ISSN 1466-2523

Animal Health Research Reviews 7(1/2); 43–59
DOI: 10.1017/S1466252307001156

Ammonia and urea transport across the rumen epithelium: a review

Khalid Abdoun[1*†], Friederike Stumpff[2†] and Holger Martens[2]

[1]*Faculty of Veterinary Medicine, University of Khartoum, P.O. Box 32, 13314 Khartoum North, Sudan and* [2]*Department of Veterinary Physiology, Freie Universität Berlin, Oertzenweg 19b, 14163 Berlin, Germany*

Received 7 June 2006; Accepted 21 August 2006

Abstract

The transport of nitrogen across the rumen epithelium is characterized by absorption of ammonia from the rumen and by an influx of urea into the rumen. The transport rates of both compounds are large and exhibit wide variation. The transport of ammonia occurs in two forms: in the lipophilic form as NH_3, the magnitude of which is linearly related to the pH in the ruminal fluid at pH values above 7, while at a physiological pH of 6.5 or lower, ammonia is predominantly absorbed as NH_4^+ via putative potassium channels in the apical membrane. The uptake of NH_4^+ depends on the potential difference of the apical membrane, Pd_a, and shows competition with K uptake. The pathway for basolateral exit of NH_4^+ is unknown. Hence, the relative transport rates of NH_3 or NH_4^+ are determined by the ruminal pH according to the Henderson–Hasselbalch equation. Transport of ammonia interacts with the transport of Na and Mg mainly via changes of the intracellular pH. Urea recycling into the rumen has been known for many years and the transport across the rumen epithelium is mediated via urea transporters in the luminal and basolateral membrane of the epithelium. Transport of urea occurs by simple diffusion, but is highly variable. A significant increase of urea influx is caused by the fermentation products CO_2 and short-chain fatty acids. Conversely, there is some evidence of inhibition of urea influx by ruminal ammonia. The underlying mechanisms of this modulation of urea transport are unknown, but of considerable nutritional importance, and future research should be directed to this aspect of ruminal transport.

Keywords: rumen, ammonia, urea, transport, ruminants

Introduction

The maintenance of an optimum nitrogen balance in ruminants for growth, pregnancy and lactation is of key economic importance, requiring a wide range of adaptive responses to supply the necessary nitrogenous metabolites for different physiological states. The splanchnic bed, comprising the gastrointestinal tract and liver, plays a pivotal role in moderating the pattern of nitrogenous nutrients available for peripheral tissues. The gastrointestinal tissues form an interface between the diet and the animal and have a direct influence on the flux of nitrogenous nutrients from the gut lumen into the bloodstream. The liver then forms the central metabolic junction, further moderating and distributing nitrogenous nutrients to peripheral tissues for maintenance or productive functions such as muscle deposition or milk synthesis.

The transport of nitrogen across the rumen epithelium is characterized by absorption of ammonia[‡] (McDonald,

*Corresponding author. E-mail: abdounn@yahoo.com
†K. Abdoun and F. Stumpff contributed equally to this review.

‡The term ammonia is used without discrimination between NH_3 and NH_4^+. Chemical symbols are used, when a specification is required.

1948) from the rumen and by an influx of urea into the rumen (see review of Harmeyer and Martens, 1980; Marini *et al.*, 2006).

Total ammonia absorption from the gastrointestinal tract represents approximately half of the absorbed N and ammonia absorption from the forestomachs amounts to roughly 50% of total flow of ammonia to the liver (Reynolds and Huntington, 1988; Huntington, 1989; Huntington *et al.*, 1996; Theurer *et al.*, 2002), although for unknown reasons, considerable variation is known to occur. Absorbed ammonia is detoxified into urea in the liver.

Total urea-N synthesis exceeds flow of ammonia-N to the liver and is explained by transfer of nitrogen from amino acids for urea production (Reynolds, 1995). The daily urea-N turnover accounts for more than 50% of the total N intake from the gastrointestinal tract (Harmeyer and Martens, 1980), most of which is recycled to ruminal tissue. In a recent study of Theurer *et al.* (2002), 77% of urea production was transferred through the rumen epithelium. This magnitude of urea recycling has also been reported in other studies (Reynolds and Huntington, 1988; Huntington *et al.*, 1996).

Urea produced in the liver passes into the digestive tract and is broken down by resident bacteria into ammonia and CO_2 (Lapierre and Lobley, 2001). The bacteria use ammonia to synthesize amino acids and peptides required for synthesis of microbial protein, which is digested and absorbed in the small intestine, thus completing the 'salvaging' of urea nitrogen (Fuller and Reeds, 1998). The salvage mechanism via urea recycling is central to the ability of the ruminant to subsist on a low protein diet since N is re-directed to productive uses via microbial protein synthesis, instead of being excreted in urine. The recycling of urea and the reuse of nitrogen for microbial protein synthesis, which covers up to 80% of protein requirement of the ruminant, is unique and one important reason for the widespread occurrence of ruminants in many habitats.

Many studies have been performed to quantify the large amounts of nitrogen passing the rumen epithelium per day as ammonia (absorption) or urea (influx) under a variety of feeding conditions (see reviews of Reynolds, 1995, 2006; Lappiere *et al.*, 2005). In contrast, surprisingly little attention has been paid to the mechanisms of ammonia and urea transport across the rumen epithelium. It is therefore the intention of this paper to review primarily data of ammonia and urea transport across the rumen epithelium. The factors that affect ammonia and urea transport will be discussed and the effects of ammonia on transport of Mg and Na will also be incorporated. Within this framework, briefly, metabolism of ammonia in the rumen, its flow to and detoxification in the liver are included and some aspects of urea recycling will be discussed.

Ammonia production in the rumen

Ammonia is generated in the rumen (and in the gut) of all animal species as a result of two main processes:

(i) microbial degradation of nitrogenous compounds such as protein, peptides, amino acids and nucleic acids; and
(ii) microbial hydrolysis of urea passing across epithelia of the gastrointestinal tract from the blood and interstitial fluids and urea flowing into the rumen via saliva.

Protein degradability in the rumen is dependent on many factors including solubility, susceptibility to microbial proteases and residence time in the rumen (Taminga, 1983). These factors combine to produce a pattern of release of peptides, amino acids and NH_3, all of which provide a source of N for microbial protein synthesis.

The relationship between N intake and ammonia irreversible loss rates within the rumen have been studied over a wide range of N source and level in the diet of sheep (Mathison and Milligan, 1971; Nolan, 1975; Nolan *et al.*, 1976; Siddons *et al.*, 1985; Kennedy *et al.*, 1986). They indicated that 50% of dietary N entering the rumen had already previously passed directly through the rumen NH_3 pool and that $3.8\,g\,day^{-1}$ ammonia-N was produced from endogenous sources (recycling) within the rumen of sheep. Most of these studies were carried out on sheep as a model for ruminants. Extrapolating these data to lactating cows, large amounts of ammonia are released and account for $38\,g\,day^{-1}$ ammonia-N at maintenance and to some $300\,g\,day^{-1}$ at a milk production of $40\,l\,day^{-1}$ assuming that 50% of dietary N passes through the ruminal ammonia pool (N intake – crude protein – according to the recommendation of the Gesellschaft für Ernährungsphysiologie, Germany).

Numerous experiments have demonstrated the impact of either concentrate- or forage-based diets on rumen ammonia levels within various time periods after feeding (Wernli and Wilkins, 1980). Effects are particularly pronounced in the case of silage feeding, where the soluble nitrogenous components are rapidly degraded in the rumen to result in peaks of ammonia concentration of 18–20 mM within 1 h after feeding from basal levels of 2–4 mM. This level can be attenuated by chemical treatment of the forage material before ensilage by using acid formaldehyde to reduce N solubility (Thompson *et al.*, 1981) or by provision of a readily fermentable carbohydrate to provide energy for N capture by the rumen microflora (Rooke *et al.*, 1987). Rumen infusion studies in which either pulsed or continuous infusions of N and energy-yielding substrates have been used (Henning *et al.*, 1993) demonstrate that providing a constant supply of energy may be a critical factor in improving nutrient utilization. In the absence of such

provision, rapid fluctuations in ammonia concentration occur. Periods during which rumen ammonia levels rise above or fall below the range thought to be optimal for microbial growth and microbial protein production of 3.5 mM (Satter and Slyter, 1974) to 6 mM (Kang-Meznarich and Broderick, 1981) result in inefficient use of N for microbial protein synthesis and loss of ammonia from the rumen by absorption across the gut wall, thereby reducing both energy and protein supply to the host animal.

Synchronization of N and energy release within the rumen in order to maximise nutrient capture by microbial populations has been an objective of ruminant feeding systems (see review of Blank *et al.*, 1998). Experiments in sheep (Sinclair *et al.*, 1993) in which diets were formulated on the basis of either asynchronous or synchronous release of nutrients have shown that manipulation of the pattern of substrate availability in this way can provide a practical method of improving the efficiency of N capture and reducing the magnitude of rumen ammonia cycling.

Ammonia is an essential source of nitrogen for microbial growth. Although early work (Bryant and Robinson, 1962) indicated that about 90% of bacterial species within the rumen could utilise ammonia as the main source of N for growth, further studies have demonstrated a potential for free amino acids and peptides to become incorporated into microbial protein without passing through the rumen ammonia pool (Cotta and Hespell, 1986; MacKie and White, 1990). More recent studies have shown that for a range of feed protein sources, a maximum of 40–70% of microbial N was derived from the rumen NH_3 pool (Hristov and Broderick, 1994).

Ammonia absorption from the rumen

Ammonia disappears from the rumen by incorporation into microbial proteins, absorption across the rumen epithelium (35–65% of the ammonia loss from the rumen), or by the outflow of ruminal fluid into the omasum (10% of the loss) (Nolan and Strachin, 1979; Siddons *et al.*, 1985; Obara *et al.*, 1991). Loss of ammonia by absorption through the rumen wall can be quantitatively very high and account for some 50% ammonia flow to the liver (see above). This absorption was first clearly demonstrated by McDonald in 1948. Since then, numerous studies have been carried out to try to determine the form (NH_3 or NH_4^+) and mechanisms governing this absorption.

The luminal uptake of ammonia across the apical membrane into the cytosol of rumen epithelial cells ($J_{Lu\rightarrow Cyt}$) is the sum of parallel movement of the non-ionized and ionized form:

$$J_{Lu\rightarrow Cyt} = {}_{Lu\rightarrow Cyt}NH_3^{+}\ {}_{Lu\rightarrow Cyt}NH_4^{+}$$

The subscripts Lu and Cyt denote lumen and cytosol, respectively. Assuming a simple diffusive movement (Lu→Cyt), the flux rates of NH_3 and NH_4^+ are determined by a product of permeability and a driving force:

$$J_{Lu\rightarrow Cyt} = P_{NH3}\cdot[NH_3] + P_{NH4}{}^{+}\cdot[NH_4^{+}]\cdot\xi$$

where P_{NH3} and $P_{NH4}{}^+$ are the permeabilities of the apical membrane to NH_3 and NH_4^+, respectively, and $[NH_4^+]$ and $[NH_3]$ refer to the concentration of the two forms of ammonia in the lumen. The driving force is given by $\xi = z\cdot F\cdot Pd_a/\{R\cdot T\cdot[\exp^{(z\cdot F\cdot Pda/R\cdot T)} - 1]\}$, in which Pd_a is the potential difference across the luminal membrane of the epithelial cell and z, F, R and T have the usual electrochemical meanings.

The relative concentrations and subsequent flux rates of NH_3 and NH_4^+ can be altered by the luminal pH according to the Henderson–Hasselbalch equation. The wide variations in the ruminal ammonia concentrations (up to 70 mM) and of pH (5.4–7.4) cause corresponding alterations of NH_3 and NH_4^+ concentrations and flux rates across the rumen epithelium (Fig. 1).

Flux of NH_3

Ammonia is a weak base with a pKa of 9 (Leng and Nolan, 1984). The Henderson–Hasselbalch equation shows that at pH values between 6 and 7, practically all the ammonia will be in its ionized form (99.9 and 98.7%, respectively) (Fig. 1A), that is, in the form that will diffuse poorly across the lipid layers of the cell membranes. It has been demonstrated that even at these neutral pH values, absorption of ammonia takes place and increases with the intraruminal ammonia concentration (Hogan, 1961; Bödeker *et al.*, 1990; Remond *et al.*, 1993a) and pH (Gärtner and v. Engelhardt, 1964). When the intraruminal pH is lowered, the permeability of the rumen wall for ammonia is depressed (Hogan, 1961; Chalmers *et al.*, 1971; Bödeker *et al.*, 1990), so that ammonia absorption remains stable despite the increase in intraruminal ammonia concentration (Hogan, 1961). These findings are generally taken as evidence that ammonia absorption across the epithelium of the rumen occurs by simple diffusion of the non-ionized lipid soluble form (NH_3).

Flux of NH_4^+

The possibility of ammonium ion (NH_4^+) absorption from the digestive contents has also been considered (Hogan, 1961; Siddons *et al.*, 1985), but as this form is weakly lipid-soluble, its movement across the membranes of the epithelial cells would require the assistance of carriers or channels. Bödeker and Kemkowski (1996) observed that the addition of NH_4^+ to the incubation

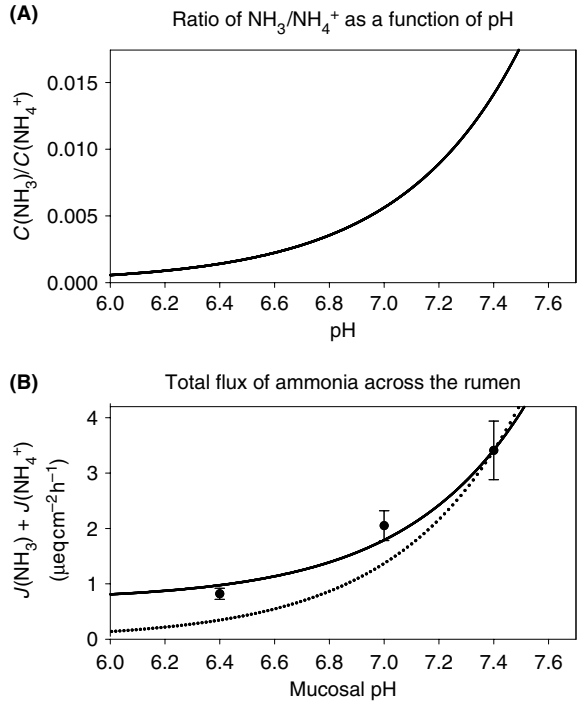

Fig. 1. (A) Correlation between pH and the ratio between ammonia and ammonium ($[NH_3]/[NH_4^+]$) as given by the Henderson–Hasselbalch equation and (B) correlation between mucosal pH and total flux of ammonia across the ruminal epithelium. (A) The concentration of the non-ionized form of ammonia [NH_3] increases 10-fold when the mucosal pH is increased from 6.4 to 7.4, while the concentration of [NH_4^+] remains almost constant. (B). The dots with error bars (⧳) designate the measured flux of both charged and uncharged ammonia ($NH_3 + NH_4^+$) through the ruminal epithelium at different values of mucosal pH. Ammonia fluxes fall off with pH and falling concentrations of NH_3, but not as rapidly as predicted by Henderson–Hasselbalch theory (dotted curve: the flux of ammonia is assumed to decrease linearly with the concentration of NH_3 as given by the Henderson–Hasselbalch equation from the measured value at pH 7.4). A better correlation of the data with theoretical predictions can be achieved if an additional flux of ammonium in its charged form (NH_4^+) at a constant rate of $0.7\,\mu eq\,cm^{-2}\,h^{-1}$ is assumed to contribute to total ammonium transport (Abdoun *et al.*, 2005) (solid curve; again, the flux data at pH 7.4 are used as a starting point). This assumption is supported by interactions of ammonium with uptake of Na via NHE. For details, see text.

solution on the mucosal side of sheep rumen epithelium *in vi*tro evoked positive short-circuit currents (I_{sc}), which were blocked by quinidine. Moreover, they reported a reduction of the transepithelial ammonia fluxes in the presence of quinidine in the incubation solution or alternately when the transepithelial potential difference was clamped to +25 mV. They suggested that NH_4^+ takes part in ammonia transport across the rumen epithelium, most probably via a K^+ channel.

Presence of K^+ channels in rumen epithelium

The ruminal epithelium was one of the first epithelia in which potassium secretion was observed *in vivo* (Sperber and Hyden, 1952; Hyden, 1961; Ferreira *et al.*, 1966a) and *in vitro* (Ferreira *et al.*, 1966b, 1972; Wolffram *et al.*, 1989; Leonhard-Marek and Martens, 1996) via an ouabain-sensitive mechanism (Harrison *et al.*, 1975). At higher concentrations of ruminal potassium, absorption of potassium can be observed that is correlated to the ruminal potassium concentration, but remains slow and much lower than absorption rates for sodium, suggesting a passive efflux pathway (Hyden, 1961; Warner and Stacy, 1972a, b).

With rising concentrations of ruminal potassium, the potential across the ruminal epithelium rises *in vivo* (Sellers and Dobson, 1960; Scott, 1966; Ferreira *et al.*, 1966a; Care *et al.*, 1984; Martens and Blume, 1986) and *in vitro* (Ferreira *et al.*, 1966b; Harrison *et al.*, 1975; Leonhard-Marek and Martens, 1996), and may limit the driving force for passive efflux of potassium from the rumen (Dobson, 1959). The notion of an electrogenic, transcellular pathway for ruminal potassium transport is supported by the observation of apical potassium secretion at low concentrations of potassium as mentioned above, and by the depolarization of the apical membrane of the ruminal epithelium in high potassium solutions with a concomitant decrease in the fractional apical resistance (Leonhard-Marek and Martens, 1996).

Elevation of ruminal potassium induces a stimulation of sodium transport out of the rumen (Hyden, 1961; Stacy and Warner, 1966; Scott, 1967; Warner and Stacy, 1972a, b; Martens and Hammer, 1981). However, attempts to block a putative Na-K-2Cl cotransport with furosemide or bumetanide in the rumen were unsuccessful (Martens *et al.*, 1988, 1991). Indeed, a fixed coupling of Na with K transport cannot explain selective stimulation of sodium absorption that is observed after abrupt transfer of animals to a high potassium diet (Sellers and Dobson, 1960), and which results in concentration of potassium in the rumen to levels that can reach 100 mM. This effect, which plays a major role in ruminant potassium homeostasis, may involve voltage-dependent stimulation of a Na-permeable cation channel (Lang and Martens, 1999; Leonhard-Marek, 2005; for a review, see Stumpff and Martens, 2006).

The model most suitable for the integration of the various data is the assumption of apical potassium channels in the ruminal epithelium and corresponding quinidine-sensitive NH_4^+ and K^+ conducting ionic channels have been demonstrated in isolated ruminal epithelial cells (Abdoun *et al.*, 2005). The permeability of the pore of potassium channels to NH_4^+ has been demonstrated numerous times (Yellen, 1987; Choe *et al.*, 2000; Hille, 2001).

Ruminal transport of ammonia

The transport of ammonia across the isolated rumen of sheep has been studied in more detail by measuring the flux of ammonia in the mucosal–serosal direction at different concentrations and pH values in order to specify the transported form of ammonia (NH_3 or NH_4^+) (Abdoun *et al.*, 2005; Fig. 1A) One approach of the study was to measure total ammonia flux at a (total) mucosal concentration of 30 mM. The mucosal pH was set to different pH values of 6.4, 6.9 and 7.4, and the corresponding flux rates of ammonia were measured. However, at acidic levels of mucosal pH, ammonia fluxes fell off less rapidly than the concentration of NH_3 (Fig. 1B), suggesting an additional flux of NH_4^+.

To assess the size of the relative contributions, concentrations of NH_3 were calculated with the Henderson–Hasselbalch equation yielding 0.07 (pH 6.4), to 0.27 (pH 6.9) or 0.68 (pH 7.4), respectively, while the concentrations of NH_4^+ remained almost unchanged. A linear correlation was obtained (see Abdoun *et al.*, 2005) between the measured total ammonia flux in $\mu eq\,cm^{-2}\,h^{-1}$ (designated as '*y*') and the concentration of mucosal NH_3 (designated as '*x*') that took the form:

$$y = 4.1\ cm\ h^{-1} \cdot x + 0.7\ \mu eq\ cm^{-2}\ h^{-1} \quad (r = 0.99).$$

The linear correlation between ammonia flux and concentration of NH_3 supports the classical notion of transport via lipid diffusion with a permeability constant $P(NH_3) = J(NH_3)/[A \cdot C(NH_3)]$ of $4.1\,cm\,h^{-1} = 1.14 \times 10^{-3}\,cm\,s^{-1}$ (where $J(NH_3)$ is the flux, A the surface area of the tissue, and $C(NH_3)$ the concentration of ammonia in the form of NH_3). Of course, it is not possible to compare this value directly to values derived from experiments with artificial lipid bilayers, since the ruminal epithelium is a multi-layered epithelium of about 70 µm thickness (Dobson, 1959) and the area is enlarged by numerous papillae. However, experiments on artificial membranes yield values that are roughly in the same order of magnitude (7.8×10^{-2} to $2.4 \times 10^{-3}\,cm\,s^{-1}$) (Lande *et al.*, 1995) and therefore we feel that simple lipid diffusion can safely be regarded as a suitable model for the uptake route of NH_3 across the ruminal epithelium until solid evidence is obtained refuting this theory.

On the contrary, the lipid diffusion model fails to explain the transport of ammonia as the concentration of NH_3 approaches zero. The *y*-intercept of $0.7\,\mu eq\,cm^{-2}\,h^{-1}$ in the absence of NH_3 should represent the flux (density) of ammonia in its charged form ($J(NH_4^+)/A$). The permeability of the ruminal epithelium for NH_4^+ is thus given by the quotient of the flux density ($0.7\,\mu eq\,cm^{-2}\,h^{-1}$) and the concentration ($30\,mmol\,l^{-1}$), yielding a value of $P(NH_4^+) = 6.46 \times 10^{-6}\,cm\,s^{-1}$ (and thus, many orders of magnitude above the value of 10^{-11} to $10^{-13}\,cm\,s^{-1}$ reported for the cation permeability of lipid membranes (Paula *et al.*, 1996)). The conclusion that an electrogenic, facilitated transport (such as by a K channel) (Abdoun *et al.*, 2005) may mediate a part of ammonia uptake by the ruminal epithelium is supported by the observation of a rise in I_{sc} of similar magnitude after increasing mucosal ammonia from 0 to 30 mM.

The fact that the permeability of the ruminal membrane for NH_3 is about 175 times as high as for NH_4^+ may lead to the incorrect assumption that it is 'negligible'. This is misleading, however, since the amount of ammonia absorbed in the ionic form is considerable due to the low concentration of NH_3 in relationship to NH_4^+ at physiological levels of pH. At neutral pH, and a total concentration of $30\,mmol\,l^{-1}$ ammonia, only $0.27\,mmol\,l^{-1}$ is in the form of NH_3 so that even if the permeability for NH_4^+ is 175 times lower than that of NH_3, the number of ions available for permeation is higher by a factor of 110. This means that for every mmol crossing the membrane as NH_3, 110/175 = 0.63 mmol or about 40% (= 0.63/1.63) of the total amount of ammonia that is transported will be transported as NH_4^+. At a slightly acidic pH of 6.4 ($C(NH_3) = 0.07\,mmol\,l^{-1}$), the concentration of NH_4^+ ($29.93\,mmol\,l^{-1}$) exceeds that of NH_3 by a factor of 428, and transport of NH_4^+ will be greater than that of NH_3 by a factor of 428/175 = 2.44. Thus, at pH 6.4, over 70% (= 2.44/3.44) of total ammonia will be absorbed as NH_4^+. Conversely, at a pH of 7.4 ($C(NH_3) = 0.68\,mmol\,l^{-1}$), the concentration of NH_4^+ ($29.32\,mmol\,l^{-1}$) is only 43 times as high as that of NH_3, and transport of NH_3 will predominate by a factor of 175/43 = 4 and 80% of total ammonia transport will occur in the form of NH_3. As to be expected from these calculations, the ruminal pH determines relative amounts of NH_3 and NH_4^+ entering the cytsol and modifying Na transport via the apical sodium–hydrogen exchanger (NHE) (see below and Abdoun *et al.* (2005)).

Effect of electrical gradients on ammonia absorption

The above-mentioned reports clearly demonstrate transepithelial movement of NH_4^+ ions. Since this form of ammonia is charged, its flux rate across the epithelium should be determined not only by the chemical, but also by the electrical gradient. Similar observations have been made in other tight epithelia such as the bladder, in which Schwartz and Tripolone (1983) reported a significant increase in the serosal to mucosal and a decrease in the mucosal to serosal ammonia flux after application of +50 mV to the serosal side (mucosal side considered ground), while the opposite changes occurred when the voltage was clamped to −50 mV. In the ruminal epithelium of sheep, ammonia fluxes from the mucosal to the serosal side were significantly higher when the transepithelial potential difference, Pd_t, was clamped to

−25 mV (polarity on the serosal side) compared to those measured at a potential difference of +25 mV (Bödeker and Kemkowski, 1996). Recent studies from our laboratory support the assumption of NH_4^+ flux across the apical membrane through a K channel (Abdoun *et al.*, 2005) driven by the potential difference of the apical membrane (Pd_a). Lang and Martens (1999) have shown that the change of Pd_t by 25 mV caused alteration of Pd_a of some 15 mV. The applied Pd_t of +25 mV in the studies of Bödeker and Kemkowski (1996) depolarizes Pd_a by some 15 mV and consequently reduces NH_4^+ uptake due to the diminished electrical driving force. By contrast, a Pd_t of −25 mV enhances NH_4^+ fluxes corresponding to the increase of Pd_a. It is worthwhile mentioning that the flow of NH_4^+ through the luminal K channel exhibits competitive interactions with luminal K (Abdoun *et al.*, 2005). Hence, NH_4^+ absorption could be reduced at a low ruminal pH (<6.4) and high K concentration and probably vice versa.

Effect of short-chain fatty acids (SCFA) and CO_2/HCO_3^- on ammonia absorption

In the short term, absorption of ammonia depends mainly on the concentration of NH_3 near the epithelium. The SCFA and the CO_2/HCO_3^- in the rumen can modulate this dependency leading to an increase in ammonia absorption (Bödeker *et al.*, 1992a, b; Remond *et al.*, 1993b). Several suggestions have been made concerning the action of these two variables (Fig. 2). Bödeker *et al.* (1992b) suggested that the interaction between SCFA and ammonia may occur just underneath the apical membrane of the epithelial cells. Since the pH inside these cells is close to 7, the SCFA absorbed in their non-ionized form will dissociate and release protons, which can be used to form NH_4^+ from the absorbed NH_3. This process would result in a decrease in the intracellular NH_3 concentration and thereby favor its absorption. Likewise, the intracellular release of HCO_3^- and H^+ from CO_2 and H_2O by the action of carbonic anhydrase could serve as a proton source for NH_4^+ formation, since the inhibition of carbonic anhydrase reduces the ammonia flux across rumen mucosa *in vitro* (Bödeker *et al.*, 1992a). The same authors also speculated about the passage of both $SCFA^-$ and HCO_3^- ions across the basolateral cell membrane in conjunction with NH_4^+, allowing electroneutral exit of all three compounds against an adverse electrical potential difference (Fig. 2).

The importance of bicarbonate in stimulating NH_3 absorption has also been demonstrated in monogastric animals (Wrong, 1978; Cohen *et al.*, 1988). They suggested that colonic HCO_3^- secretion titrates luminal NH_4^+ to NH_3, permitting NH_3 to diffuse from the lumen, while HCO_3^- is titrated to carbon dioxide which also diffuses from the lumen. Because HCO_3^- is secreted into the rumen via anion exchange (Gäbel *et al.*, 2002), the

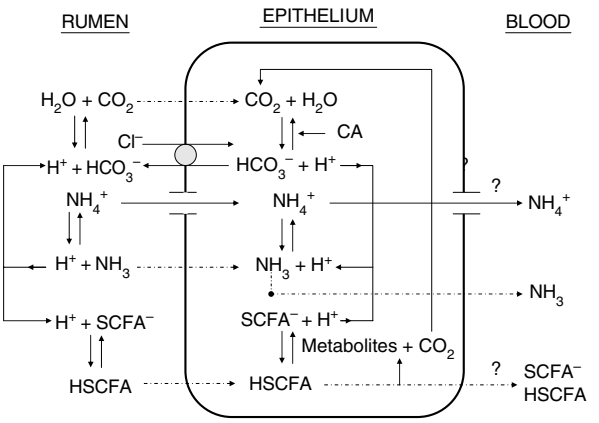

Fig. 2. Scheme of pathways of NH_3 and NH_4^+ across the rumen epithelium and modulation by CO_2/HCO_3^- and SCFA. The luminal uptake of NH_4^+ and NH_3 is mediated by a putative K channel and by diffusion, respectively. The relative transport rates of both forms depend on the ruminal pH and the concentration of protons just above the luminal membrane. Availability of protons can be reduced by reaction with secreted HCO_3^- ($H^+ + HCO_3^- \leftrightarrow H_2O + CO_2$) or by protonation of SCFA ($H^+ + SCFA^- \leftrightarrow HSCFA$). In both cases, the NH_3 concentration increases and hence NH_3 uptake. Intracellularly NH_3 will be protonated to NH_4^+ by protons of dissociation of HSCFA or H_2CO_3 (catalyzed by the carboanhydrase = CA). This intracellular protonation of NH_3 maintains the NH_3 gradient and uptake across the luminal membrane. The exit of both forms of ammonia across the basolateral membrane is not clear. Modified from Bödeker *et al.* (1992a, b).

demonstrated negative effect of an inhibition of carbonic anhydrase on ammonia transport by Bödeker *et al.* (1992a) can be explained by a combination of two factors: reduced intracellular titration of NH_3 to NH_4^+, as mentioned above, and reduced luminal titration of NH_4^+ to NH_3.

The effects of SCFA and CO_2 on ammonia absorption may also be explained by their action on the subepithelial blood flow (Dobson, 1979) and irrigated capillary surface area (Thorlacius, 1972). However, Remond *et al.* (1993b) observed that when butyric acid was supplied to the rumen contents or when CO_2 was blown in, the increase in ammonia flux across the rumen wall was always lower than the increase in ruminal blood flow produced by either of the two treatments.

Ammonia transporting proteins

The available data about ammonia transport across the rumen epithelium support the assumption of NH_4^+ transport via an apical K channel und diffusion of lipophilic NH_3 through the membranes of the epithelium. In contrast to the kidney, where transport of ammonia takes place against a concentration gradient and thus, has to be coupled to transport of other ions, ruminal ammonia

transport is passive and can utilize the pronounced gradient for ammonium across the tissue.

Despite this, a possible role for other membrane proteins known to facilitate transport of ammonia should be discussed. Thus, a role for the Na^+/H^+ exchanger (NHE3) in the transport of ammonia has been suggested (Nagami, 1988; Karim *et al.*, 2005; Cermak *et al.*, 2002). Although the rumen is known to express NHE3 (Etschmann *et al.*, 2006), stimulation of Na transport by NH_4^+ as found in the rumen does not fit this model (Abdoun *et al.*, 2005) and appears to play no role in this tissue. Transport of ammonia can also take place via K^+/H^+ (NH_4^+) exchange (Amlal and Soleimani, 1997; Karim *et al.*, 2005), but there is no evidence for an exchanger of this type in the rumen (Schweigel and Martens, 2003).

In the kidney, substitution of NH_4^+ for K^+ in Na-K-2Cl accounts for the major fraction of ammonia transport in the thick ascending limb of Henle's loop (Karim *et al.*, 2005). In the rumen, bumetanide and furosemide do not show effects on Na transport (Martens *et al.*, 1988; Martens *et al.*, 1991). It should also be noted that amiloride blocks stimulation of Na transport by NH_4^+ at acidic pH (Abdoun *et al.*, 2005), speaking against a role for Na-K-2Cl. The same study shows electrogenic effects of ammonia that cannot be explained by assumption of electroneutral uptake.

A lively debate surrounds the role of the ubiquitous ammonium transporter/methylpermease/Rhesus (Amt/MEP/Rh) protein family (for reviews, see Weiner (2004) and Winkler (2006)). Amt/MEP/Rh proteins exhibit a characteristic extracellular high-affinity ammonium ion-binding site adapted to the function of scavenging ammonia from a site with low concentration (μM) for release at a site of higher concentration, allowing survival of microorganisms in an ammonium-depleted environment (Marini *et al.*, 2000; Winkler, 2006). In experimental situations with application of higher concentrations of ammonia (>5 mM), pronounced endogenous conductances for NH_4^+ (Burckhardt and Frömter, 1992) typically complicate efforts to observed specific RhBG- or RhCG-mediated NH_4Cl-dependent transport (Mak *et al.*, 2006; Mayer *et al.*, 2006). However, it is possible to distinguish between the two since ammonia transport via mammalian Rh proteins is electroneutral and typically saturates before electrogenic transport via ionic channels begins to play a major role. Recent research indicates that Rh proteins facilitate dissociation of NH_4^+ at the channel mouth, so that uncharged NH_3 and not NH_4^+ is transported into the cytosol (Khademi *et al.*, 2004; Winkler, 2006). Both exchange of NH_4^+ for H^+ (Mak *et al.*, 2006) and conductance for NH_3 (Winkler, 2006) should inhibit, and not stimulate, Na transport via NHE as observed in the rumen at acidic pH values (Abdoun *et al.*, 2005).

For the reasons outlined above, Rh proteins do not appear to be suitable candidates to explain NH_4^+ efflux from the rumen. However, the demonstration of ammonia transporter proteins Rh B and Rh C in the intestine of the mouse (Handlogten *et al.*, 2005) raises the question whether these proteins may play a role in the regulation of NH_3 transport across the ruminal wall, and encourages corresponding studies in the rumen epithelium.

Ammonia metabolism in the rumen mucosa

The ability of the rumen mucosa to metabolise ammonia to glutamic acid by the use of α-ketoglutaric acid has been demonstrated by McLaren *et al.* (1961) and by Hoshino *et al.* (1966). The presence in rumen mucosa of glutamate-oxalacetate transaminase was reported by Chalupa *et al.* (1970a); however, the nature of this synthesis reaction was reported to be reductive desamination rather than transamination, and NADH-glutamate dehydrogenase represented the major system for glutamate synthesis from ammonia in rumen mucosa (Chalupa *et al.*, 1970b).

More recently (Bödeker *et al.*, 1992b) reported that 54% of the ammonia taken up by the sheep rumen mucosa from the mucosal solution was not accounted for in the serosal solution in experiments performed during summer, whereas in tissues from sheep slaughtered in winter mucosal disappearance and serosal appearance of ammonia were equal. Moreover, we have reported a higher mucosal disappearance and a lower serosal appearance rate of ammonia across the isolated rumen epithelium of concentrate-fed sheep compared to those fed solely on hay (Abdoun *et al.*, 2003). The gap between disappearance and appearance of ammonia can be explained by diet-dependent detoxification of ammonia in the epithelium, which indeed has been observed by Nocek *et al.* (1980). It has been suggested by these authors that such a mechanism would be a useful adaptation to variable intake of nitrogen and hence, different ammonia concentrations and absorption rates.

Effects of ammonia on ruminal transport mechanisms

In our studies with isolated rumen epithelium, we have shown that increasing the luminal (mucosal) ammonia concentration elevated the short-circuit current (I_{sc}) and the conductance (G_t) across the rumen epithelium in sheep (Abdoun *et al.*, 2006). Furthermore, we reported an inhibitory effect of luminal ammonia on the electroneutral Na transport via Na^+/H exchange (NHE) across the rumen epithelium of hay-fed sheep at pH 7.4, which is completely abolished at 30 mM. In contrast, at pH 6.4 we reported a stimulatory effect of luminal ammonia on Na transport via NHE (Abdoun *et al.*, 2005).

The pH-dependent inversion of the effect of mucosal ammonia on NHE can easily be explained by the

predominantly absorbed form of ammonia. At a pH 7.4, ammonia is mostly transported as NH_3, which diffuses into the cytosol, binds H^+ and reduces proton availability for NHE. Conversely, at a pH of 6.4, concentration and influx of NH_3 are extremely low, and uptake of NH_4^+ occurs through the apical K channel with subsequent dissociation and release of H^+ in the more alkalic cytosol, thus stimulating NHE activity and Na transport. It should be emphasized that the stimulatory effect of ammonia on Na transport might be of significance *in vivo*, because the physiological pH is some 6.5 or even lower in high producing cows.

Physiologically, this ammonia-dependent stimulated Na absorption should help to restore osmotic pressure in the ruminal fluid after a meal. Hypertonic ruminal fluid increases water influx into the rumen (Dobson *et al.*, 1976), decreases salivary flow (Warner and Stacy, 1977), food intake (Carter and Grovum, 1990) and SCFA absorption (Bennink *et al.*, 1978), and thus, ammonia-stimulated Na absorption may be of practical importance for all feeding conditions with rapid breakdown of protein. Interestingly, ammonia has been shown to *stimulate* Na flux across the rumen epithelium in concentrate-fed and urea-fed sheep even at a relatively high luminal pH of 7.4 (Abdoun *et al.*, 2003), a pH value at which ammonia *inhibits* Na fluxes across the rumen of hay-fed animals. Note that this difference reflects a shift in the relative permeabilities of the rumen for NH_3 and NH_4^+ and that this shift cannot be explained by an increase in the surface area of the tissue (e.g. by growth of papillae). However, at this point, it is not clear if the permeability of the membrane for NH_3 is decreased (for instance, by changes in thickness or lipid raft composition (Kikeri *et al.*, 1989; Singh *et al.*, 1995)) or if the relative permeability of the membrane for NH_4^+ is increased (up-regulation of the activity or expression of an ammonium transporting protein).

It has been known for some time that ammonia inhibits Mg absorption (Martens and Rayssiguier, 1980; Care *et al.*, 1984) and urea secretion (v. Engelhardt *et al.*, 1978; Remond *et al.*, 1993b) across the rumen epithelium. Both the depolarization of the apical membrane by influx of NH_4^+ and the change of pH (mediated primarily by influx of NH_3 at neutral pH in non-adapted animals) should contribute to this effect (Martens and Schweigel, 2000). The negative effect of a sudden increase of ruminal ammonia concentration on Mg absorption disappeared within 3–4 days indicating mechanism(s) of adaptation (Gäbel and Martens, 1986; see review of Martens and Schweigel, 2000). The very tentative assumption that the permeability of the membrane for NH_3 may be reduced by adaptational processes (Kikeri *et al.*, 1989; Singh *et al.*, 1995) is in line both with this observation, and with the previous observations concerning interaction of ammonia with Na uptake in adapted animals (Abdoun *et al.*, 2003).

Ammonia toxicity and ruminal pH

Ammonia toxicity occurred when circulating blood ammonia-N concentrations exceeded 0.57 mM (Webb *et al.*, 1972). Ammonia toxicity can arise from feeding urea or ammonium salts but there is no evidence of ammonia poisoning from protein feeding even when the concentration of ammonia in rumen liquor reaches values of over 142.8 mM (Briggs *et al.*, 1957).

Because rumen pH has a major impact on ammonia absorption, the incidence of ammonia toxicity is related to the pH of the ruminal fluid. Signs of toxicity were observed when the pH in the rumen was above 7.3 and the increase in peripheral blood ammonia was due to the inability of the liver to detoxify the increased quantity of ammonia arising from the increased rate of absorption at high pH (Coombe *et al.*, 1960; Hogan, 1961; Ortolani *et al.*, 2000). In addition, it was concluded that only non-ionized ammonia molecules were able to penetrate the ruminal wall proportional to their concentration, a correlation which was found to be correct for pH 6.9–7.5 but not from pH 6.75 to 6.9 (Gärtner and v. Engelhardt, 1964). As mentioned above, ammonia is mostly absorbed in the form of NH_4^+ at pH 6.4, while the uptake of NH_3 exceeded that of NH_4^+ by a factor of 4 at pH 7.4 (Abdoun *et al.*, 2005). However, due to the low permeability of the membrane for the charged ion, ammonia as NH_4^+ is trapped in the rumen at low pH and saturation effects within the ruminal proteins responsible for facilitated diffusion, such as within the multi-ion pore of channels selective for potassium, should limit the uptake of ammonia via this route (Hille and Schwarz, 1978; Hille, 2001; Abdoun *et al.*, 2005). Conversely, the route via lipid diffusion does not saturate. Accordingly, risk of ammonia toxicity may rise with ruminal ammonia concentration, but only if ruminal pH reaches values of over 7.3 (Coombe *et al.*, 1960; Hogan, 1961; Ortolani *et al.*, 2000).

It has been shown that when ammonium acetate was given, rumen pH remained fairly stable and rumen ammonia-N concentrations greater than 140 mM were tolerated without toxic manifestations. However, when urea was administered, the pH increased rapidly and rumen ammonia-N concentrations less than 70 mM resulted in appreciable ammonia absorption and as little as 7 mM was frequently highly toxic to sheep that had been kept on a diet of poor quality grass hay previous to dosing (Webb *et al.*, 1972). In this context, it is important to remember that at constant concentration of total ammonia, the concentration of NH_3 rises by a factor of 3 when pH rises from 6.5 to 7, by a factor of 10 when pH rises from 6.5 to 7.5, and by a factor of 20 if pH increases to 7.8. Thus, ruminal pH is a very critical factor in determining the toxicity of ammonia, leading to an increased concentration of ammonia in portal vein blood overloading the liver and causing a rise in the ammonia concentration of circulating peripheral

blood (Coombe *et al.*, 1960; Hogan, 1961; Ortolani *et al.*, 2000).

Ammonia metabolism in the liver

Measurements of net portal drained viscera (PDV) ammonia-N flux over a wide range of nitrogen intakes and different diets (Fig. 3A) suggest that ammonia-N absorption by PDV is not closely correlated with dietary nitrogen supply.

Net PDV ammonia nitrogen flux is, however, highly correlated with the digestible nitrogen intake (Reynolds *et al.*, 1991, 1992a). Quantities of ammonia absorbed by PDV seem to be determined not only by intake of digestible nitrogen but also by the nature of carbohydrate and protein consumed.

All ammonia absorbed by PDV is subsequently removed by the liver so that splanchnic flux is very low or negative (Chalmers *et al.*, 1971). Hepatic removal of ammonia and conversion to urea has been proposed to involve concomitant catabolism of amino acids, to provide via aspartate the other N atom in urea (Parker *et al.*, 1995). However, the relationship between hepatic removal of ammonia and amino acids is described as weak (Lapierre *et al.*, 2005). Furthermore, in sheep infused with ammonium bicarbonate, there was only a limited requirement for additional amino acid catabolism to support the extra urea synthesis (Milano *et al.*, 2000). In addition, the utilization of ammonia for urea synthesis in hepatocytes isolated from sheep liver was stimulated by propionate (Garwacki *et al.*, 1990).

Ammonia removed by the liver is converted into urea or glutamine. Half of the extra ammonia removed by the liver was, apparently, utilized by periportal glutamate dehydrogenase and aspartate aminotransferase for sequential glutamate and aspartate synthesis and converted to urea as the 2-amino moiety of aspartate (Milano *et al.*, 2000). Any ammonia which escapes conversion to urea in periportal hepatocytes is converted to glutamine in perivenous hepatocytes. The amide-N of glutamine is then removed and metabolized to urea by periportal hepatocytes during subsequent passages through the liver (Hussinger *et al.*, 1992).

Ammonia absorbed across the PDV is derived in part from urea transferred into the gut lumen, and a substantial cycling of urea and ammonia between the PDV and liver occurs in ruminants (Huntington, 1986). In growing cattle fed diets high in rumen soluble nitrogen, excessive ammonia absorption has been associated with increased net removal of amino acids by the liver (Huntington, 1989; Reynolds *et al.*, 1991). This response has been attributed to an increase in urea cycle requirements for cytosolic aspartate and glutamate, which cannot be met by mitochondrial capture of ammonia as glutamate (Reynolds, 1992).

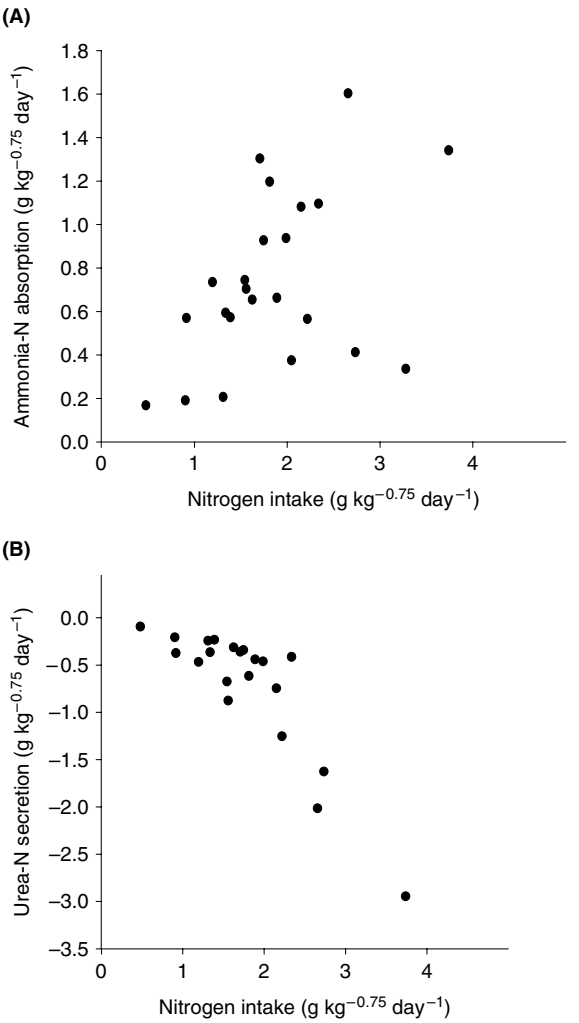

Fig. 3. The relationship between nitrogen intake ($g\,kg^{-0.75}\,day^{-1}$) and the transport rates of ammonia nitrogen (absorption) and urea nitrogen (secretion) across the digestive tract ($g\,kg^{-0.75}\,day^{-1}$). Each point represents animal means from individual experiments. There is evidently no close correlation between the nitrogen intake and ammonia absorption from the digestive tract. Calculation of a statistical correlation requires a careful meta-analysis of the data, which was beyond the scope of this review. However, it is quite evident that urea recycling is increased with nitrogen intake. Data are from the experiments of Huntington and Prior (1983), Harmon *et al.* (1993), Reynolds *et al.* (1992b), Guerino *et al.* (1991), Reynolds and Tyrrell (1991), Eisemann and Nienaber (1990), Reynolds and Huntington (1988), Sniffen and Jacobson (1974), Wolf *et al.* (1972), Wilton *et al.* (1988), Fitch *et al.* (1989), Huntington (1989), Maltby *et al.* (1991), Maltby *et al.* (1993a), Maltby *et al.* (1993b) and Reynolds *et al.* (1991).

Over a wide range of portal ammonia concentrations on a variety of different diets, the liver is able to extract almost 100% of the portal ammonia (Parker *et al.*, 1995). The upper limit of the capacity of ruminant liver to remove ammonia is 1.2–1.5 $\mu mol\,min^{-1}\,g^{-1}$

(Linzell *et al.*, 1971; Symonds *et al.*, 1981; Orzechowski *et al.*, 1987), which is higher than the range of 0.2–0.8 $\mu mol\,min^{-1}\,g^{-1}$ of flow of ammonia to the liver over a wide variety of nutritional regimens. Therefore, the capacity of ruminant hepatocytes to detoxify ammonia directly to urea appears to be well adapted to large changes in portal ammonia concentration and is only exceeded when ammonia loads on the liver are abnormal (Symonds *et al.*, 1981; Fernandez *et al.*, 1990).

Ammonia is extremely toxic in non-hepatic tissues and causes changes in cerebral metabolism by affecting the glutamate transporter (GLAST) and thus reducing the capacity of astrocytes for reuptake of glutamate (Chan *et al.*, 2000), which results in tetany and death when circulating ammonia concentrations exceed 0.7 mM (Symonds *et al.*, 1981). Most investigators have reported physiological arterial ammonia concentrations in the 0.1 mM range using specific enzyme assays which follow the reaction with glutamate dehydrogenase (Bergmeyer and Beutler, 1985).

Recycling of urea

Since the work of Simmonet *et al.* (1957), numerous studies have shown that blood urea can cross the rumen epithelium. This process is nutritionally beneficial for ruminants, since the bacteria present in the rumen are able to use urea nitrogen for synthesis of proteins, the amino acid of which is subsequently available for postruminal absorption. The quantities of nitrogen recycled vary widely, and may account for up to 25% of the nitrogen ingested (Obara *et al.*, 1991) or up to 90% of urea turnover (see review of Harmeyer and Martens, 1980; Marini *et al.*, 2006). However, the absolute amount of recycled urea appears to be relative constant (Marini *et al.*, 2006).

Removal of absorbed ammonia plus excess amino acids and their conversion to urea by the liver represents a major cross-road in terms of whole body N exchange. At this metabolic junction, hepatic urea genesis represents 0.81 of digested N (Lapierre *et al.*, 2005). Therefore, a large part of this synthesized urea must be cycled rather than lost in urine. The quantities of nitrogen recycled in this way vary widely, and may account for up to 90% of urea turnover (see review of Harmeyer and Martens, 1980; Marini *et al.*, 2006). There is a negative venous-arterial flux of urea across the PDV. This denotes a net transfer of urea from the blood into the gut lumen. Net PDV urea N flux is clearly correlated with dietary nitrogen intake (Fig. 3B). On average, 0.47 of hepatic urea genesis, the equivalent of 0.34 of the digested N is returned to the gut via the PDV (Lapierre *et al.*, 2005). This N recycling represents an important fraction of the total flow of N through the digestive tract.

In practice, the true contribution of urea to gut N inflow should be even higher as the portal flux measurements do not include saliva flow. This latter input varies with the type of diet given and represents 0.22 of total gut entry rate with a concentrate-based ration, but 0.47 with a forage-based diet (Huntington, 1989). Such recycling will be effective only if the urea returned to the gut is used for anabolic purposes, i.e. as a precursor for microbial protein synthesis. The magnitude of this contribution will depend, among other things, on the site of return to the gut, as urea which enters the rumen is more likely to act as precursor-N for microbial protein synthesis with subsequent digestion and absorption across the small intestine. In contrast, while urea returned to the hind gut can also be used for microbial protein synthesis, these proteins will not be absorbed. The use of urea-N to support microbial protein synthesis within the small intestine and to supply amino acids to the host animals has recently been of considerable interest in non-ruminants (including pigs and human; Metges *et al.*, 1999; Torrallardona *et al.*, 2003). In ruminants, however, the contribution from this site is probably minor compared to that from the rumen (Lapierre and Lobley, 2001). In dairy cows, based on [^{15}N] urea kinetics, urea returned to the gut represented 0.67 of whole body urea production. This value is higher than the average obtained from PDV flow and includes the contribution from saliva. Of this return, 0.54 was used for anabolic purposes, 0.38 was reabsorbed as ammonia and returned to the urea cycle, and 0.08 was lost in the faeces (Lapierre *et al.*, 2004).

Ruminal urea transport

Diffusion of urea through the rumen epithelium has been demonstrated many years ago *in vivo* and *in vitro* (Gärtner *et al.*, 1961; v. Engelhardt und Nickel, 1965). The work of Houpt and Houpt (1968) showed that urea transfer across the rumen wall was linearly related to the rumen-blood concentration gradient, and it has been generally accepted that urea crosses the rumen epithelium by simple diffusion. Intraruminal hydrolysis of urea by bacterial urease therefore facilitates the movement of urea through the rumen wall by maintaining concentration gradient favorable to diffusion. Hence, it has been shown that the inhibition of urease activity in the rumen causes a decrease in the transepithelial flux of urea (Houpt and Houpt, 1968; Remond *et al.*, 1993b). In addition, the urea flux is dependent on the permeability of the epithelium. Since damage to the structure of the stratum corneum results in a marked increase in urea transport, urea diffusion seems to be strongly limited by the low permeability of this epithelial layer (Houpt and Houpt, 1968).

Factors influencing urea recycling/flux

Stimulation

When the feed is supplemented with a rapidly fermentable energy source (sucrose, extruded barley), the daily flux of urea across the rumen wall can be increased (Kennedy and Milligan, 1980; Reynolds and Huntington, 1988; Theurer *et al.*, 2002) or even doubled (Obara *et al.*, 1991; Norton *et al.*, 1982). As the blood urea level decreases when these energy supplements are added, the increase in urea flux must be mainly due to modified epithelial surface area and/or permeability, in response to intraruminal volatile fatty acids (VFAs) levels or CO_2 production. VFA and CO_2 production indicates fermentation in the rumen, which can only be maintained at optimal ammonia concentration for microbial growth (Satter and Slyter, 1974). Urea influx into the rumen significantly contributes to sustain sufficient ammonia concentration for optimal fermentation. Vice versa, reflux of urea was reduced in unfed sheep (Harmeyer and Martens, 1980).

Indeed, the transfer of urea across the rumen wall varies in the course of a feeding cycle (Remond *et al.*, 1993a), and so is evidently governed by a system of short-term regulation. Bubbling CO_2 in the rumen significantly increases urea flux across the rumen wall (Thorlacius *et al.*, 1971; v. Engelhardt *et al.*, 1978; Remond *et al.*, 1993b). Likewise, increasing the butyric acid concentration in an isolated pouch of the rumen favors urea transfer (v. Engelhardt *et al.*, 1978). The action of these two intraruminal factors does not involve modifications to the ruminal urease activity (Thorlacius *et al.*, 1971; Remond *et al.*, 1993b). Although CO_2 and butyric acid stimulate sub-epithelial blood flow (Dobson, 1984), the permeability of the capillary walls to urea is too low for the blood flow to affect urea diffusion (Landis and Pappenheimer, 1963). In addition, according to the results of Dobson *et al.* (1971), urea clearance seems virtually independent of blood flow.

Inhibition

Increasing intraruminal ammonia concentration decreases the urea flux across the rumen wall (v. Engelhardt *et al.*, 1978; Remond *et al.*, 1993b). According to Remond *et al.* (1993b), ammonia absorption may be responsible for reducing urea flux. The effect of ammonia on urease activity is long term (Cheng and Wallace, 1979) rather than short term, and the mechanism by which ammonia regulates the transepithelial flux of urea during short-term variations is not yet known.

Further observations

Other factors may also be involved in the regulation of transepithelial urea flux. Increasing osmotic pressure in an isolated pouch of the rumen with mannitol (Houpt, 1970) stimulates urea transfer. However, Remond *et al.* (1993b) raised the intraruminal osmotic pressure with NaCl injections, and observed no effect on urea flux.

Hormonal regulation of the urea flux has also been considered. According to Houpt (1970), vasopressin may modify the permeability of the rumen wall to urea, which has recently been shown to increase urea transport in MDCK monolayers (Potter *et al.*, 2006). However, Thorlacius *et al.* (1971) observed no modification of the urea clearance in response to vasopressin injection. The work of Harrop and Phillipson (1970) and Remond *et al.* (1993b) also suggests that gastrin might play a role in the regulation of the urea flux across the rumen wall. Generally, the effects of hormones and second messengers on transport mechanisms of the rumen epithelium have hardly been studied and are not well understood (see the literature in Schweigel *et al.*, 2005).

Urea transport proteins and tentative model of ruminal urea transport

Previously, movement of urea across biological membranes was considered to occur by lipid phase diffusion. However, the urea permeability of erythrocyte membranes and of certain plasma membranes in the terminal part of the kidney collecting duct were found to be much higher than could be explained by passive lipid diffusion alone (Marsh and Knepper, 1992). This inconsistency prompted the notion that urea crosses biological membranes by a carrier-mediated mechanism (urea transporter: UT). Recent studies have clearly shown that UT mRNA and protein are present in the rumen and colon epithelium of sheep (Ritzhaupt *et al.*, 1997, 1998). Protein corresponding to UT-B has been reported in rumen of cattle (Marini and Van Amburgh, 2003) and the entire gastrointestinal tract of sheep, while bands corresponding to UT-A could only be found in sheep duodenum (Marini *et al.*, 2004; Marini *et al.*, 2006). The structure of the bovine UT-B gene has been determined (Stewart *et al.*, 2005). This study also demonstrates that in analogy to sheep, only UT-B, but not UT-A, is expressed by the bovine rumen.

In a recent study in our laboratory, the serosal to mucosal urea fluxes across the isolated rumen epithelium did not show any correlation to the variation in tissue conductance (G_t). This means that the shunt pathway does not play a major role in urea transport across the rumen epithelium, and that transcellular urea flux predominates. In addition, the concentration-dependent flux density ($J/A \cdot C$) of urea over the membrane was

found to be considerably lower than the corresponding values for the flux of NH_3 from our previous study (Abdoun *et al.*, 2005). These data allow further deliberations. As stated above, our lack of knowledge about the precise dimensions of the ruminal epithelial barrier do not permit an estimate for the permeability coefficient of urea. However, if the permeability is given by $P=J/A \cdot C$ (J=flux, A=area and C=concentration), the permeability ratio $P(NH_3)/P$(Urea) between two substances such as NH_3 and urea can be calculated with reasonable accuracy since the unknown eithelial area is cancelled out of the equation, and compared to the same quotients from experiments performed on artificial lipid bilayers (Lande *et al.*, 1995). The permeability ratio $P(NH_3)/P$(Urea) of ruminal epithelium was found to be two orders of magnitude above that of the artificial bilayer systems, which can be taken as further piece of evidence suggesting that assumption of simple diffusion is not sufficient to explain the observations.

This conclusion is consistent with recent studies about the localization of the proposed urea transporter in the rumen epithelium of cows. Stewart *et al.* (2005) demonstrated staining of urea transporter in membranes of all epithelial layers of bovine rumen with the exception of the stratum corneum.

In combination with immunolocalization studies of urea transporter proteins of Stewart *et al.* (2005), the physiological findings about the flux of urea across the rumen epithelium allow the proposal of a putative model of urea transport. Under *in vivo* conditions, urea is taken up with the urea transporter across the basolateral membrane of the stratum basale into the epithelium. The flux of urea through the different layers of the rumen epithelium is mediated via the urea transporter (Stewart *et al.*, 2005) or via diffusion through the gap junctions (Graham and Simmons, 2004). The localization of urea transporter in the membrane of the stratum granulosum permits the extrusion of urea into the lumen. At the present time no explanation can be given, how ammonia, CO_2 or SCFA change urea flux rates according to the proposed model of urea transport.

Despite these uncertainties, the improved knowledge about urea transport across the rumen epithelium allows some conclusions and suggestions for future research. It is unlikely that the activity of ruminal urease limits urea back flow, because this enzyme has a capacity which clearly exceeds the amount of urea flowing back into the rumen (Bloomfield *et al.*, 1960). Furthermore, blood flow probably seems not to be a limiting factor. It is interesting to note that urea transport is modified by the fermentation products ammonia, CO_2 and SCFA. It has been convincingly demonstrated that these fermentation products significantly influence electroneutral Na transport via NHE indicating changes of intracellular pH and availability of H^+ for NHE (Gäbel *et al.*, 1991; Sehested *et al.*, 1999; Abdoun *et al.*, 2003, 2005). Any direct link or transfer of this knowledge to urea transport is very speculative at this time, but the shift of urea recycling to the forestomachs by increasing the intake of concentrate of an isocaloric and isonitrogenous diet (Theurer *et al.*, 2002) in steers suggests an important effect of fermentation at least in short-term regulation of urea transport.

In conclusion, large amounts of ammonia and urea are transported per day across the rumen epithelium: ammonia is absorbed from and urea is recycled into the rumen. The present data support the assumption of absorption of NH_3 by simple diffusion, which dominates at ruminal pH values above 7.0. At physiological pH values – pH 6.5 or lower – ammonia is predominantly absorbed as NH_4^+ through a putative K channel. No data are available regarding ammonia transporting proteins. Ammonia is metabolized during the passage across the epithelium at various rates, which are probably influenced by the diet.

Urea recycling is one of the important strategies of the ruminants to use food with a low protein content and to survive under harsh feeding conditions. The flow of urea into the rumen exhibits wide variations, which are not well understood at the present time. The recent detection of the urea transporter in the rumen epithelium and the well-established *in vivo* and *in vitro* techniques for studying urea transport open up perspectives for a better understanding of the modulation of urea transport. Since interaction between fermentation products and urea transport is established in a descriptive way, future research should be directed to modulation of urea transport by VFA or CO_2 for a better understanding of these effects. Still not clear are the effects of ruminal ammonia concentration on urea flux. It could be that these effects of ruminal ammonia on urea transport depends on the pH and/or feeding conditions as it has been demonstrated for Na transport (Abdoun *et al.*, 2003, 2005).

Acknowledgments

K. Abdoun is a recipient of a scholarship of the Alexander von Humboldt Foundation (Georg-Foster). Our studies were supported by the Deutsche Forschungsgemeinschaft and the Margarete-Markus-Charity.

References

Abdoun K, Wolf K, Arndt G and Martens H (2003). Effect of ammonia on Na^+ transport across isolated rumen epithelium of sheep is diet dependent. *British Journal of Nutrition* **90**: 751–758.

Abdoun K, Stumpff F, Wolf K and Martens H (2005). Modulation of electroneutral Na transport in sheep rumen epithelium by luminal ammonia. *American Journal of Physiology, Gastrointestinal and Liver Physiology* **289**: G508–G520.

Abdoun K, Wolf K and Martens H (2006). Interaction between ammonia, sodium and chloride transport across the

rumen epithelium of sheep. *Small Ruminant Research* **63**: 91–99.

Amlal H and Soleimani M (1997). K^+/NH_4^+ antiporter: a unique ammonium carrying transporter in the kidney inner medulla. *Biochimica et Biophys Acta* **1323**: 319–333.

Bennink MR, Tyler TR, Ward GM and Johnson DE (1978). Ionic milieu of bovine and ovine rumen as affected by diet. *Journal of Dairy Science* **61**: 315–323.

Bergmeyer HU and Beutler H-O (1985). In: Bergmeyer HU (ed.) *Methods of Enzymatic Analysis*, 3rd edn. Weinheim, Germany: VCH Publishing Group, Vol. VIII, pp. 454–461.

Blank R, Südekum K-H, Immig I and Kleinmans J (1998). Synchroner Abbau von Kohlenhydraten und Rohprotein in den Vormägen – eine neue Variable für die Rationsgestaltung? *Übersichten Tierernährung* **26**: 157–188.

Bloomfield RA, Garner GB and Muhrer ME (1960). Kinetics of urea metabolism in sheep. *Journal of Animal Science* **19**: 1248 (Abstract).

Bödeker D and Kemkowski J (1996). Participation of NH_4^+ in total ammonia absorption across the rumen epithelium of sheep (*Ovis aries*). *Comparative Biochemical Physiolog* A **114**: 305–310.

Bödeker D, Winkler A and Holler H (1990). Ammonia absorption from the isolated reticulo-rumen of sheep. *Experimental Physiology* **75**: 587–795.

Bödeker D, Oppelland G and Holler H (1992a). Involvement of carbonic anhydrase in ammonia flux across rumen mucosa *in vitro*. *Experimental Physiology* **77**: 517–519.

Bödeker D, Shen Y, Kemkowski J and Höller H (1992b). Influence of short-chain fatty acids on ammonia absorption across the rumen wall of sheep. *Experimental Physiology* **77**: 369–376.

Briggs PK, Hogan JP and Reid RL (1957). Effect of volatile fatty acids, lactic acid and ammonia on rumen pH in sheep. *Australian Journal of Agricultural Research* **8**: 674–690.

Bryant MP and Robinson IM (1962). Some nutritional characteristics of predominant culturable ruminal bacteria. *Journal of Bacteriology* **84**: 605–614.

Burckhardt BC and Frömter E (1992). Pathways of NH_3/NH_4^+ permeation across *Xenopus laevis* oocyte cell membrane. *Pflügers Archiv: European Journal of Physiology* **420**: 83–86.

Care AD, Brown RC, Farrar AR and Pickard DW (1984). Magnesium absorption from the digestive tract of sheep. *Quarterly Journal of Experimental Physiology* **69**: 577–587.

Carter RR and Grovum WL (1990). A review of the physiological significance of hypertonic body fluids on feed intake and ruminal function: salivation, motility and microbes. *Journal of Animal Science* **68**: 2811–2832.

Cermak R, Minck K, Lawnitzak C and Scharrer E (2002). Ammonia inhibits sodium and chloride absorption in rat distal colon. *Experimental Physiology* **87**: 311–319.

Chalmers MI, Jaffray AE and White F (1971). Movements of ammonia following intraruminal administration of urea or casein. *Proceedings of the Nutritional Society* **30**: 7–17.

Chalupa WJ, Opliger CP and Lavker R (1970a). Ammonia metabolism in rumen bacteria and mucosa from sheep fed soy protein or urea. *Journal of Nutrition* **100**: 161–169.

Chalupa WJ, Opliger CP and Lavker R (1970b). Detoxification of ammonia in sheep fed soy protein or urea. *Journal of Nutrition* **100**: 170–176.

Chan H, Hazell AS, Desjardins P and Butterworth RF (2000). Effect of ammonia on glutamate transporter (GLAST) protein and mRNA in cultured rat cortical astrocytes. *Neurochemistry International* **37**: 243–248.

Cheng KJ and Wallace RJ (1979). The mechanism of passage of endogenous urea through the rumen wall and the role of ureolytic epithelial bacteria in the urea flow. *British Journal of Nutrition* **42**: 553–557.

Choe H, Sackin H and Palmer LG (2000). Permeation properties of inward-rectifier potassium channels and their molecular determinants. *Journal of General Physiology* **115**: 391–404.

Cohen RM, Stephenson RL and Feldman GM (1988). Bicarbonate secretion modulates ammonium absorption in rat distal colon *in vivo*. *American Journal of Physiology* **254**: F657–F667.

Coombe JB, Tribe DE and Morrison JWC (1960). Some experimental observation on the toxicity of urea to sheep. *Australian Journal of Agricultural Research* **11**: 247–256.

Cotta MA and Hespell RB (1986). Protein and amino acid metabolism of rumen bacteria. In: Milligan LP, Grovum WL and Dobson A (eds) *Control of Digestion and Metabolism in Ruminants*. Washington, DC: Butterworths, pp. 122–136.

Dobson A (1959). Active transport through the epithelium of the reticulo-rumen sac. *Journal of Physiology* **146**: 235–251.

Dobson A (1979). The choice of models relating tritiated water absorption to subepithelial blood flow in the rumen of sheep. *Journal of Physiology* **297**: 111–121.

Dobson A (1984). Blood flow and absorption from the rumen. *Quarterly Journal of Experimental Physiology* **69**: 599–606.

Dobson A, Sellers AF and Thorlacius SO (1971). Limitation of diffusion by blood flow through bovine ruminal epithelium. *American Journal of Physiology* **220**: 1337–1343.

Dobson A, Sellers AF and Gatewood VH (1976). Absorption and exchange of water across rumen epithelium. *American Journal of Physiology* **231**: 1588–1594.

Eisemann JH and Nienaber JA (1990). Tissue and whole-body oxygen uptake in fed and fasted steers. *British Journal of Nutrition* **64**: 399–411.

v. Engelhardt W and Nickel W (1965). Die Permealität der Pansenwand für Harnstoff, Antipyrin und Wasser. *European Journal of Physiology* **286**: 57–75.

v. Engelhardt W, Hinderer S and Wipper E (1978). Factors influencing the endogenous urea-N secretion and utilization in the gastrointestinal tract. In: Osboum DF, Beever DE and Thomson DJ (eds) *Ruminants Digestion and Feed Evaluation*. London: Agricultural Research Council, pp. 4.1–4.12.

Etschmann B, Heipertz KS, von der Schulenburg A and Schweigel M (2006). A vH^+-ATPase is present in cultured sheep ruminal epithelial cells. *American Journal of Physiology Gastrointest Liver Physiology*, Epub ahead of print.

Fernandez JM, Croon WJ, Tate LP and Johnson AD (1990). Subclinical ammonia toxicity in steers: effects on hepatic and portal-drained visceral flux of metabolites and regulatory hormones. *Journal of Animal Science* **68**: 419–429.

Ferreira HG, Harrison FA, Keynes RD and Nauss AH (1966a). Observations on the potential across the rumen of sheep. *Journal of Physiology* **187**: 615–630.

Ferreira HG, Harrison FA and Keynes RD (1966b). The potential and short-circuit current across isolated rumen epithelium of the sheep. *Journal of Physiology* **187**: 631–644.

Ferreira HG, Harrison FA, Keynes RD and Zurich L (1972). Ion transport across an isolated preparation of sheep rumen epithelium. *Journal of Physiology* **222**: 77–93.

Fitch NA, Gill M, Lomax MA and Beever DE (1989). Nitrogen and glucose metabolism by the liver of forage- and forage-concentrate fed cattle. *Proceedings of the Nutrition Society* **48**: 76A.

Fuller MF and Rees PJ (1998). Nitrogen recycling in the gut. *Annual Review of Nutrition* **18**: 385–411.

Gäbel G and Martens H (1986). The effect of ammonia on magnesium metabolism in sheep. *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **55**: 278–287.

Gäbel G, Vogler S and Martens H (1991). Short-chain fatty acids and CO_2 as regulators of Na^+ and Cl^- absorption in isolated sheep rumen mucosa. *Journal of Comparative Physiology* B **161**: 419–426.

Gäbel G, Aschenbach JR and Müller F (2002). Transfer of energy substrates across the ruminal epithelium: implications and limitations. *Animal Health Research Reviews* **3**: 15–30.

Gärtner K and v. Engelhardt W (1964). Experiments concerning the resorption mechanism of ammonia through the ruminal mucosa of ruminants. *Deutsche Tierärztliche Wochenschrift* **71**: 57–60.

Gärtner K, Decker P and Hill H (1961). Untersuchungen über die Passage von Harnstoff und Ammoniak durch die Pansenwand von Ziegen. *European Journal of Physiology* **274**: 281–288.

Garwacki S, Wiechetek M, Karlik W, Souffrant WB and Krawielitzki K (1990). Effect of propionate on the utilization of nitrogen from $^{15}NH_4Cl$ for urea synthesis in hepatocytes isolated from sheep liver. *International Journal of Biochemistry* **22**: 1185–1188.

Graham C and Simmons NL (2004). Functional organization of the bovine rumen epithelium. *American Journal of Physiology* **288**: R173–R181.

Guerino F, Huntington GB and Erdman RA (1991). The net portal and hepatic flux of metabolites and oxygen consumption in growing beef steers given postruminal casein. *Journal of Animal Science* **69**: 387–395.

Handlogten ME, Hong SP, Zhang L, Vander AW, Steinbaum ML, Campbell-Thompson M and Weiner ID (2005). Expression of the ammonia transporter proteins Rh B glycoprotein and Rh C glycoprotein in the intestinal tract. *American Journal of Physiology* **288**: G1036–G1047.

Harmeyer J and Martens H (1980). Some aspects of urea metabolism in ruminants with particular reference to the goat. *Journal of Dairy Science* **63**: 1707–1728.

Harmon DL, Kreikemeier KK and Gross KL (1993). Influence of addition of monensin to an alfalfa hay diet on net portal and hepatic nutrient flux in steers. *Journal of Animal Science* **71**: 218–225.

Harrison FA, Keynes RD, Rankin JC and Zurich L (1975). The effect of ouabain on ion transport across isolated sheep rumen epithelium. *Journal of Physiology* **249**: 669–677.

Harrop CJF and Phillipson AT (1970). The effect of diet and pentagastrin on thew influx of urea into the rumen of sheep. *Proceedings of the Nutrition Society* **30**: 3A

Henning JP, Steyn DG and Meissner HH (1993). Effect of synchronization of energy and nitrogen supply on ruminal characteristics and microbial growth. *Journal of Animal Science* **71**: 2516–2528.

Hille B (2001). *Ion Channels of Excitable Membranes*, 3rd edn. Sunderland, USA: Sinauer Associates, Inc., Chapter 15, pp. 482.

Hille B and Schwarz W (1978). Potassium channels as multi-ion single-file pores. *Journal of General Physiology* **72**: 409–442.

Hogan JP (1961). The absorption of ammonia through the rumen of the sheep. *Australian Journal of Biological Science* **14**: 448–460.

Hoshino S, Sarumaru K and Morimoto K (1966). Ammonia anabolism in ruminants. *Journal of Dairy Science* **49**: 1523–1528.

Houpt TR (1970). Transfer of urea and ammonia to the rumen. In: Phillipson AT (ed.) *Physiology of Digestion and Metabolism in the Ruminant*. Newcastle-upon-Tyne, UK: Oriel Press, pp. 119–131.

Houpt TR and Houpt KA (1968). Transfer of urea nitrogen across the rumen wall. *American Journal of Physiology* **214**: 1296–1303.

Hristov M and Broderick GA (1994). *In vitro* determinations of rumination protein degradability using [^{15}N] ammonia to correct for microbial nitrogen uptake. *Journal of Animal Science* **72**: 1344–1354.

Huntington GB (1986). Uptake and transport of non protein nitrogen by the ruminant gut. *Federation Proceedings* **45**: 2272–2276.

Huntington GB (1989). Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high concentrate diet. *Canadian Journal of Animal Science* **69**: 215–223.

Huntington GB and Prior RL (1983). Digestion and absorption of nutrients by beef heifers fed a high concentrate diet. *Journal of Nutrition* **113**: 2280–2288.

Huntington GB, Zetina EJ, Whitt JM and Potts W (1996). Effects of dietary concentrate level on nutrient absorption, liver metabolism, and urea kinetics of beef steers fed isonitrogenous and isoenergetic diets. *Journal of Animal Science* **74**: 908–916.

Hussinger D, Lamers WH and Moorman AFM (1992). Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. *Enzyme* **46**: 72–93.

Hyden S. (1961). Observations on the absorption of inorganic ions from the reticulo-rumen of the sheep. *Kungl. Lantbrukshögkolans Annaler* **27**: 273–285.

Kang-Meznarich JH and Broderick GA (1981). Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein formation. *Journal of Animal Science* **51**: 422–431.

Karim Z, Szutkowska M, Vernimmen C and Bichara M (2005). Renal handling of NH_3/NH_4^+: recent concepts. *Nephron Physiology* **101**: 77–81.

Kennedy PM and Milligan LP (1980). The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants, a review. *Canadian Journal of Animal Science* **60**: 205–221.

Kennedy PM, Early RJ, Chrispherson RJ and Milligan LP (1986). Nitrogen transformations and duodenal amino acid content in sheep given four forage diets and exposed to warm and cold ambient temperatures. *Canadian Journal of Animal Science* **66**: 951–957.

Khademi S, O'Connell J, 3rd, Remis J, Robles-Colmenares Y, Miercke LJ and Stroud RM (2004). Mechanism of ammonia transport by Amt/MEP/Rh: structure of AmtB at 1.35 A. *Science* **305**: 1587–1594.

Kikeri D, Sun A, Zeidel ML and Hebert SC (1989). Cell membranes impermeable to NH_3. *Nature* **339**: 478–480.

Lande MB, Donovan JM and Zeidel ML (1995). The relationship between membrane fluidity and permeabilities to water, solutes, ammonia and protons. *Journal of General Physiology* **106**: 67–84.

Landis EM and Pappenheimer JR (1963). Exchanges of substances through the capillary walls. In: Hamilton WF (ed.) *Handbook of Physiology*, Vol. 2, Section 2, Circulation. Washington, DC: American Physiological Society, pp. 961.

Lang I and Martens H (1999). Na transport in sheep rumen is modulated by voltage-dependent cation conductance in apical membrane. *American Journal of Physiology* **277**: G609–G618.

Lapierre H and Lobley GE (2001). Nitrogen recycling in the ruminant, a review. *Journal of Dairy Science* **84** (**suppl. E**): E223–E236.

Lapierre H, Ouellet DR, Berthiaume R, Girard C, Dubreuil P, Babkine M and Lobley GE (2004). Effect of urea supplementation on urea kinetics and splanchnic flux of amino acids in dairy cows. *Journal of Animal and Feed Sciences* **13** (**suppl. 1**): 319–322.

Lapierre H, Berthiaume R, Raggio G, Thivierge MC, Doepel L, Pacheco D, Dubreuil P and Lobley GE (2005). The rout of absorbed nitrogen into milk protein. *Journal of Animal Science* **80**: 11–22.

Leng RA and Nolan JV (1984). Symposium: protein nutrition of the lactating dairy cows. *Journal of Dairy Science* **67**: 1072–1089.

Leonhard-Marek S and Martens H (1996). Effects of potassium on magnesium transport across rumen epithelium. *American Journal of Physiology* **271**: G1034–G1038.

Leonhard-Marek S, Stumpff F, Brinkmann I, Breves G and Martens H (2005). Basolateral Mg^{2+}/Na^{+} exchange regulates apical nonselective cation channel in sheep rumen epithelium via cytosolic Mg^{2+}. *American Journal of Physiology: Gastrointestinal Liver Physiology* **288**: G630–G645.

Linzell JL, Setchell BP and Lindsay DB (1971). The isolated perfused liver of sheep: assessment of its metabolic, synthetic and secretory functions. *Quarterly Journal of Experimental Physiology* **56**: 53–71.

MacKie RI and White BA (1990). Recent advances in rumen microbial ecology and metabolism: Potential impact on nutrient impact. *Journal of Dairy Science* **73**: 2971–2995.

Mak DO, Dang B, Weiner ID, Foskett JK and Westhoff CM (2006). Characterization of ammonia transport by the kidney Rh glycoproteins RhBG and RhCG. *American Journal of Physiology: Renal Physiology* **290**: F297–F305.

Maltby SA, Lomax MA, Beever DE and Pippard CJ (1991). The effect of increased ammonia and amino acid supply on post-prandial portal-drained viscera and hepatic metabolism in growing steers fed maize silage. In: Wenk C and Boessinger M (eds) *Energy Metabolism of Farm Animals*. European Association of Animal Production Publication no. 58, Zurich, Switzerland: ETH-Zentrum, pp. 20–23.

Maltby SA, Beever DE, Lomax MA, Crompton LA and Pippard CJ (1993a). The influence of diet and increased ammonia supply on energy and nitrogen metabolism across splanchnic tissues in growing cattle. *Animal Production* **56**: 431 (Abstract).

Maltby SA, Crompton LA, Lomax MA, Beever DE and Pippard CJ (1993b). The effect of increased ammonia supply on post-prandial hepatic metabolism in growing steers fed either forage or cereal-based diets. *Proceedings of the Nutrition Society* **52**: 295A.

Marini AM, Matassi G, Raynal V, Andre B, Cartron JP and Cherif-Zahar B (2000). The human Rhesus-associated RhAG protein and a kidney homologue promote ammonium transport in yeast. *Nature Genetics* **26**: 341–344.

Marini JC and Van Amburgh ME (2003). Nitrogen metabolism and recycling in Holstein heifers. *Journal of Animal Science* **81**: 545–552.

Marini JC, Klien JD, Sands JM and Van Amburg ME (2004). Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. *Journal of Animal Science* **82**: 1157–1164.

Marini JC, Sands JM and Van Amburgh ME (2006). Urea transporters and urea recycling in ruminants. In: Sejrsen K, Hvelplund T and Nielsen MO (eds) *Ruminant Physiology*. The Netherlands: Wageningen Academic Publishers, pp. 155–171.

Marsh MJ and Knepper MA (1992). Renal handling of urea. In: Windhager EE (ed.). *Hand Book of Physiology, Renal Physiology*. Oxford, UK: Oxford University Press, pp. 1317–1347.

Martens H and 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 and Hammer U (1981). Magnesium and sodium absorption from the isolated sheep rumen during intravenous aldosterone infusion (author's transl.). *Deutsche tierärztliche Wochenschrift* **88**: 404–407.

Martens H and Rayssiguier Y (1980). Magnesium metabolism and hypomagnesaemia. In: Ruckebusch Y and Thivend P (eds) *Digestive Physiology and Metabolism in Ruminants*. Clermont-Ferrand, France: MTP Press, pp. 447–466.

Martens H and Schweigel M (2000). Pathophysiology of grass tetany and other hypomagnesemias. Implications for clinical management, review. *Veterinary Clinics of North America: Food Animal Practice* **16**: 339–368.

Martens H, Heggemann G and Regier K (1988). Studies on the effect of K, Na, NH_4^{+}, VFA and CO_2 on the net absorption of magnesium from the temporarily isolated rumen of heifers. *Zentralblatt für Veterinärmedizin. Reihe* A **35**: 73–80.

Martens H, Gäbel G and Strozyk B (1991). Mechanism of electrically silent Na and Cl transport across the rumen epithelium of sheep. *Experimental Physiology* **76**: 113–114.

Mathison GW and Milligan LP (1971). Nitrogen metabolism in sheep. *British Journal of Nutrition* **25**: 351–366.

Mayer M, Schaaf G, Mouro I, Lopez C, Colin Y, Neumann P, Cartron JP and Ludewig U (2006). Different transport mechanisms in plant and human AMT/Rh-type ammonium transporters. *Journal of General Physiology* **127**: 133–144.

McDonald IW (1948). The absorption of ammonia from the rumen of the sheep. *Biochemical Journal* **42**: 584–587.

McLaren GA, Anderson GC, Martin WG and Cooper WK (1961). Fixation of ammonia nitrogen by rumen mucosa. *Journal of Animal Science* **20**: 942–943.

Metges CC, Petzke KJ, El-Khoury AE, Henneman L, Grant I, Bedri S, Regan MM, Fuller MF and Young VR (1999). Incorporation of urea and ammonia nitrogen into ileal and fecal microbial proteins and plasma free amino acids in normal men and ileostomates. *American Journal of Clinical Nutrition* **70**: 1046–1058.

Milano GD, Hotston-Moore A and Lobley GE (2000). Influence of hepatic ammonia removal on ureagenesis, amino acid utilization and energy metabolism in the ovine liver. *British Journal of Nutrition* **83**: 307–315.

Nagami GT (1988). Luminal secretion of ammonia in the mouse proximal tubule perfused *in vitro*. *Journal of Clinical Investigation* **81**: 159–164.

Nocek JE, Herbein J and Polan CE (1980). Influence of ration physical form, ruminal degradable nitrogen and age on rumen epithelial propionate and acetate transport and some enzyme activities. *Journal of Nutrition* **110**: 2355–2364.

Nolan JV (1975). Quantitative models of nitrogen metabolism in sheep. In: McDonald IW and Warner ACI (eds) *Digestion and Metabolism in the Ruminants*. Armidale: University of New England, Australia, pp. 416–431.

Nolan JV and Strachin S (1979). Fermentation and nitrogen dynamics in Merino sheep given a low-quality-roughage diet. *British Journal of Nutrition* **42**: 63–80.

Nolan JV, Norton BW and Leng RA (1976). Further studies on the dynamics of nitrogen metabolism in sheep. *British Journal of Nutrition* **35**: 127–147.

Norton BW, Mackintosh JB and Armstrong DG (1982). Urea synthesis and degradation in sheep given pelleted-grass diets containing flaked barley. *British Journal of Nutrition* **48**: 249–264.

Obara Y, Dellow DW and Nolan JV (1991). Effects of energy-rich supplements on nitrogen kinetics in ruminants. In: Tsuda T, Sasaki Y and Kawashima R (eds) *Physiological Aspects of Digestion and Metabolism in Ruminants*. San Diego, CA: Academic Press, pp. 515–539.

Ortolani EL, Moris CS and Rodrigues Filho JA (2000). Ammonia toxicity from urea in a Brazilian dairy goat flock. *Veterinary and Human Toxicology* **42**: 87–89.

Orzechowski A, Motyl T, Pierzynowski G and Barej W (1987). Hepatic capacity for ammonia removal in sheep. *Journal of Veterinary Medicine* **34**: 108–112.

Parker DS, Lomax MA, Seal CJ and Wilton JC (1995). Metabolic implications of ammonia production in the ruminant, review. *Proceedings of the Nutritional Society* **54**: 549–563.

Paula S, Volkov AG, Van Hoek AN, Heines TH and Deamer DW (1996). Permeation of protons, potassium ions, and small polar molecules through phospholipid bilayers as a function of membrane thickness. *Biophysical Journal* **70**: 339–348.

Potter EA, Stewart G and Smith CP (2006). Urea flux across MDCK-mUT-A2 monolayers is acutely sensitive to AVP, cAMP, and $[Ca^{2+}]i$. *American Journal of Physiology* **291**: R122–R128.

Remond D, Chaise JP, Delval E and Poncet C (1993a). Net transfer of urea and ammonia across the ruminal wall of sheep. *Journal of Animal Science* **71**: 2785–2792.

Remond D, Poncet C and Lefaivre J (1993b). Technical note: ruminal vein catheterization and continuous blood flow measurement in ruminal arteries of sheep. *Journal of Animal Science* **71**: 1276–1280.

Reynolds CK (1992). Metabolism of nitrogenous compounds by ruminant liver, review. *Journal of Nutrition* **122** (**suppl. 3**): 850–854.

Reynolds CK (1995). Quantitative aspects of liver metabolism in ruminants. In: Engelhardt Wv, Leonhard-Marek S, Breves G and Giesecke D (eds) *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*. Stuttgart, Germany: Ferdinand Enke Verlag, pp. 351–372.

Reynolds CK (2006). Splanchnic amino acid metabolism in ruminants. In: Sejrsen K, Hvelplund T and Nielsen MO (eds) *Ruminant Physiology*. Wageningen, The Netherlands Wageningen Academic Publisher, pp. 225–248.

Reynolds CK and Huntington GB (1988). Partition of portal-drained visceral net flux in beef steer. 1. Blood flow and net flux of oxygen, glucose and nitrogenous compounds across stomach and post-stomach tissues. *British Journal of Nutrition* **60**: 539–551.

Reynolds CK and Tyrrell HF (1991). Effects of mesenteric vein L-alanine infusion on liver metabolism in beef heifers fed on diets differing in forage: concentrate ratio. *British Journal of Nutrition* **66**: 437–450.

Reynolds CK, Tyrrell HF and Reynolds PJ (1991). Effect of diet forage -to- concentrate ratio and intake on energy metabolism in growing beef heifers: net nutrient metabolism by visceral tissues. *Journal of Nutrition* **121**: 1004–1015.

Reynolds CK, Casper DP, Harmon DL and Milton CT (1992a). Effect of CP and ME intake on visceral nutrient metabolism in beef steers. *Journal of Animal Science* **70** (**suppl. 1**): 315 (Abstract).

Reynolds CK, Lapierre H, Tyrrell HF, Elsasser TH, Staples RC, Gaudreau P and Brazeau P (1992b). Effect of growth-hormone releasing factor and feed intake on energy metabolism in growing beef steers: net nutrient metabolism by the portal-drained viscera and liver. *Journal of Animal Science* **70**: 752–763.

Ritzhaupt A, Breves G, Schroder B, Winckler CG and Shirazi-Beechey SP (1997). Urea transport in gastrointestinal tract of ruminants: effect of dietary nitrogen. *Biochemical Society Transaction* **25**: S490 (Abstract).

Ritzhaupt A, Wood IS, Jackson AA, Moran BJ and Shirazi-Beechey SP (1998). Isolation of a RT-PCR fragment from human colon and sheep rumen RNA with nucleotide sequence similarity to human and rat urea transporter isoforms. *Biochemical Society Transaction* **26**: S122 (Abstract).

Rooke JA, Lee NH and Armstrong DG (1987). The effects of intraruminal infusions of urea, casein, glucose syrup and a mixure of casein and glucose syrup on nitrogen digestion in the rumen of cattle receiving grass-silage diets. *British Journal of Nutrition* **57**: 89–98.

Satter LD and Slyter LL (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro*. *British Journal of Nutrition* **32**: 199–208.

Schwartz JH and Tripolone M (1983). Characteristics of NH_4^+ and NH_3 transport across the isolated turtle urinary bladder. *American Journal of Physiology* **245**: F210–F216.

Schweigel M and Martens H (2003). Anion-dependent Mg^{2+} influx and a role for a vacuolar H^+-ATPase in sheep ruminal epithelial cells. *American Journal of Physiology* **285**: G45–G53.

Schweigel M, Freyer M, Leclercq S, Etschmann B, Lodemann U, Bottcher A and Martens H (2005). Luminal hyperosmolarity decreases Na transport and impairs barrier function of sheep rumen epithelium. *Journal of Comparative Physiology* B **175**: 575–591.

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

Scott D (1967). The effects of potassium supplements upon the absorption of potassium and sodium from the sheep rumen. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* **52**: 382–391.

Sehested J, Diernaes L, Moller PD and Skadhauge E (1999). Transport of butyrate across the isolated bovine rumen epithelium. Interaction with sodium, chloride and bicarbonate. *Comparative Biochemistry and Physiology* A **123**: 399–408.

Sellers AF and Dobson A (1960). Studies on reticulo-rumen sodium and potassium concentration and electrical potentials in sheep. *Research in Veterinary Science* **1**: 95.

Siddons RC, Nolan JV, Beever DE and McRae JC (1985). Nitrogen digestion and metabolism in sheep consuming diets containing contrasting forms and levels of N. *British Journal of Nutrition* **54**: 545–561.

Simmonet H, Le Bars H and Molle J (1957). Le cycle de l'urée administrée par voie buccale chez les ruminants (Urea cycle after oral administration to ruminants). *Séance Académique Science* **244**: 943–945.

Sinclair LA, Garnsworthy PC, Newbold JR and Buttery PJ (1993). Effect of synchronising the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. *Journal of Agricultural Science* **120**: 251–263.

Singh SK, Binder HJ, Geibel JP and Boron WF (1995). An apical permeability barrier to NH_3/NH_4^+ in isolated, perfused

colonic crypts. *Proceedings of the National Academy of Sciences, USA* **92**: 11573–11577.

Sniffen CJ and Jacobson DR (1975). Net amino acid absorption in steers fed alfalfa hay cut at two stages of maturity. *Journal of Dairy Science* **58**: 371–385.

Sperber I and Hyden S (1952). Transport of chloride through the ruminal mucosa. *Nature* **169**: 587.

Stacy BD and Warner AC (1966). Balances of water and sodium in the rumen during feeding: osmotic stimulation of sodium absorption in the sheep. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* **51**: 79–93.

Stewart GS, Graham C, Cattell S, Smith TPL, Simmons NL, Smith CP (2005). UT-B is expressed in bovine rumen: potential role in ruminal urea transport. *American Journal of Physiology* **289**: R605–R612.

Stumpff F and Martens H (2006). A role for magnesium in the regulation of ruminal sodium transport. In: McAlpine G (ed.) *Focus on Signal Transduction Research*. Hauppauge, NY: Nova Science Publishers, Inc., Chapter 2, pp. 36–66.

Symonds HW, Mather DL and Collis KA (1981). The maximum capacity of the liver of the adult dairy cow to metabolize ammonia. *British Journal of Nutrition* **46**: 481–486.

Taminga S (1983). Recent advances in our knowledge on protein digestion and absorption in ruminants. In: Arnal M, Pion R and Bonin D (eds) *Proceedings of the 4th International Symposium of European Association of Animal Production (EAAP), Protein Metabolism and Nutrition*. Paris: INRA, pp. 263–287.

Theurer CB, Huntington GB, Huber JT, Swingle RS and Moore JA (2002). Net absorption and utilization of nitrogenous compounds across ruminal, intestinal, and hepatic tissues of growing beef steers fed dry-rolled or steam-flaked sorghum grain. *Journal of Animal Science* **80**: 525–532.

Thompson DJ, Beever DE, Lonsdale CR, Haines MJ, Cammell SB and Austin RA (1981). The digestion by cattle of grass silage made with formic acid and formic acid-formaldehyde. *British Journal of Nutrition* **46**: 193–207.

Thorlacius SO (1972). Effect of stream-volatile fatty acids and carbon dioxide on blood content of rumen papillae of the cow. *American Journal of Veterinary Research* **33**: 427–430.

Thorlacius SO, Dobson A, Sellers AF (1971). Effect of carbon dioxide on urea diffusion through bovine ruminal epithelium. *American Journal of Physiology* **220**: 162–170.

Torrallardona D, Harris CI and Fuller MF (2003). Pigs' gastrointestinal microflora provide them with essential amino acids. *Journal of Nutrition* **133**: 1127–1131.

Warner AC and Stacy BD (1972a). Water, sodium and potassium movements across the rumen wall of sheep. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* **57**: 103–119.

Warner AC and Stacy BD (1972b). Intraruminal and systemic responses to variations in intake of sodium and potassium by sheep. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* **57**: 89–102.

Warner AC and Stacy BD (1977). Influence of ruminal and plasma osmotic pressure on salivary secretion in sheep. *Quarterly Journal of Experimental Physiology* **62**: 133–142.

Webb DW, Bartley EE and Meyer MR (1972). A comparison of nitrogen metabolism and ammonia toxicity from ammonium acetate and urea in cattle. *Journal of Animal Science* **35**: 1263–1270.

Weiner ID (2004). The Rh gene family and renal ammonium transport. *Current Opinion in Nephroogyland Hypertension* **13**: 533–540.

Wernli CG and Wilkins RJ (1980). Nutritional studies with sheep fed conserved grass. 1. Silage and dried grass offered ad libitum without supplements. *Quarterly Journal of Experimental Physiology* **60**: 89–94.

Westhoff CM, Ferreri-Jacobia M, Mak DO and Foskett JK (2002). Identification of the erythrocyte Rh blood group glycoprotein as a mammalian ammonium transporter. *Journal of Biological Chemistry* **277**: 12499–12502.

Wilton JC, Gill M and Lomax MA (1988). Uptake of ammonia across the liver of forage-fed cattle. *Proceedings of the Nutrition Society* **47**: 153A.

Winkler FK (2006). Amt/MEP/Rh proteins conduct ammonia. *European Journal of Physiology* **451**: 701–707.

Wolf JE, Bergman EN and Williams HH (1972). Net metabolism of plasma amino acids by liver and portal drained viscera of fed sheep. *American Journal of Physiology* **223**: 438–446.

Wolffram S, Frischknecht R and Scharrer E (1989). 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.

Wrong OM (1978). Nitrogen metabolism in the gut. *American Journal of Clinical Nutrition* **31**: 1587–1593.

Yellen G (1987). Permeation in potassium channels: implications for channel structure. *Annual Review of Biophysics and Biophysical Chemistry* **16**: 227–246.