

## Metabolic Fates of Ammonia-N in Ruminal Epithelial and Duodenal Mucosal Cells Isolated from Growing Sheep

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### ABSTRACT

The objective of this experiment was to determine the capability of ruminant gut tissues to detoxify ammonia-N using short-term incubations of isolated cells in vitro. Ruminal epithelial cells (REC) and duodenal mucosal cells (DMC) were isolated from growing Texel-Polypay ram lambs ( $n = 4$ ) fed a pelleted forage:concentrate-based diet. Immediately after isolation, primary cells were incubated for 60 min with glucose (1mM), glutamate (1mM), [<sup>15</sup>N]ammonium chloride (5, 10, 20, or 40 mM), and 1 of 4 combinations of substrates (1 mM each) that could support urea synthesis [control, *N*-carbamoylglutamate (NCG); NCG + ornithine (ONCG); and ONCG + aspartate (AONCG)]. Treatments were arranged in a 4 × 4 factorial design. Incorporation of ammonia-<sup>15</sup>N into alanine, citrulline, arginine, and urea was determined by gas chromatography-mass spectrometry. For both cell types, ammonia-N transfer to alanine was lower when incubation medium contained NCG compared with control, whereas use of ammonia-N for net alanine synthesis increased quadratically with ammonia concentration regardless of substrate treatment. For REC, ammonia-N was not incorporated into citrulline, arginine, or urea, nor into arginine or urea by DMC. Ammonia-N use for net citrulline synthesis exhibited an inverse relationship with ammonia concentration, decreasing linearly as media ammonia concentration increased. Thus, alanine synthesis may be a significant metabolic pathway for ruminant gut tissues to detoxify ammonia-N when it is presented luminally at high concentrations as compared with detoxification by the ornithine-urea cycle. Furthermore, DMC do exhibit a metabolic capability to incorporate ammonia-N into citrulline, but low or absent activity of downstream enzymes of the ornithine-urea cycle appears to limit ammonia-N transfers to urea.

(Key words: sheep, rumen, duodenum, epithelial cells, mucosal cells, ammonia)[Au: only 4 key words/phrases permitted; please delete 2]

**Abbreviation key:** AONCG = ONCG + aspartate, DMC = duodenal mucosal cells, GC-MS = gas chromatography/mass spectrometry, NCG = *N*-carbamoylglutamate, ONCG = NCG + ornithine, REC = ruminal epithelial cells, *t*-BDMS = *t*-butyldimethylsilyl.

### INTRODUCTION

Extensive dietary protein catabolism by rumen microbiota and subsequent ammonia absorption result in decreased efficiency of dietary protein use for productive purposes in ruminant animals. Enhancing rumen microbial protein production, increasing urea recycling to the rumen, optimizing amino acid balance for intestinal absorption, and decreasing first-pass metabolism of absorbed amino acids have been areas of research targeted to improve the net efficiency of N usage in ruminants. Recent studies (Wu, 1995; Mouille et al., 1999; Oba et al., 2004a) indicate that ammonia-N detoxification pathways exist in gut tissues, thus providing another target for nutritional or physiological approaches to reduce ammonia absorption and enhance net efficiency of N use in ruminants.

Our previous study indicated that ruminant gut tissues are capable of synthesizing urea from arginine or from ammonia when stimulated by *N*-carbamoylglutamate (NCG), a stable analog of *N*-acetylglutamate (Oba et al., 2004a). However, that study did not conclusively demonstrate that ammonia-N is assimilated into urea, but rather that urea is net-released by ruminant gut cells. This could have occurred via complete function of the ornithine-urea cycle or by action of arginase on arginine. Nonetheless, our results agreed with work with pig intestinal (Wu, 1995) and rat colonic (Mouille et al., 1999) cells in which ammonia was detoxified to urea and citrulline, respectively. Another potential metabolic route for ammonia detoxification is via amination of keto-acids to form nonessential amino acids (e.g., alanine). Indeed, net absorption of alanine by the portal-drained viscera is

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greater than for other amino acids in sheep (Wolff et al., 1972) and steer (Seal and Parker, 1996), potentially with N derived from absorbed ammonia. To date, however, the metabolic capability of ruminant gut tissues to detoxify ammonia-N via synthesis of citrulline, arginine, urea, or alanine has not been explored.

The overall aim of the present study was to determine metabolic fates of ammonia-N in ruminant gut tissues. Specific objectives were to confirm our previous observations that ruminant gut tissues possess a complete ornithine-urea cycle pathway for de novo synthesis of urea from [<sup>15</sup>N]-ammonia, and to determine whether ammonia-N is assimilated into alanine by ruminal epithelial (REC) and duodenal mucosal cells (DMC) of ruminant sheep. To establish the existence and activity of the pathways leading to urea synthesis, combinations of substrates that contribute to the ornithine-urea cycle pathway (ammonia, NCG, ornithine, aspartate) were provided to cells and the relative partition of [<sup>15</sup>N]-ammonia into alanine, citrulline, ornithine, arginine, and urea determined by gas chromatography-mass spectrometry (GC-MS).

## MATERIALS AND METHODS

### Animals and Cell Isolation

All animal procedures were approved by the Beltsville Agricultural Research Center Institutional Animal Care and Use Committee (protocol #02-008). Ruminal epithelium cells (REC) and duodenal mucosal cells (DMC) were isolated from 4 growing Texel-Polypay crossbred ram lambs purchased from a commercial sheep farm in Maryland. Lambs were housed in individual pens at the USDA-ARS research facility (Beltsville, MD), and fed ad libitum a pelleted diet composed of 55% forage and 45% concentrate (Table 1) for at least 2 wk before slaughter. Daily DM intake and gains, and BW at slaughter were  $1.5 \pm 0.1$  kg/d,  $0.33 \pm 0.11$  kg/d, and  $34.6 \pm 2.9$  kg, respectively. Gut cells were isolated separately for each sheep following the procedures described by Baldwin and McLeod (2000) and Oba et al. (2004b). Cell viability (trypan blue dye exclusion) averaged 79.0% for REC and 81.6% for DMC.

### Incubations

For all experiments, 2 flasks were prepared as time-zero controls to allow correction for endogenous metabolites and for determination of background abundance of <sup>15</sup>N. Ammonium chloride (99 atom % <sup>15</sup>N) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Incubation medium (2.5 mL; Krebs-Ringer plus 25 mM HEPES and 0.12 M sodium bicarbonate)

**Table 1.** Ingredients and nutrient composition of experimental diet (% of dietary DM except for DM).

Ingredient	
Alfalfa hay	55.0
Ground corn	40.0
Soybean meal	3.5
Ammonium chloride	0.5
Premix of salt and trace mineral <sup>1</sup>	0.5
Dicalcium phosphate	0.45
Premix of vitamin A, D, and E <sup>2</sup>	0.05
Chemical composition	
DM	88.8
NDF	34.5
ADF	23.7
CP	15.8
Ether extracts	2.5
Calcium	1.2
Phosphorus	0.42

<sup>1</sup>Premix of salt and trace mineral contains minimum of 92.0% NaCl; 8000 ppm Zn; 5500 ppm Fe; 2400 ppm Mn; 670 ppm Cu; 67 ppm I; 67 ppm Co; and 1.6 ppm Se.

<sup>2</sup>Premix of vitamins contains 5,291 kIU/kg of vitamin A, 1,322 kIU/kg of vitamin D, and 11,023 IU/kg of vitamin E.

was oxygenated with O<sub>2</sub>:CO<sub>2</sub> (95:5) and adjusted to pH 7.4. Incubations were initiated by addition of 0.5 mL of cell suspension ( $1 \times 10^7$  viable cells) to freshly gassed (20 s under 95:5 O<sub>2</sub>:CO<sub>2</sub>) media, and flasks were placed into a reciprocal-action shaking water bath at 37°C. After 60 or 90 min of incubation, 0.2 mL of concentrated HClO<sub>4</sub> was injected into the flasks to terminate the incubation, followed by addition of 0.3 mL of 5.8 M K<sub>2</sub>CO<sub>3</sub> to neutralize the medium.

**Experiment 1.** Primary REC and DMC were incubated in triplicate for 90 min in the presence of 5 mM ammonium chloride ([<sup>15</sup>N] or [<sup>14</sup>N]) and 5 mM glucose. The parallel incubations with unlabelled (<sup>14</sup>N) ammonium chloride were used for determination of unlabelled metabolite concentrations and release rates by an isotope dilution technique (Calder et al., 1999). For these unlabelled incubations, medium was clarified of cellular debris by centrifugation (2300 × g for 7 min), and to a known weight (2 g) of clarified medium was added a known weight (0.5 g) of a solution containing a mixture of tracer standards ([<sup>15</sup>N]glutamate, [<sup>15</sup>N]aspartate, and [<sup>15</sup>N]alanine, each at 250 nmol). For incubations containing [<sup>15</sup>N]ammonium chloride, clarified medium was analyzed for <sup>15</sup>N-containing end-products to determine the contribution of ammonia-N to the synthesis of glutamate, aspartate, and alanine.

**Experiment 2.** Primary REC and DMC were incubated for 60 min in basal medium containing glucose (1 mM), glutamate (1 mM), and [<sup>15</sup>N]ammonium chloride (5, 10, 20, or 40 mM), plus 1 of 4 combinations of substrates to support urea synthesis via the ornithine-urea cycle [control, NCG, NCG + ornithine (ONCG), and ONCG + aspartate (AONCG); 1 mM each]. Glu-

cose and glutamate were included in the basal medium to act as substrates for de novo synthesis of *N*-acetylglutamate, ornithine, and aspartate. The NCG is a stable analog of *N*-acetylglutamate, an allosteric activator of carbamoyl phosphate synthetase (Wu et al., 2004). Treatments were arranged in a 4 × 4 factorial design, and 3 flasks containing no ammonium chloride were prepared for each substrate combination treatment to determine metabolite production rates from nonammonia-N.

**Experiment 3.** The REC and DMC were incubated for 60 min in medium containing 10 mM [<sup>15</sup>N]ammonium chloride plus either 1 mM glucose, 1 mM glutamate, or both substrates, to determine the specific effects of glucose and glutamate on ammonia-N metabolism.

For experiments 2 and 3, a known weight (0.5 g) of a mixture containing tracer standards ([5,5-D<sub>2</sub>]citrulline, [U-<sup>13</sup>C]arginine, [2,3,3,3-D<sub>4</sub>]alanine, and [<sup>13</sup>C, <sup>15</sup>N<sub>2</sub>]urea, each at 125 nmol) was added to a known weight (2 g) of clarified medium for determination of metabolite concentrations and release rates by the isotope dilution technique. To enhance GC-MS measurements at such low substrate concentrations, the tracer standard mixture also contained known amounts of unlabeled citrulline, arginine, alanine, and urea (250 nmol/g each) to raise unlabelled concentrations to within the standard curve range. The amounts of added unlabeled metabolites were, for citrulline, arginine, alanine, and urea, 4-, 0.4-, 1.25-, and 15-fold greater than their production rates, respectively.

### Sample Analysis

Concentrations and [<sup>15</sup>N] enrichments of analytes in the cell-free media were determined by GC-MS (HP6890 coupled to an HP5973 Mass Selective Detector, Agilent, Palo Alto, CA). In experiment 1, samples were applied to an H<sup>+</sup>-form cation exchange resin (Lobley et al., 1995). Isolated amino acids and urea were converted to the *t*-butyldimethylsilyl (*t*-BDMS) derivative (Calder and Smith, 1988) before GC-MS under electrical impact mode. Net incorporation of ammonia-<sup>15</sup>N into metabolites was calculated as the product of [<sup>15</sup>N] enrichment and the metabolite concentration at the end of incubations. In experiments 2 and 3, for citrulline, urea, and alanine determinations, media samples were sequentially applied to a Na<sup>+</sup>-form cation exchange resin to remove arginine (Brosnan et al., 1996) followed by application to an H<sup>+</sup>-form cation exchange resin (Lobley et al., 1995). Isolated citrulline, urea, and alanine were converted to the *t*-BDMS derivative (Calder and Smith, 1988) before GC-MS under electrical impact mode. The 2 cation resin steps were

necessary to separate arginine from citrulline because *t*-BDMS arginine yields the same ion fragments as *t*-BDMS citrulline under electron impact. For arginine determinations, media samples were applied to the H<sup>+</sup>-form cation exchange resin, and the isolated arginine converted to the methyl ester trifluoroacetyl derivative (Castillo et al., 1993) before GC-MS analysis under the chemical ionization mode. Metabolite concentrations were determined by isotope dilution (Calder et al., 1999), and standard curves constructed to account for isotopomer spillover and for determination of [<sup>15</sup>N] enrichment. Fragment ions containing the labeled nitrogen atom from ammonia were monitored for citrulline ([M+1]) and alanine ([M+1]), whereas for arginine and urea, the ions at [M+1] and [M+2] were both monitored to assess the potential incorporations of [<sup>15</sup>N]ammonia via carbamoyl-phosphate and via aspartate.

Data were analyzed separately for REC and DMC using the Fit model procedure of JMP (SAS Institute, Inc., Cary, NC). For experiment 2, the model included ammonia concentration, substrate combination, their interactions as fixed effects, and animal as a random effect. When the main effect of substrate combination was significant, treatment means were compared by *t*-test to determine the effects of NCG addition (NCG, ONCG, and AONCG vs. control), effects of ornithine addition (ONCG and AONCG vs. NCG), and effects of aspartate addition (AONCG vs. ONCG). Furthermore, linear and quadratic effects of ammonia concentrations were also determined. For experiment 3, the model included the fixed effect of substrate and the random effect of animal, and orthogonal contrasts were used to determine the main effects of glucose, glutamate, and the interactions.

## RESULTS

### Experiment 1

For REC, ammonia-N incorporation into alanine, aspartate, and glutamate was 0.52 (±0.23), 0.17 (±0.03), and 0.46 (±0.08) nmol/10<sup>6</sup> cells per 90 min, respectively. Corresponding REC <sup>15</sup>N enrichments (atom % excess) were 0.99% (±0.32) for alanine, 1.28% (±0.21) for aspartate, and 1.87% (±0.19) for glutamate. For DMC, ammonia-N incorporation into alanine, aspartate, and glutamate was 1.22 (±0.38), 0.58 (±0.09), and 1.72 (±0.32) nmol/10<sup>6</sup> cells per 90 min, respectively. Corresponding DMC <sup>15</sup>N enrichments were 11.8% (±2.7) for alanine, 14.1% (±0.7) for aspartate, and 14.0% (±1.2) for glutamate.

### Experiment 2

Ammonia-N incorporation rates into metabolites are expressed in nmol/10<sup>6</sup> cells per 60 min. For REC, use of

ammonia-N for net alanine synthesis increased (from 0.31 to 0.78 nmol;  $P < 0.001$ , quadratic; Table 2) for control as ammonia concentration increased from 5 to 40 mM. No interactions between ammonia-N concentration and substrate treatment were observed. Ammonia-N incorporation into alanine by REC decreased in the presence of NCG compared with the controls ( $P < 0.05$ ; Figure 1). By contrast, ammonia-N was not incorporated into citrulline, arginine, or urea; thus, it appears that ammonia is not a substrate for the ornithine-urea cycle in REC or this pathway is incomplete in REC.

For DMC, use of ammonia-N for net alanine synthesis increased (from 0.73 to 1.35 nmol;  $P < 0.001$ ; quadratic) as ammonia concentration increased from 5 to 40 mM. An interaction between ammonia-N concentration and substrate treatment was not observed. Similar to REC, ammonia-N incorporation into alanine by DMC decreased in the presence of NCG compared with the controls ( $P < 0.001$ ; Figure 2). In contrast to REC, ammonia- $^{15}\text{N}$  was incorporated into citrulline but not into arginine or urea. The latter indicates that DMC may have limited or no activity of arginino-succinate synthetase for completion of the cycle. In the presence of NCG and ornithine, there was a 2-fold higher ( $P < 0.01$ ) incorporation of ammonia- $^{15}\text{N}$  into citrulline compared with when only NCG was provided (0.35 vs. 0.74 nmol; SE = 0.20; Figure 3). However, ammonia- $^{15}\text{N}$  incorporation into citrulline decreased linearly (SE = 0.23;  $P < 0.001$ ) for all treatments when ammonia concentrations were raised from 5 to 40 mM.

### Experiment 3

Ammonia-N incorporation rates into metabolites are expressed in nmol/ $10^6$  cells per 60 min. When REC or DMC were incubated in 10 mM ammonia and in the absence of substrates for the ornithine-urea cycle, ammonia-N assimilation into alanine was higher when 1 mM glucose was present ( $P < 0.01$ ), but not when 1 mM glutamate was present (Table 3). Glucose addition to the medium increased ammonia-N assimilation into alanine by 18% for REC and by 100% for DMC (Figure 4).

## DISCUSSION

Previously, we observed that ovine DMC and REC, incubated in the presence of ammonia, ornithine, and aspartate (5 mM each), net released greater amounts of urea into the medium when NCG was added to the incubations (Oba et al., 2004a). These data suggested, but did not directly prove, that ammonia-N contributes

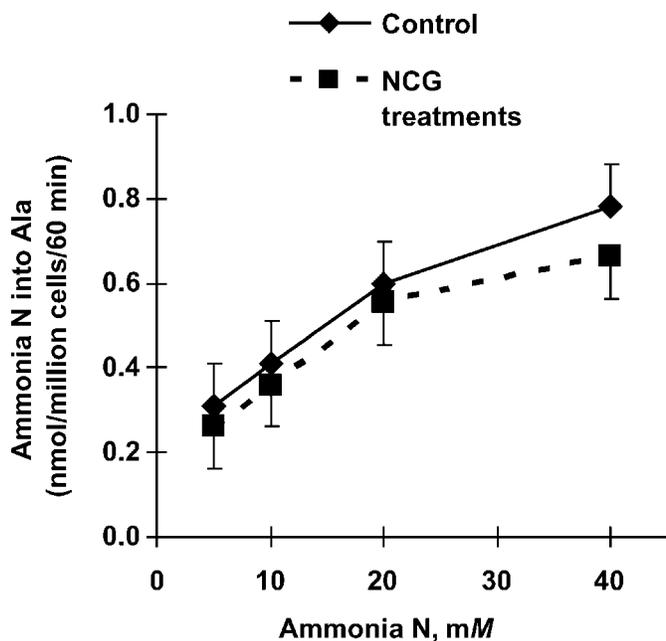
**Table 2.** Ammonia- $^{15}\text{N}$  incorporation into alanine and citrulline (nmol/ $10^6$  cells per 60 min) by ruminal epithelial cells and duodenal mucosal cells incubated with [ $^{15}\text{N}$ ]ammonium chloride (0, 5, 10, 20, or 40 mM) and 1 of 4 combinations of substrates that could support urea synthesis.

Ammonia, mM:	Substrate <sup>1</sup>												P-value							
	Control				NCG				AONCG				Sub	L	Q					
	5	10	20	40	5	10	20	40	5	10	20	40								
Rumen [ $^{15}\text{N}$ ]alanine	0.31	0.41	0.60	0.78	0.28	0.40	0.63	0.68	0.24	0.33	0.52	0.63	0.27	0.35	0.51	0.68	0.10	*	***	***
Duodenum [ $^{15}\text{N}$ ]alanine	0.87	1.16	1.39	1.53	0.76	1.00	1.24	1.34	0.66	0.94	1.21	1.32	0.64	0.89	1.13	1.22	0.45	***	***	***
Duodenum [ $^{15}\text{N}$ ]citrulline	0.48	0.37	0.30	0.25	0.48	0.48	0.31	0.18	0.98	0.83	0.71	0.48	0.76	0.62	0.45	0.16	0.23	***	***	0.48

<sup>1</sup>Substrates: NCG = *N*-carbamoylglutamate, AONCG = NCG + ornithine, AONCG = NCG + aspartate; 1 mM each.

<sup>2</sup>Sub = Effect of combinations of substrate to support urea synthesis; L = effect of ammonia dose (linear); Q = effect of ammonia dose (quadratic).

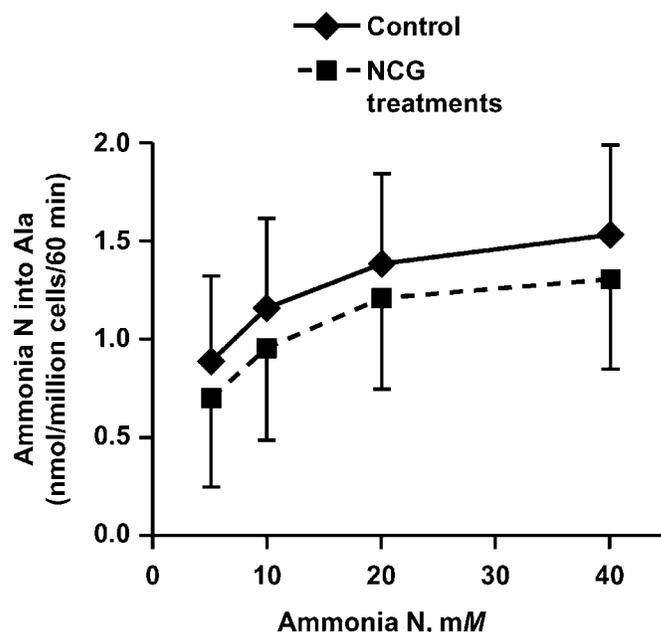
\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



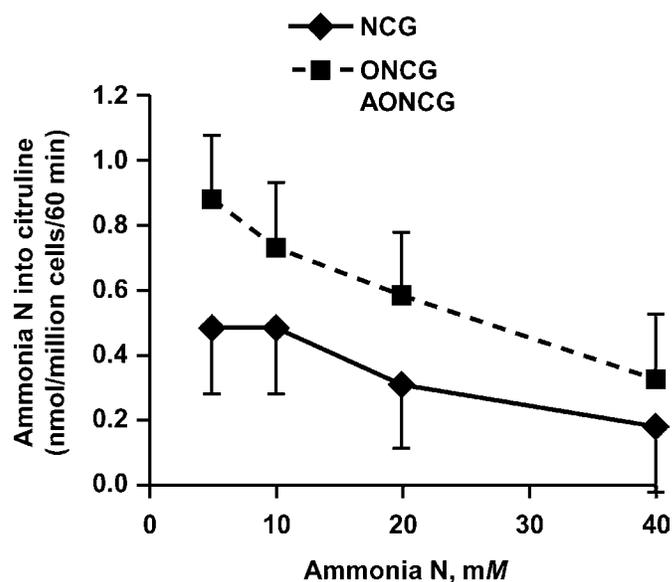
**Figure 1.** Ammonia- $^{15}\text{N}$  incorporation into alanine (nmol/ $10^6$  cells per 60 min) by ruminal epithelial cells incubated with [ $^{15}\text{N}$ ]ammonium chloride (5, 10, 20, or 40 mM) and 1 of 4 combinations of substrates that could support urea synthesis [control, *N*-carbamoylglutamate (NCG), NCG + ornithine (ONCG), ONCG + aspartate (AONCG)]. The comparison of treatments containing NCG (NCG, ONCG, and AONCG) vs. control is shown ( $P < 0.03$ ). Effect of ammonia dose: linear,  $P < 0.001$ ; quadratic,  $P = 0.001$ .

to urea synthesis, and that stimulation of carbamoyl phosphate synthetase by NCG promotes urea synthesis by the ornithine-urea cycle (Oba et al., 2004a). An aim of the current study was to provide direct proof of a complete pathway (i.e., ornithine-urea cycle) for urea synthesis. Herein, we monitored the incorporation of [ $^{15}\text{N}$ ]ammonia into intermediates and products of the cycle. [ $^{15}\text{N}$ ]Ammonia was not found to be incorporated into arginine or urea by DMC or REC; therefore, it appears unlikely that ruminant gut tissues possess a complete ornithine-urea cycle. Rather, our previous observations of urea release by gut cells are probably the result of direct catabolism of arginine derived from cellular protein degradation or from the medium. Indeed, gut tissues of several species, including ruminants, possess significant arginase activity (Aminlari and Vaseghi, 1992), which would yield urea upon catabolism of arginine. What remains inexplicable, however, is our previous observation that NCG stimulated urea production. To our knowledge, there is no evidence in the literature indicating that NCG stimulates protein degradation (yielding arginine) or activates arginase activity.

The current results indicate that certain enzymatic steps of the ornithine-urea cycle are present and that



**Figure 2.** Ammonia- $^{15}\text{N}$  incorporation into alanine (nmol/ $10^6$  cells per 60 min) by duodenal mucosal cells incubated with [ $^{15}\text{N}$ ]ammonium chloride (5, 10, 20, or 40 mM) and 1 of 4 combinations of substrates that could support urea synthesis [control, *N*-carbamoylglutamate (NCG), NCG + ornithine (ONCG), ONCG + aspartate (AONCG)]. The comparison of treatments containing NCG (NCG, ONCG, and AONCG) vs. control is shown ( $P < 0.001$ ). Effect of ammonia dose: linear,  $P < 0.001$ ; quadratic,  $P = 0.001$ .



**Figure 3.** Ammonia- $^{15}\text{N}$  incorporation into citrulline (nmol/ $10^6$  cells per 60 min) by duodenal mucosal cells incubated with [ $^{15}\text{N}$ ]ammonium chloride (5, 10, 20, or 40 mM) and 1 of 4 combinations of substrates that could support urea synthesis [control, *N*-carbamoylglutamate (NCG), NCG + ornithine (ONCG), ONCG + aspartate (AONCG)]. The comparison of ONCG and AONCG vs. NCG is shown ( $P < 0.01$ ). Effect of ammonia dose: linear,  $P < 0.001$ .

**Table 3.** Effects of glucose (1 mM) and glutamate (1 mM) on ammonia-<sup>15</sup>N incorporation into alanine and citrulline (nmol/10<sup>6</sup> cells per 60 min) by ruminal epithelial cells and duodenal mucosal cells.

	Control	Glutamate	Glucose	Both	SE	P-value		
						Glutamate	Glucose	Interaction
Rumen								
[ <sup>15</sup> N]alanine	0.33	0.32	0.36	0.41	0.06	0.45	**	0.14
Duodenum								
[ <sup>15</sup> N]alanine	0.52	0.55	0.98	1.16	0.37	0.33	***	0.45
[ <sup>15</sup> N]citrulline	0.38	0.48	0.45	0.37	0.21	0.77	0.86	0.15

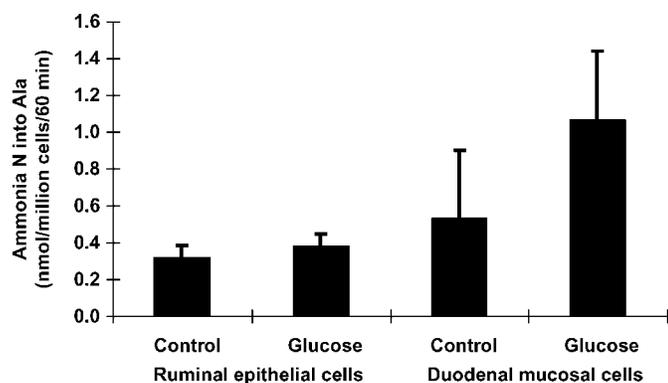
\*\**P* < 0.01; \*\*\**P* < 0.001.

these can be activated or upregulated in ruminant DMC. When NCG and ornithine were included in incubations, ammonia-N assimilation into citrulline was greater compared with when only NCG was provided. This observation suggests that the availability of ornithine may be a limiting factor for citrulline synthesis by ruminant DMC. Ornithine is the direct precursor for citrulline synthesis (Cynober et al., 1995) and, indeed, ornithine availability has been shown to be limiting for citrulline synthesis by enterocytes of pre-weaned piglets (Wu et al., 1994). However, we observed that ammonia-N assimilation into citrulline decreased linearly as ammonia-N concentration increased in the incubation medium. If, as we proposed, citrulline synthesis provides a mechanism to protect against tissue ammonia toxicity, then increased ammonia-<sup>15</sup>N incorporation into citrulline would have been expected. Our results question the role of citrulline synthesis in ammonia-N disposal by ruminant gut tissues. By contrast, Mouille et al. (1999) observed increased citrulline production by isolated rat colonocytes (by 3- to 10- fold) when ammonium chloride was present at 10 and 50 mM. Those data suggested a role for ammonia assimilation into citrulline for ammonia-

N disposal, but only at high concentrations of ammonia. One possible explanation for the differences between studies may relate to our choice of glucose, glutamate, and ornithine as sole substrates for citrulline synthesis, compared with arginine in the study by Mouille et al. (1999). In addition, it has been shown that glutamine is the primary substrate for citrulline synthesis by the small intestines of pigs (Wu et al., 1994) and rats (Windmueller and Spaeth, 1981). Therefore, ornithine produced from catabolism of arginine or glutamine may be channeled preferentially toward citrulline synthesis although DMC apparently do have some metabolic capacity to use exogenous ornithine for citrulline synthesis.

Our results (experiment 1) demonstrated that ammonia-N is assimilated into alanine, aspartate, and glutamate by ruminant gut cells. However, because aspartate and glutamate are net catabolized by the small intestinal mucosa (Wu, 1998), these 2 amino acids were not considered vehicles for ammonia disposal, and we did not determine their net release by cells in experiments 2 and 3. Herein, we specifically evaluated ammonia-N assimilation into alanine based on observations in sheep and cattle that the gut tissues net synthesize alanine for release into the portal circulation (Wolff et al., 1972; Seal and Parker, 1996). Another reason that we did not determine ammonia-N assimilation into glutamate and aspartate in experiments 2 and 3 was that we included these metabolites in incubation media as substrates at concentrations far above those expected to be produced, making the enrichment of these metabolites with <sup>15</sup>N too low to be detected. Nonetheless, greater <sup>15</sup>N enrichment of glutamate compared with that of alanine, found in experiment 1, indicates that glutamate-N contributes to a portion of alanine-N, and that glutamate dehydrogenase may be the first step of ammonia-N assimilation into alanine.

Ammonia-N assimilation into alanine increased in the presence of physiological (gut luminal) concentrations of ammonia (5 to 20 mM; Gustafsson and Palmquist, 1993), supporting our view that net alanine syn-

**Figure 4.** Effects of glucose (1 mM) on ammonia-<sup>15</sup>N incorporation into alanine (nmol/10<sup>6</sup> cells per 60 min) by ruminal epithelial cells (*P* < 0.01) and duodenal mucosal cells (*P* < 0.001).

thesis by the gut tissues is probably an important pathway for ammonia-N disposal. As expected, ammonia-N use for alanine synthesis decreased precipitously when DMC and REC were incubated with NCG. Because NCG is a stable analog of the carbamoyl phosphate synthetase activator *N*-acetylglutamate, it was expected that ammonia-N would be channeled toward carbamoyl phosphate. By contrast, glucose addition to the media increased ammonia-<sup>15</sup>N incorporation into alanine by DMC and REC. Compared with their controls, the stimulatory effect of glucose addition on ammonia-N assimilation into alanine was greater for DMC (+100%) compared with REC (+18%), suggesting that glucose is a primary source of the carbon skeleton for alanine synthesis by DMC. However, it is not possible to determine from the current experiment whether the extent of ammonia-N assimilation into alanine depends on glucose concentration.

The ability to reduce net ammonia-N absorption has the potential to improve the efficiency of nitrogen use by decreasing amino acid oxidation in the liver. Increased urea production by the liver of ruminants is associated with greater removal of  $\alpha$ -amino nitrogen. It has been proposed that the additional removal of amino acids serves to supply nitrogen for urea synthesis via aspartate, whereas absorbed ammonia contributes the second nitrogen in urea via carbamoyl-phosphate (Reynolds, 1992; Parker et al., 1995). Consistent with this hypothesis is the observation by Lobley et al. (1995) that leucine oxidation by the liver, to supply nitrogen via aspartate to urea synthesis, is increased upon infusion of ammonium chloride into the mesenteric vein of sheep. In this respect, alanine synthesis from ammonia by the gut tissues may reduce the ammonia load on the liver, in consequence avoiding the need for oxidation of additional amino acids by the liver.

## CONCLUSIONS

Alanine synthesis may be a more significant pathway for ruminant DMC to detoxify ammonia-N compared with the ornithine-urea cycle when luminal ammonia concentrations reach high levels. In addition, the role of glucose in ammonia detoxification by ruminant gut tissues warrants further investigation to better clarify the specific requirements of these tissues for glucose. Lastly, although DMC were observed to possess the metabolic machinery to incorporate ammonia-N into citrulline, our observations that ammonia-N assimilation into citrulline is decreased when ammonia concentrations are raised puts into question the role of citrulline synthesis, particularly as the pathway citrulline to arginine (and urea) was apparently ab-

sent. The current research was intended to evaluate gut cell metabolism under simple incubation conditions and with single or few nutrient substrates that might otherwise complicate interpretations. Further research is needed, however, to evaluate the biological significance and regulatory mechanisms of ammonia-N metabolism under physiological conditions involving the array of nutrients to which gut tissues are normally exposed.

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