminal epithelial cells [1]. Interestingly and typically [5, 33, 55, 56, 165], the open probability of these channels [1] rose with the concentration of the permeant ion. This property of potassium channels may help to explain why the cell is depolarized with increasing levels of potassium and falling levels of sodium [39, 53, 76] despite the fact that in low potassium ringer, the conductance for sodium exceeds that for potassium.

The assumption of transcellular flux via potassium channels integrates certain observations in a satisfactory way, since uptake of NH_4^+ is favored by the electrochemical gradient across the apical membrane of the rumen, whereas passive influx of K^+ into the cytosol is not possible except at high ruminal potassium concentrations due to the high cytosolic concentration of potassium. This is very much in line with the *in vivo* observations of ammonia transport at low pH and low concentrations of ammonia [17], while very high concentrations of potassium are necessary in order to observe sizable transport rates for potassium [160]. However, the failure to observe a clear effect of quinidine on potassium transport across the rumen *in vivo* does leave some room for a debate that should include the very wide range of quinidine effects on cellular metabolism [35]. Despite this caveat, the *in vivo* and *in vitro* evidence for the existence of potassium channels with a substantial contribution to total conductance of the apical and basolateral membrane of the ruminal epithelium is solid, and a viable model is needed to explain why nevertheless, potassium movements across the ruminal wall remain small [69, 136, 160].

An attempt to integrate the various observations can be made by considering the interaction of the electrogenic sodium conductance with potassium transport (Figure 7). As stated before, this pathway is stimulated by the depolarization of the apical membrane [72]

and part of the potential generated by an increase of potassium in the rumen should be due to stimulation of this sodium conductance. Now, the paradoxical observations fall into place: potassium depolarizes the apical membrane, opens the sodium conductance [72, 77], the transepithelial potential increases, the electrochemical driving force for the efflux of potassium from the rumen falls. Therefore, we see an increase in sodium transport, a rise in transepithelial potential, but potassium efflux from the rumen remains limited.

A) Low ruminal potassium



B) High ruminal potassium

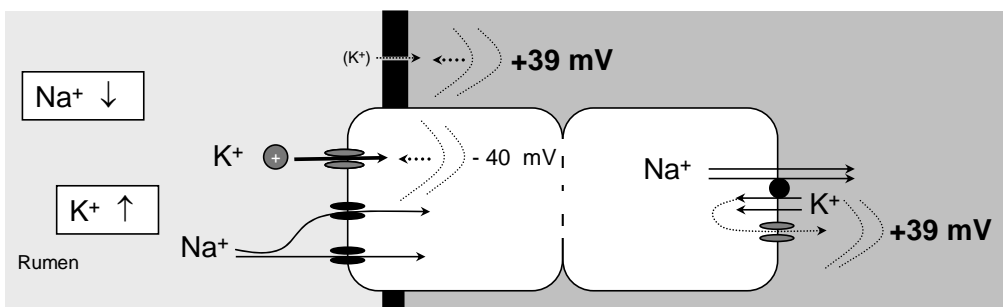


Fig 7: Uptake of K^+ by the ruminal epithelium: Flux of K^+ across the ruminal epithelium is passive and proportional to the transepithelial electrochemical driving force. With increasing concentration of ruminal K^+ , the efflux of K^+ from the rumen into the plasma increases. However, stimulation of electrogenic Na^+ transport with depolarization of the apical membrane and generation of a strong, lumen positive transepithelial potential limits the amount of K^+ that leaks out of the rumen. *In vivo*, transepithelial potential can reach values of 60 – 70 mV.

A ROLE FOR MAGNESIUM IN RUMINAL OSMOREGULATION

Numerous studies demonstrate that rising levels of potassium stimulate the absorption of sodium across the rumen *in vivo* [59, 130, 144, 158, 160]. Harrison notes that *in vitro* stimulation of Na^+ transport by elevation of K^+ only succeeded under open circuit conditions [52], and thus, a potential dependent mechanism appears to be involved. This effect cannot be attributed to a rise in osmotic pressure, since the effects of urea or mannitol on sodium absorption were discrete [160]. As has been outlined above, a high increase in osmotic pressure due to administration of non-ionic osmotic agents can even reduce Na^+ absorption, and induce water influx into the rumen [44, 78, 122]. Conversely, potassium stimulates absorption of sodium even in the absence of an osmotic gradient [85].

In light of the information we have at this point, it is possible to develop a fairly consistent model that may describe the events that occur when sheep or cows are transferred

from a low potassium diet of hay to a diet higher in potassium, such as fresh spring grass (Figure 8). As the concentration of potassium in the rumen increases, the conductance of the apical membrane for potassium rises [1, 55] and the apical membrane is depolarized [76], the binding affinity of divalent cations at the external mouth of the non-selective cation channel is loosened, and simultaneously, uptake of magnesium decreases. Now, the non-selective cation channel begins to open [77, 147].

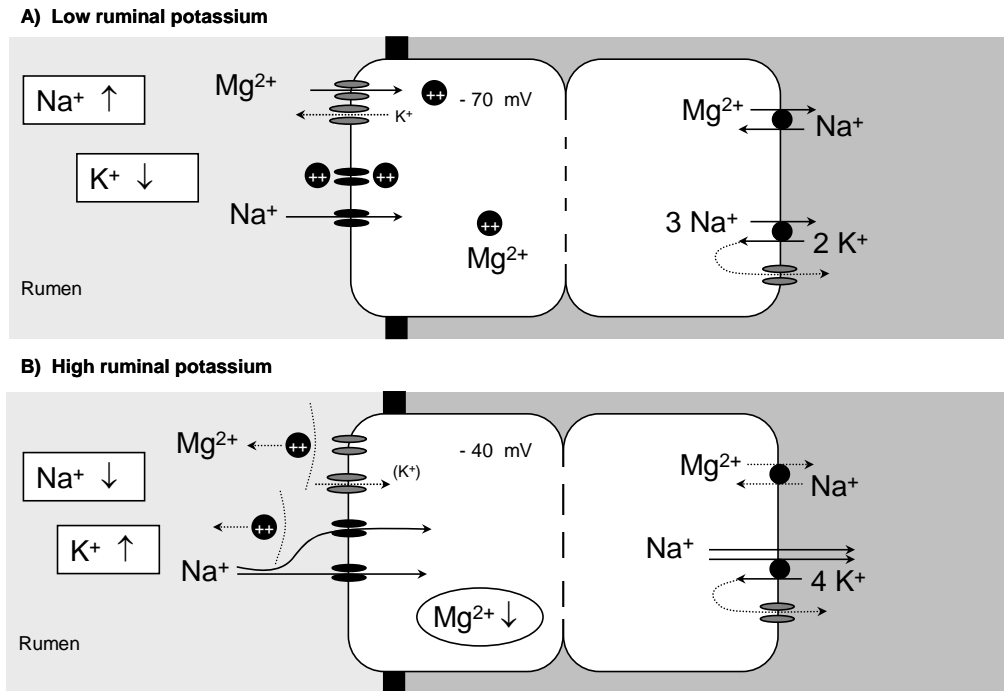


Fig 8: Stimulation of ruminal sodium transport by elevation of ruminal potassium: A) Divalent cations block many of the non-selective cation channels in the apical membrane, limiting electrogenic sodium influx. Despite a large concentration of apical Na⁺, total sodium flux is low. After elevation of the ruminal K⁺ concentration (B), the apical membrane is depolarized, uptake of Mg²⁺ decreases and cytosolic Mg²⁺ concentration drops. In addition, depolarization relieves the block of the non-selective cation from the external side. Electrogenic Na⁺ transport rises creating an additional depolarizing stimulus and reducing the electrochemical gradient for the uptake of K⁺.

As Na⁺ rushes in, the apical membrane is depolarized even further and a catastrophic cascade of events would unfold but for the fact that at a certain potential level, residual internal Mg²⁺ begins to block the pore from the inside, thus limiting a further depolarization. It appears conceivable that at elevated cytosolic concentrations of sodium, basolateral Na⁺/Mg²⁺ exchange slows down so that internal Mg²⁺ levels begin to rise again despite reduced uptake. And thus, this model can predict that at medium levels of potassium, sodium transport is stimulated, while at very high levels of potassium, sodium transport is depressed [160].

An intriguing feature of this model is that as the transepithelial potential depolarizes due to the electrogenic efflux of sodium, the driving force for the flux of potassium through leaks from the rumen such as the paracellular pathway is decreased. Again, we can predict what happens when potassium levels rise: as sodium transport is depressed, potassium conductance begins to rise and this is, indeed, what is observed [160]. Thus, the introduction of potassium

channels in the apical membrane of the rumen does make “sense” from an evolutionary point of view – or did, until potash fertilizers came up [140].

CONCLUSION

Ruminants can deal with abrupt and extremely large rises in potassium intake due to their ability to utilize the rumen as an additional reservoir for buffering K^+ until renal excretion of K^+ has set in. Ruminal concentrations of potassium can rise to values of 100 mmol/l in these situations, with sodium content decreasing in proportion so that osmolarity remains constant throughout. This is essential since rising osmolarity would interfere with ruminal fermentation processes and food uptake by the animal.

In contrast to the pronounced ability to maintain physiological ruminal osmolarity and plasma potassium levels, hypomagnesemia is a known complication with economic significance after transfer of ruminants to a high potassium diet. The pathogenesis of this condition has been shown to involve a decrease in Mg^{2+} absorption by the rumen after exposure to high ruminal potassium.

We feel that the two phenomena may be causally linked via a signaling cascade that involves depolarization of the apical membrane by high potassium, reduced uptake of magnesium, and opening of a sodium conductance with subsequent rise in sodium transport out of the ruminal pouch (Figure 9). In evolutionary terms, the potassium-induced reduction in digestibility of Mg^{2+} may be balanced by the increased ability to quickly ingest very large amounts of fresh grass after a longer period of famine.

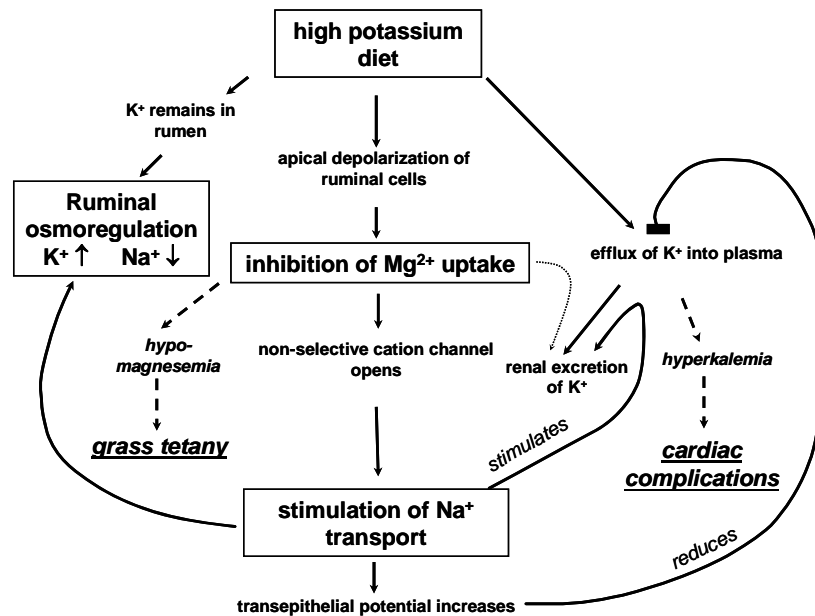


Fig 9: A role for magnesium in ruminal osmoregulation: The adverse effects of an inhibition of transcellular ruminal Mg^{2+} transport are balanced by the positive effects of a stimulation of Na^+ efflux from the rumen, namely, inhibition of K^+ efflux into the plasma, stimulation of renal K^+ excretion, and regulation of ruminal osmolarity.

REFERENCES

- [1] Abdoun K, Stumpff F, Wolf K, and Martens H. Modulation of electroneutral Na transport in sheep rumen epithelium by luminal ammonia. *Am J Physiol Gastrointest Liver Physiol* 289: G508-520, 2005.
- [2] Abdoun K, Wolf K, Arndt G, and Martens H. Effect of ammonia on Na⁺ transport across isolated rumen epithelium of sheep is diet dependent. *Br J Nutr* 90: 751-758, 2003.
- [3] Albitz R, Magyar J, and Nilius B. Block of single cardiac sodium channels by intracellular magnesium. *Eur Biophys J* 19: 19-23, 1990.
- [4] Antonov SM and Johnson JW. Permeant ion regulation of N-methyl-D-aspartate receptor channel block by Mg(2+). *Proc Natl Acad Sci U S A* 96: 14571-14576, 1999.
- [5] Aqvist J and Luzhkov V. Ion permeation mechanism of the potassium channel. *Nature* 404: 881-884, 2000.
- [6] Ash RW and Dobson A. The Effect of Absorption on the Acidity of Rumen Contents. *J Physiol* 169: 39-61, 1963.
- [7] Awayda MS, Bengrine A, Tobey NA, Stockand JD, and Orlando RC. Nonselective cation transport in native esophageal epithelia. *Am J Physiol Cell Physiol* 287: C395-402, 2004.
- [8] Bach A, Calsamiglia S, and Stern MD. Nitrogen metabolism in the rumen. *J Dairy Sci* 88 Suppl 1: E9-21, 2005.
- [9] Bailey CB. Saliva secretion and its relation to feeding in cattle. 4. The relationship between the concentrations of sodium, potassium, chloride and inorganic phosphate in mixed saliva and rumen fluid. *Br J Nutr* 15: 489-498, 1961.
- [10] Balsler JR, Roden DM, and Bennett PB. Single inward rectifier potassium channels in guinea pig ventricular myocytes. Effects of quinidine. *Biophys J* 59: 150-161, 1991.
- [11] Beja-Pereira A, Luikart G, England PR, Bradley DG, Jann OC, Bertorelle G, Chamberlain AT, Nunes TP, Metodiev S, Ferrand N, and Erhardt G. Gene-culture coevolution between cattle milk protein genes and human lactase genes. *Nat Genet* 35: 311-313, 2003.
- [12] Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* 70: 567-590, 1990.
- [13] Bergman EN, Reid RS, Murray MG, Brockway JM, and Whitelaw FG. Interconversions and production of volatile fatty acids in the sheep rumen. *Biochem J* 97: 53-58, 1965.
- [14] Blair-West JR, Coghlan JP, Denton DA, Goding JR, and Wright RD. The effect of aldosterone, cortisol, and corticosterone upon the sodium and potassium content of sheep's parotid saliva. *J Clin Invest* 42: 484-496, 1963.
- [15] Boda JM, McDonald PG, and Walker JJ. Effects of the Addition of Fluids to the Empty Rumen on the Flow Rate and Chemical Composition of Bovine Mixed Saliva. *J Physiol* 177: 323-336, 1965.
- [16] Bödeker D and Kemkowski J. Participation of NH₄⁺ in total ammonia absorption across the rumen epithelium of sheep (*Ovis aries*). *Comp Biochem Physiol A Physiol* 114: 305-310, 1996.

-
- [17] Bödeker D, Winkler A, and Holler H. Ammonia absorption from the isolated reticulo-rumen of sheep. *Exp Physiol* 75: 587-595, 1990.
- [18] Care AD, Brown RC, Farrar AR, and Pickard DW. Magnesium absorption from the digestive tract of sheep. *Q J Exp Physiol* 69: 577-587, 1984.
- [19] Care AD, Farrar AR, and Pickard DW. Factors affecting the absorption of magnesium from the rumen in sheep. *Journal of Physiology* 325, 1982.
- [20] Care AD and van't Klooster AT. In Vivo Transport of Magnesium and Other Cations across the Wall of the Gastro-Intestinal Tract of Sheep. *J Physiol* 177: 174-191, 1965.
- [21] Carr DH and Titchen DA. Post prandial changes in parotid salivary secretion and plasma osmolality and the effects of intravenous of saline solutions. *Q J Exp Physiol Cogn Med Sci* 63: 1-21, 1978.
- [22] Carter RR, Allen OB, and Grovum WL. The effect of feeding frequency and meal size on amounts of total and parotid saliva secreted by sheep. *Br J Nutr* 63: 305-318, 1990.
- [23] Cassida KA and Stokes MR. Eating and resting salivation in early lactation dairy cows. *J Dairy Sci* 69: 1282-1292, 1986.
- [24] Denton DA. The effect of variation in the concentration of individual extracellular electrolytes on the response of the sheep's parotid gland to Na⁺ depletion. *J Physiol* 140: 129-147, 1958.
- [25] Dewhurst JK, Harrison FA, and Keynes RD. Renal excretion of potassium in the sheep. *J Physiol* 195: 609-621, 1968.
- [26] Diakov A and Korbmacher C. A novel pathway of epithelial sodium channel activation involves a serum- and glucocorticoid-inducible kinase consensus motif in the C terminus of the channel's alpha-subunit. *J Biol Chem* 279: 38134-38142, 2004.
- [27] Dobson A. Active transport through the epithelium of the reticulo-rumen sac. *J Physiol* 146: 235-251, 1959.
- [28] Dobson A. The forces moving sodium ions through rumen epithelium. *J Physiol* 128: 39-40P, 1955.
- [29] Dobson A and Phillipson AT. The forces moving chloride ions through rumen epithelium. *J Physiol* 125: 26-27P, 1954.
- [30] Dobson A, Scott D, and Bruce JB. Changes in sodium requirement of the sheep associated with changes of diet. *Q J Exp Physiol Cogn Med Sci* 51: 311-323, 1966.
- [31] Dobson A, Sellers AF, and Gatewood VH. Dependence of Cr-EDTA absorption from the rumen on luminal osmotic pressure. *Am J Physiol* 231: 1595-1600, 1976.
- [32] Donovan GA, Steenholdt C, McGehee K, and Lundquist R. Hypomagnesemia among cows in a confinement-housed dairy herd. *J Am Vet Med Assoc* 224: 96-99, 54, 2004.
- [33] Doyle DA. Structural themes in ion channels. *Eur Biophys J* 33: 175-179, 2004.
- [34] Dua K and Care AD. Impaired absorption of magnesium in the aetiology of grass tetany. *Br Vet J* 151: 413-426, 1995.
- [35] Dua K and Care AD. Lack of effect of quinidine on divalent mineral absorption from the reticulorumen of sheep. *Res Vet Sci* 56: 114-115, 1994.
- [36] Emanovic D, Harrison FA, Keynes RD, and Rankin JC. The effect of acetazolamide on ion transport across isolated sheep rumen epithelium. *Journal of Physiology* 254: 803-812, 1976.
- [37] Ferreira HG, Harrison FA, and Keynes RD. The potential and short-circuit current across isolated rumen epithelium of the sheep. *J Physiol* 187: 631-644, 1966.

-
- [38] Ferreira HG, Harrison FA, Keynes RD, and Nauss AH. Observations on the potential across the rumen of sheep. *J Physiol* 187: 615-630, 1966.
- [39] Ferreira HG, Harrison FA, Keynes RD, and Zurich L. Ion transport across an isolated preparation of sheep rumen epithelium. *J Physiol* 222: 77-93, 1972.
- [40] Gäbel G, Butter H, and Martens H. Regulatory role of cAMP in transport of Na⁺, Cl⁻ and short-chain fatty acids across sheep ruminal epithelium. *Exp Physiol* 84: 333-345, 1999.
- [41] Gäbel G and Martens H. The effect of ammonia on magnesium metabolism in sheep. *J Anim Physiol Anim Nutr* 55: 278-287, 1986.
- [42] Gäbel G, Martens H, Suendermann M, and Galfi P. The effect of diet, intraruminal pH and osmolarity on sodium, chloride and magnesium absorption from the temporarily isolated and washed reticulo-rumen of sheep. *Q J Exp Physiol* 72: 501-511, 1987.
- [43] Gäbel G and Sehested J. SCFA transport in the forestomach of ruminants. *Comp Biochem Physiol A Physiol* 118: 367-374, 1997.
- [44] Gäbel G, Suendermann M, and Martens H. The influence of osmotic pressure, lactic acid and pH on ion and fluid absorption from the washed and temporarily isolated reticulo-rumen of sheep. *J Vet Med A* 34: 220-226, 1987.
- [45] Gäbel G, Vogler S, and Martens H. Short-chain fatty acids and CO₂ as regulators of Na⁺ and Cl⁻ absorption in isolated sheep rumen mucosa. *J Comp Physiol [B]* 161: 419-426, 1991.
- [46] Giebisch G. Renal potassium transport: mechanisms and regulation. *Am J Physiol* 274: F817-833, 1998.
- [47] Goff JP. Macromineral disorders of the transition cow. *Vet Clin North Am Food Anim Pract* 20: 471-494, v, 2004.
- [48] Graham C and Simmons NL. Functional organization of the bovine rumen epithelium. *Am J Physiol Regul Integr Comp Physiol* 288: R173-181, 2005.
- [49] Greene LW, Webb KE, Jr., and Fontenot JP. Effect of potassium level on site of absorption of magnesium and other macroelements in sheep. *J Anim Sci* 56: 1214-1221, 1983.
- [50] Grings EE and Males JR. Effects of potassium on macromineral absorption in sheep fed wheat straw-based diets. *J Anim Sci* 64: 872-879, 1987.
- [51] Günzel D, McGuigan JA, and Schlue WR. Use of Mg²⁺ and Ca²⁺ macroelectrodes to measure binding in extracellular-like physiological solutions. *Front Biosci* 10: 905-918, 2005.
- [52] Harrison FA. Ion transport across rumen and omasum epithelium. *Philos Trans Royal Soc Lond B Biol Sci* 262: 301-305, 1971.
- [53] Harrison FA, Keynes RD, Rankin JC, and Zurich L. The effect of ouabain on ion transport across isolated sheep rumen epithelium. *J Physiol* 249: 669-677, 1975.
- [54] Head MJ and Rook JA. Hypomagnesaemia in dairy cattle and its possible relationship to ruminal ammonia production. *Nature* 176: 262-263, 1955.
- [55] Hille B. Ion Channels of Excitable Membranes. *Sinauer Associates, Inc*: Chapter 15, pg. 482, 2001.
- [56] Hille B and Schwarz W. Potassium channels as multi-ion single-file pores. *J Gen Physiol* 72: 409-442, 1978.
- [57] Hoover WH and Miller TK. Rumen digestive physiology and microbial ecology. *Vet Clin North Am Food Anim Pract* 7: 311-325, 1991.

-
- [58] Hungate RE, Bryant MP, and Mah RA. The rumen bacteria and protozoa. *Annu Rev Microbiol* 18: 131-166, 1964.
- [59] Hyden S. Observations on the absorption of inorganic ions from the reticulo-rumen of the sheep. *Kugl Lantbrukshögskolans Annaler* 27: 273-285, 1961.
- [60] Janis CM, Damuth J, and Theodor JM. Miocene ungulates and terrestrial primary productivity: Where have all the browsers gone? *Proc Natl Acad Sci U S A* 97: 7899-7904, 2000.
- [61] Jittakhot S, Schonewille JT, Wouterse HS, Yuangklang C, and Beynen AC. The relationships between potassium intakes, transmural potential difference of the rumen epithelium and magnesium absorption in wethers. *Br J Nutr* 91: 183-189, 2004.
- [62] Johnson JW and Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 325: 529-531, 1987.
- [63] Johnson JW and Ascher P. Voltage-dependent block by intracellular Mg^{2+} of N-methyl-D-aspartate-activated channels. *Biophys J* 57: 1085-1090, 1990.
- [64] Kamel KS, Harvey E, Douek K, Parmar MS, and Halperin ML. Studies on the pathogenesis of hypokalemia in Gitelman's syndrome: role of bicarbonaturia and hypomagnesemia. *Am J Nephrol* 18: 42-49, 1998.
- [65] Kamel KS, Oh MS, and Halperin ML. Bartter's, Gitelman's, and Gordon's syndromes. From physiology to molecular biology and back, yet still some unanswered questions. *Nephron* 92 Suppl 1: 18-27, 2002.
- [66] Katz B. Les constantes électriques de la membrane du muscle. *Arch Sci Physiol* 2: 285-299, 1949.
- [67] Kay RN. The rate of flow and composition of various salivary secretions in sheep and calves. *J Physiol* 150: 515-537, 1960.
- [68] Khalbuss WE, Alkiek R, Marousis CG, and Orlando RC. Potassium conductance in rabbit esophageal epithelium. *Am J Physiol* 265: G28-34, 1993.
- [69] Khorasani GR, Janzen RA, McGill WB, and Kennelly JJ. Site and extent of mineral absorption in lactating cows fed whole-crop cereal grain silage of alfalfa silage. *J Anim Sci* 75: 239-248, 1997.
- [70] Koeppen BM and Stanton BA. Renal Physiology. (Third edition, Mosby Inc, St. Louis, Missouri, ISBN 0-323-01242-01246). 2001.
- [71] Kunzelmann K and Mall M. Electrolyte transport in the mammalian colon: mechanisms and implications for disease. *Physiol Rev* 82: 245-289, 2002.
- [72] Lang I and Martens H. Na^+ transport in sheep rumen is modulated by voltage-dependent cation conductance in apical membrane. *Am J Physiol* 277: G609-618, 1999.
- [73] Leonhard-Marek S. Divalent cations reduce the electrogenic transport of monovalent cations across rumen epithelium. *J Comp Physiol [B]* 172: 635-641, 2002.
- [74] Leonhard-Marek S, Breves G, and Busche R. Effect of chloride on pH microclimate and electrogenic Na^+ absorption across the rumen epithelium of goat and sheep. *Am J Physiol Gastrointest Liver Physiol*, 2006.
- [75] Leonhard-Marek S, Gäbel G, and Martens H. Effects of short chain fatty acids and carbon dioxide on magnesium transport across sheep rumen epithelium. *Exp Physiol* 83: 155-164, 1998.
- [76] Leonhard-Marek S and Martens H. Effects of potassium on magnesium transport across rumen epithelium. *Am J Physiol* 271: G1034-1038, 1996.

-
- [77] Leonhard-Marek S, Stumpff F, Brinkmann I, Breves G, and Martens H. Basolateral Mg^{2+}/Na^{+} exchange regulates apical nonselective cation channel in sheep rumen epithelium via cytosolic Mg^{2+} . *Am J Physiol Gastrointest Liver Physiol* 288: G630-645, 2005.
- [78] Lodemann U and Martens H. Effects of diet and osmotic pressure on Na^{+} transport and tissue conductance of sheep isolated rumen epithelium. *Exp Physiol* 91: 539-550, 2006.
- [79] Luthi D, Günzel D, and McGuigan JA. Mg-ATP binding: its modification by spermine, the relevance to cytosolic Mg^{2+} buffering, changes in the intracellular ionized Mg^{2+} concentration and the estimation of Mg^{2+} by ^{31}P -NMR. *Exp Physiol* 84: 231-252, 1999.
- [80] Martens H and Blume I. Effect of intraruminal sodium and potassium concentrations and of the transmural potential difference on magnesium absorption from the temporarily isolated rumen of sheep. *Q J Exp Physiol* 71: 409-415, 1986.
- [81] Martens H and Blume I. Studies on the absorption of sodium and chloride from the rumen of sheep. *Comp Biochem Physiol A* 86: 653-656, 1987.
- [82] Martens H and Gäbel G. Transport of Na and Cl across the epithelium of ruminant forestomachs: rumen and omasum. A review. *Comp Biochem Physiol A* 90: 569-575, 1988.
- [83] Martens H, Gäbel G, and Strozyk B. Mechanism of electrically silent Na and Cl transport across the rumen epithelium of sheep. *Exp Physiol* 76, 1991.
- [84] Martens H, Gäbel G, and Strozyk H. The effect of potassium and the transmural potential difference on magnesium transport across an isolated preparation of sheep rumen epithelium. *Q J Exp Physiol* 72: 181-188, 1987.
- [85] Martens H and Hammer U. [Magnesium and sodium absorption from the isolated sheep rumen during intravenous aldosterone infusion (author's transl)]. *Dtsch Tierarztl Wochenschr* 88: 404-407, 1981.
- [86] Martens H and Harmeyer J. Magnesium transport by isolated rumen epithelium of sheep. *Res Vet Sci* 24: 161-168, 1978.
- [87] Martens H, Heggemann G, and Regier K. Studies on the effect of K, Na, NH_4^{+} , VFA and CO_2 on the net absorption of magnesium from the temporarily isolated rumen of heifers. *Zentralbl Veterinarmed A* 35: 73-80, 1988.
- [88] Martens H, Kubel OW, Gäbel G, and Honig H. Effects of low sodium intake on magnesium metabolism in sheep. *J agric Sci*: 237-243, 1987.
- [89] Martens H and Rayssiguier Y. Magnesium metabolism and hypomagnesaemia. In: Proceedings of the 5th International Symposium on Ruminant Physiology (ed. Ruckebush, Y and Thivend, P.). 447-466, 1980.
- [90] Martens H and Schweigel M. Pathophysiology of grass tetany and other hypomagnesemias. Implications for clinical management. *Vet Clin North Am Food Anim Pract* 16: 339-368, 2000.
- [91] Martens H and Stossel EM. Magnesium absorption from the temporarily isolated rumen of sheep: no effect of hyper- or hypomagnesaemia. *Q J Exp Physiol* 73: 217-223, 1988.
- [92] McCoy MA. Hypomagnesaemia and new data on vitreous humour magnesium concentration as a post-mortem marker in ruminants. *Magnes Res* 17: 137-145, 2004.

- [93] McDonald IW. The absorption of ammonia from the rumen of the sheep. *Biochem J* 42: 584-587, 1948.
- [94] McLean AF, Buchan W, and Scott D. Magnesium absorption in mature ewes infused intrarumenally with magnesium chloride. *Br J Nutr* 52: 523-527, 1984.
- [95] Monteilh-Zoller MK, Hermosura MC, Nadler MJ, Scharenberg AM, Penner R, and Fleig A. TRPM7 provides an ion channel mechanism for cellular entry of trace metal ions. *J Gen Physiol* 121: 49-60, 2003.
- [96] Morris JG and Gartner RW. The effect of potassium on the sodium requirements of growing steers with and without alpha-tocopherol supplementation. *Br J Nutr* 34: 1-14, 1975.
- [97] Muller F, Aschenbach JR, and Gäbel G. Role of Na^+/H^+ exchange and HCO_3^- transport in pH_i recovery from intracellular acid load in cultured epithelial cells of sheep rumen. *J Comp Physiol [B]* 170: 337-343, 2000.
- [98] Mutoh K and Wakuri H. Early organogenesis of the caprine stomach. *Nippon Juigaku Zasshi* 51: 474-484, 1989.
- [99] Nadler MJ, Hermosura MC, Inabe K, Perraud AL, Zhu Q, Stokes AJ, Kurosaki T, Kinet JP, Penner R, Scharenberg AM, and Fleig A. LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability. *Nature* 411: 590-595, 2001.
- [100] Nilius B, Vennekens R, Prenen J, Hoenderop JG, Bindels RJ, and Droogmans G. Whole-cell and single channel monovalent cation currents through the novel rabbit epithelial Ca^{2+} channel ECaC. *J Physiol* 527 Pt 2: 239-248, 2000.
- [101] Nilius B, Vennekens R, Prenen J, Hoenderop JG, Droogmans G, and Bindels RJ. The single pore residue Asp542 determines Ca^{2+} permeation and Mg^{2+} block of the epithelial Ca^{2+} channel. *J Biol Chem* 276: 1020-1025, 2001.
- [102] Nocek JE and Polan CE. Influence of ration form and nitrogen availability on ruminal fermentation patterns and plasma of growing bull calves. *J Dairy Sci* 67: 1038-1042, 1984.
- [103] Oba M, Baldwin RL, Owens SL, and Bequette BJ. Metabolic fates of ammonia-N in ruminal epithelial and duodenal mucosal cells isolated from growing sheep. *J Dairy Sci* 88: 3963-3970, 2005.
- [104] Odette O. Grass tetany in a herd of beef cows. *Can Vet J* 46: 732-734, 2005.
- [105] Owens FN, Secrist DS, Hill WJ, and Gill DR. Acidosis in cattle: a review. *J Anim Sci* 76: 275-286, 1998.
- [106] Owsianik G, Talavera K, Voets T, and Nilius B. Permeation and selectivity of TRP channels. *Annu Rev Physiol* 68: 685-717, 2006.
- [107] Pfeffer E, Thompson A, and Armstrong DG. Studies on intestinal digestion in the sheep. 3. Net movement of certain inorganic elements in the digestive tract on rations containing different proportions of hay and rolled barley. *Br J Nutr* 24: 197-204, 1970.
- [108] Phillipson AT. Absorption of acetate from the rumen of sheep. *J Physiol* 109: Proc, 31, 1949.
- [109] Powell DW, Morris SM, and Boyd DD. Water and electrolyte transport by rabbit esophagus. *Am J Physiol* 229: 438-443, 1975.
- [110] Qian A, Antonov SM, and Johnson JW. Modulation by permeant ions of $\text{Mg}(2+)$ inhibition of NMDA-activated whole-cell currents in rat cortical neurons. *J Physiol* 538: 65-77, 2002.

-
- [111] Rabinowitz L. Aldosterone and potassium homeostasis. *Kidney Int* 49: 1738-1742, 1996.
- [112] Rabinowitz L, Green DM, Sarason RL, and Yamauchi H. Homeostatic potassium excretion in fed and fasted sheep. *Am J Physiol* 254: R357-380, 1988.
- [113] Rahnema SH and Fontenot JP. Effects of intravenous infusion of high levels of potassium and sodium on mineral metabolism in sheep. *J Anim Sci* 68: 2833-2838, 1990.
- [114] Rossier BC. The epithelial sodium channel: activation by membrane-bound serine proteases. *Proc Am Thorac Soc* 1: 4-9, 2004.
- [115] Rossier BC, Pradervand S, Schild L, and Hummler E. Epithelial sodium channel and the control of sodium balance: interaction between genetic and environmental factors. *Annu Rev Physiol* 64: 877-897, 2002.
- [116] Rübhelke MK. In vitro Untersuchungen des Pansenepithels von Schafen zur Charakterisierung eines elektrogenen, calcium-sensitiven Natriumtransportes (Inaugural-Dissertation, Institut für Veterinär-Physiologie, Freie Universität Berlin). Berlin, 1998 (Journal-Nr: 2169), 1998.
- [117] Sakmann B and Trube G. Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *J Physiol* 347: 641-657, 1984.
- [118] Sargison ND, Macrae AI, and Scott PR. Hypomagnesaemic tetany in lactating Cheviot gimmers associated with pasture sodium deficiency. *Vet Rec* 155: 674-676, 2004.
- [119] Sawchenko PE and Friedman MI. Sensory functions of the liver--a review. *Am J Physiol* 236: R5-20, 1979.
- [120] Schröder B, Vossing S, and Breves G. In vitro studies on active calcium absorption from ovine rumen. *J Comp Physiol [B]* 169: 487-494, 1999.
- [121] Schultheiss G and Martens H. Ca-sensitive Na transport in sheep omasum. *Am J Physiol* 276: G1331-1344, 1999.
- [122] Schweigel M, Freyer M, Leclercq S, Etschmann B, Lodemann U, Bottcher A, and Martens H. Luminal hyperosmolarity decreases Na transport and impairs barrier function of sheep rumen epithelium. *J Comp Physiol [B]* 175: 575-591, 2005.
- [123] Schweigel M, Lang I, and Martens H. Mg(2+) transport in sheep rumen epithelium: evidence for an electrodiffusive uptake mechanism. *Am J Physiol* 277: G976-982, 1999.
- [124] Schweigel M and Martens H. Anion-dependent Mg²⁺ influx and a role for a vacuolar H⁺-ATPase in sheep ruminal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 285: G45-53, 2003.
- [125] Schweigel M and Martens H. [Electrophysiologic changes in rumen epithelium in their effect on magnesium transport--a review]. *Berl Munch Tierarztl Wochenschr* 113: 97-102, 2000.
- [126] Schweigel M, Park HS, Etschmann B, and Martens H. Characterization of the Na⁺-dependent Mg²⁺ transport in sheep ruminal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 290: G56-65, 2006.
- [127] Schweigel M, Vormann J, and Martens H. Mechanisms of Mg(2+) transport in cultured ruminal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 278: G400-408, 2000.

- [128] Scott D. Absorption of chloride from the rumen of the sheep. *Res Vet Sci* 11: 291-293, 1970.
- [129] Scott D. Changes in mineral, water and acid base balance associated with feeding and diet. Digestion and metabolism in the ruminant: Proceedings of the IV International Symposium on Ruminant Physiology (1974). ed: I.W. McDonald and A.C.I. Warner. The University of New England Publishing Unit (1975), 1975.
- [130] Scott D. The effects of potassium supplements upon the absorption of potassium and sodium from the sheep rumen. *Q J Exp Physiol Cogn Med Sci* 52: 382-391, 1967.
- [131] Scott D. The effects of sodium depletion and potassium supplements upon electrical potentials in the rumen of the sheep. *Q J Exp Physiol Cogn Med Sci* 51: 60-69, 1966.
- [132] Sehested J, Diernaes L, Moller PD, and Skadhauge E. Interaction between absorption of sodium and acetate across the rumen epithelium of cattle. *Acta Vet Scand Suppl* 89: 107-108, 1993.
- [133] Sehested J, Diernaes L, Moller PD, and Skadhauge E. Ruminal transport and metabolism of short-chain fatty acids (SCFA) in vitro: effect of SCFA chain length and pH. *Comp Biochem Physiol A Mol Integr Physiol* 123: 359-368, 1999.
- [134] Sehested J, Diernaes L, Moller PD, and Skadhauge E. Transport of butyrate across the isolated bovine rumen epithelium--interaction with sodium, chloride and bicarbonate. *Comp Biochem Physiol A Mol Integr Physiol* 123: 399-408, 1999.
- [135] Sehested J, Diernaes L, Moller PD, and Skadhauge E. Transport of sodium across the isolated bovine rumen epithelium: interaction with short-chain fatty acids, chloride and bicarbonate. *Exp Physiol* 81: 79-94, 1996.
- [136] Sellers AF and Dobson A. Studies on reticulo-rumen sodium and potassium concentration and electrical potentials in sheep. *Res Vet Sci* 1: 95, 1960.
- [137] Sellers AF, Gitis TL, and Roepke MH. Studies of electrolytes in body fluids of dairy cattle. III. Effects of potassium on electrolyte levels in body fluids in midlactation. *Am J Vet Res* 12: 296-301, 1951.
- [138] Silanikove N. The struggle to maintain hydration and osmoregulation in animals experiencing severe dehydration and rapid rehydration: the story of ruminants. *Exp Physiol* 79: 281-300, 1994.
- [139] Silanikove N and Tadmor A. Rumen volume, saliva flow rate, and systemic fluid homeostasis in dehydrated cattle. *Am J Physiol* 256: R809-815, 1989.
- [140] Sjollem B. On the nature and therapy of grass staggers. *Vet Rec* 10: 425, 1930.
- [141] Sjollem B. Untersuchungen über die Ursache der Grastetanie und der großen Frequenzzunahme dieser Krankheit. *Deutsche Tierärztliche Wochenschrift* 15, 1932.
- [142] Skou JC. The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim Biophys Acta* 23: 394-401, 1957.
- [143] Sperber I and Hyden S. Transport of chloride through the ruminal mucosa. *Nature* 169: 587, 1952.
- [144] Stacy BD and Warner AC. Balances of water and sodium in the rumen during feeding: osmotic stimulation of sodium absorption in the sheep. *Q J Exp Physiol Cogn Med Sci* 51: 79-93, 1966.
- [145] Stevens CE. Transport of sodium and chloride by the isolated rumen epithelium. *Am J Physiol* 206: 1099-1105, 1964.

-
- [146] Stumpff F, Brinkmann I, and Martens H. Regulation of a non-selective cation channel in cells of the ovine rumen epithelium by PGE2 and Forskolin. *Pflügers Arch* 449 (Suppl.): S116, 2005.
- [147] Stumpff F, Brinkmann I, Schweigel M, and Martens H. High potassium diet, sodium and magnesium in ruminants: the story is not over. *Production diseases in farm animals*: 284-285, 2004.
- [148] Stumpff F, Martens H, and Schweigel M. A patch-clamp and furaptra study of electrodiffusive Mg uptake into cells of the ruminal epithelium. *Acta Physiol* 186 (Suppl): 232 (PW203A-217), 2006.
- [149] Suttle NF and Field AC. Studies on magnesium in ruminant nutrition. 8. Effect of increased intakes of potassium and water on the metabolism of magnesium, phosphorus, sodium, potassium and calcium in sheep. *Br J Nutr* 21: 819-831, 1967.
- [150] Tomas FM and Potter BJ. The site of magnesium absorption from the ruminant stomach. *Br J Nutr* 36: 37-45, 1976.
- [151] Tsuchiya Y, Nakashima S, Banno Y, Suzuki Y, and Morita H. Effect of high-NaCl or high-KCl diet on hepatic Na⁺- and K⁺-receptor sensitivity and NKCC1 expression in rats. *Am J Physiol Regul Integr Comp Physiol* 286: R591-596, 2004.
- [152] Ussing HH and Zerahn K. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol Scand* 23: 110-127, 1951.
- [153] van de Graaf SF, Hoenderop JG, and Bindels RJ. Regulation of TRPV5 and TRPV6 by associated proteins. *Am J Physiol Renal Physiol* 290: F1295-1302, 2006.
- [154] Voets T, Janssens A, Prenen J, Droogmans G, and Nilius B. Mg²⁺-dependent gating and strong inward rectification of the cation channel TRPV6. *J Gen Physiol* 121: 245-260, 2003.
- [155] Wadhwa DR and Care AD. The absorption of calcium ions from the ovine reticulo-rumen. *J Comp Physiol [B]* 170: 581-588, 2000.
- [156] Wallace RJ. Ruminal microbial metabolism of peptides and amino acids. *J Nutr* 126: 1326S-1334S, 1996.
- [157] Warner AC and Stacy BD. Influence of ruminal and plasma osmotic pressure on salivary secretion in sheep. *Q J Exp Physiol Cogn Med Sci* 62: 133-142, 1977.
- [158] Warner AC and Stacy BD. Intraruminal and systemic responses to variations in intake of sodium and potassium by sheep. *Q J Exp Physiol Cogn Med Sci* 57: 89-102, 1972.
- [159] Warner AC and Stacy BD. Solutes in the Rumen of the Sheep. *Q J Exp Physiol Cogn Med Sci* 50: 169-184, 1965.
- [160] Warner AC and Stacy BD. Water, sodium and potassium movements across the rumen wall of sheep. *Q J Exp Physiol Cogn Med Sci* 57: 103-119, 1972.
- [161] Warnock DG. Renal genetic disorders related to K⁺ and Mg²⁺. *Annu Rev Physiol* 64: 845-876, 2002.
- [162] Wilkens M, Kunert-Keil C, and Schröder B. Expression of the epithelial Ca²⁺ channels TRPV5 and TRPV6 in ovine Ca²⁺ transporting tissues. *Acta Physiol* 186(Suppl): 232 (PW203A-215), 2006.
- [163] Wolfram S, Frischknecht R, and Scharrer E. Influence of theophylline on the electrical potential difference and ion fluxes (Na, Cl, K) across the isolated rumen epithelium of sheep. *Zentralbl Veterinarmed A* 36: 755-762, 1989.

- [164] Wylie MJ, Fontenot JP, and Greene LW. Absorption of magnesium and other macrominerals in sheep infused with potassium in different parts of the digestive tract. *J Anim Sci* 61: 1219-1229, 1985.
- [165] Yellen G. The voltage-gated potassium channels and their relatives. *Nature* 419: 35-42, 2002.