Hepatic detoxification of ammonia in the ovine liver: possible consequences for amino acid catabolism

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The effects of either low (25 μmol/min) or high (235 μmol/min) infusion of NH_4Cl into the mesenteric vein for 5 d were determined on O_2 consumption plus urea and amino acid transfers across the portal-drained viscera (PDV) and liver of young sheep. Kinetic transfers were followed by use of $^{15}NH_4Cl$ for 10 h on the fifth day with simultaneous infusion of [1-^{13}C]leucine to monitor amino acid oxidation. Neither PDV nor liver blood flow were affected by the additional NH_3 loading, although at the higher rate there was a trend for increased liver O_2 consumption. NH_3-N extraction by the liver accounted for 64–70% of urea-N synthesis and at the lower infusion rate the additional N required could be more than accounted for by hepatic removal of free amino acids. At the higher rate of NH_3 administration additional sources of N were apparently required to account fully for urea synthesis. Protein synthesis rates in the PDV and liver were unaffected by NH_3 infusion but both whole-body ($P < 0.05$) and splanchnic tissue leucine oxidation were elevated at the higher rate of administration. Substantial synthesis of [^{15}N]glutamine occurred across the liver, particularly with the greater NH_3 supply, and enrichments exceeded considerably those of glutamate. The [^{15}N]urea synthesized was predominantly as the single labelled, i.e. [$^{14}N^{15}N$], species. These various kinetic data are compatible with the action of ovine hepatic glutamate dehydrogenase (*EC* 1.4.1.2) in periportal hepatocytes in the direction favouring glutamate deamination. Glutamate synthesis and uptake is probably confined to the perivenous cells which do not synthesize urea. The implications of NH_3 detoxification to the energy and N metabolism of the ruminant are discussed.

Ammonia: Ureagenesis: Liver: Amino acids: Sheep

During growth, conversion of feed-N into animal protein is a relatively inefficient process in all mammals, including ruminants (Lobley, 1992), with a substantial portion eliminated as urea synthesized in the liver. There are several roles for ureagenesis, including removal of amino acid-N surplus to anabolic requirements (see Meijer *et al.* 1990) and regulation of acid–base balance (Atkinson & Bourke, 1984). One additional function involves removal of gastrointestinal-tract (GIT)-derived NH_3 such that the peripheral concentrations are sufficient to provide substrate for reactions such as synthesis of nucleic acid bases but below those toxic to the central nervous system (Summerskill & Wolpert, 1970). NH_3 removed from the portal vein by the liver enters the ornithine (urea) cycle directly by condensation with mitochondrial CO_2 to form carbamoyl phosphate under the regulated action of carbamoyl phosphate synthetase (CPS; *EC* 6.3.4.16; reviewed by Meijer *et al.* 1990). This only supplies one N atom to urea with the other contributed by cytoplasmic aspartate. The immediate N-donor to aspartate is probably glutamate which is involved in a wide range of transamination reactions and although, in theory, many metabolites may contribute to urea synthesis probably only amino acids are absorbed in quantities sufficient to provide the additional N.

NH_3-stimulated ureagenesis may, therefore, require input of amino acid-N with consequent penalties on protein gain. Some evidence to support this comes from studies where the relationships between urea production and hepatic removal of NH_3 and amino acids have been compared. In cattle, for example, the potential contribution of extracted NH_3-N to urea-N release ranges from 0·27 to 0·88 (Wilton *et al.* 1988; Fitch *et al.* 1989; Huntington, 1989; Maltby *et al.* 1991; Reynolds *et al.* 1991*b*) while in response to increased NH_3 extraction more free amino acids are apparently removed by the liver, with the quantities involved either sufficient (Huntington, 1989), inadequate (Reynolds *et al.* 1991*b*) or in excess (Maltby *et al.* 1991) as supplies of the additional N for ureagenesis. For cattle fed on rations based largely on forages there is greater portal absorption and hepatic removal of NH_3 which is accompanied by extra extraction of amino acids across the liver (Huntington, 1989; Maltby *et al.* 1991; Reynolds & Tyrrell, 1991; Reynolds *et al.* 1991*b*). Such a mechanism may contribute to the poorer N retention often observed for ruminants offered roughage as opposed to concentrate diets.

With the exception of Wilton *et al.* (1988), however, all the above experiments have involved changes in either diet quality or quantity and, thus, factors other than NH_3 absorption may have been altered. Furthermore, these results are based on mass transfers across the liver and do not necessarily reflect the actual movement and fate of NH_3-N. Thus the greater hepatic uptake of amino acids in response to increased NH_3 removal may not automatically result in elevated amino acid catabolism because return to the circulation as other forms, e.g. peptides, proteins, oxo-acids, may occur.

It is also possible for NH_3-N to be transferred to aspartate, and thus contribute both N atoms to urea, by, for example, the action of glutamate dehydrogenase (GLDH; *EC* 1.4.1.2; amination of 2-oxoglutarate) and under such circumstances the need to invoke additional N (from amino acids) would be unnecessary. Were this or similar mechanisms to occur then, following administration of an $^{15}NH_3$ source, the relative fluxes of NH_3-N transfer into urea can be assessed from the $[^{14}N^{14}N]:[^{15}N^{15}N]$urea ratios produced. On the other hand, if NH_3 detoxification does require net input from essential amino acids then associated irreversible oxidation of their C skeletons should occur with a consequent penalty on peripheral protein anabolism. The present study examines the hypotheses that, first, NH_3 detoxification in sheep involves catabolism of amino acids by examining oxidation of [1-^{13}C]leucine and, second, that the claimed normal direction of the GLDH reaction, towards 2-oxoglutarate formation, precludes the synthesis of urea in which both N atoms are derived from NH_3. The experimental model involves sheep offered a pelleted lucerne (*Medicago sativa*) diet with chronic supply of NH_3 to the liver augmented by 50% through intramesenteric vein infusion. Consequent changes in bioenergetics plus the net mass and isotopic transfers of $^{15}NH_3$ and products were monitored.

MATERIALS AND METHODS

Animals and surgical preparations

Four Suffolk cross wether lambs (30–35 kg live weight (LW), 6–9 months old) were prepared with indwelling catheters in the aorta, portal, hepatic and mesenteric (2) veins under general anaesthesia with halothane–nitrous oxide. Briefly, the surgical procedure followed that described by Katz & Bergman (1969*a*) except that the hepatic portal vein catheter was inserted via the liver similar to the hepatic vein procedure and thus was retrograde to the flow. For the mesenteric vein catheters direct insertion by the Herd & Barger (1964) method, as modified by Fleet & Mepham (1983), was performed. All catheters were a combination of silicone rubber for the portion inside the vessels and polyvinyl chloride (PVC), thereafter. This reduced thrombogenicity and yet avoided the

diffusion of gases which occurs with porous silicone rubber when exteriorized. Full details of the surgical procedure and catheter preparation are available on request. Catheters were sterilized with ethylene oxide before surgery. Sheep were allowed 3 weeks recovery before commencement of experimental observations and during this period appropriate veterinary treatment of antibiotics and analgesics was administered. Catheters (PVC) were inserted into each external jugular vein as described previously (Harris *et al.* 1992) before each experimental period.

Diets and design

Animals were adjusted to metabolism cages and offered 800 g lucerne pellets/d (10·5 MJ metabolizable energy/kg dry matter (DM); 25 g N/kg DM; DM = 0·89), supplied as twenty-four 1 h portions from automated feeders.

For each sheep the design involved 5 d of a low NH_4Cl infusion (0·4 M; 25 μmol/min) into the mesenteric vein. This was followed after 3 d by a 5 d high NH_4Cl infusion (2·5 M; 235 μmol/min). Measurement of $^{15}NH_3$ and [1-^{13}C]leucine kinetics were performed on day 5 of each level of infusion. The low and high NH_4Cl infusions represented approximately 0·06 and 0·55 of estimated NH_3 absorption across the GIT. The design was not randomized because of initial uncertainty as to catheter patency under the increased NH_3 supply.

On the fifth day the natural abundance NH_4Cl infusate was replaced by a solution containing sodium *p*-aminohippurate (PAH; 8 g/200 ml) plus either, for the low NH_3 infusion, 0·9 g $^{15}NH_4Cl$ (98·4 atoms %; Isotec Inc., Miamisburg, OH, USA) or, for the high NH_3 infusion, 1·86 g enriched plus 6·05 g natural abundance NH_4Cl for the first two sheep. These amounts were adjusted to 0·96 g and 6·95 g for the higher rate of infusion for the latter pair of animals on the grounds of economy; it had been established that adequate enrichments of the main metabolites would still be achieved. These solutions were infused for 10 h (20 g/h). At the same time 200 g of a solution containing 0·5 g [1-^{13}C]leucine (99 atoms %, Tracer Technologies Ltd, Somerville, MA, USA) and 50000 IU sodium heparin in sterile saline (9 g NaCl/l) was infused into the jugular vein, also at a rate of 20 g/h.

Blood samples were withdrawn continuously between 5 and 9 h of stable isotope infusion from the aorta, hepatic and portal veins by means of a peristaltic pump set to collect at 10 ml/h. The collection lines were passed through ice, to reduce risk of coagulation, and blood pumped directly into 10 ml syringes stored in ice-water. Samples were collected hourly.

Measurements of respiratory gas exchange under the basal dietary conditions were performed in an automated confinement respiration chamber (Blaxter *et al.* 1972) over three successive days and within 7 d of the second infusion period.

Analytical measurements

The contents of each syringe were mixed thoroughly by hand-rolling before any sample removal. Blood pH, pCO_2, pO_2 and haemoglobin concentration were measured immediately with a Radiometer ABL3 Blood Gas Analyzer (Radiometer, Copenhagen, Denmark) and packed cell volume was determined by haematocrit. Two 0·7 ml portions were then stored for measurement of blood CO_2 enrichment, as described previously (Harris *et al.* 1992). Blood (5 ml) was then centrifuged at 1300 ***g*** for 15 min to prepare plasma. NH_3 concentration in plasma was determined enzymically (Mondzac *et al.* 1965) using a Kone Dynamic Selective Analyzer (Kone Instruments, Espoo, Finland). All subsequent operations were then performed gravimetrically. To 1 g blood was added 11·5 g trichloroacetic acid (100 g/l) and this was analysed for PAH (Harris *et al.* 1992) and involved an acid deacetylation step at 90° for 2 h. The completeness of the reaction was confirmed by tests with synthesized *N*-acetyl-PAH (results not shown).

The remaining blood was then haemolysed by the addition of an equal weight of a solution of 300 μM-L-norleucine and 30 μM-4-methyl-2-oxo-pentanoate (MOP). NH_3 concentration and enrichment were determined by a procedure similar to that of Nissim *et al.* (1985). The glutamate content, and thus the amount of NH_3 present in the blood sample, was determined, relative to the known additions of L-norleucine and L-aspartate, by HPLC analysis based on pre-column derivatization with *o*-phthalylaldehyde (Turnell & Cooper, 1982) of 0·25 ml of the extract while the remainder was used for enrichment analysis by gas chromatography–mass spectrometry (GCMS). Urea concentrations were determined on the haemolysed blood by a Technicon automated procedure based on that of Marsh *et al.* (1965). Approximately 1·4 g haemolysed blood was freeze-dried and then resuspended in 0·5 ml 70 g/l sulphosalicylic acid, centrifuged and the supernatant fraction clarified through 0·22 μm nylon filters using a microcentrifuge filter system (Sigma Chemical Co., Poole, Dorset). Approximately 80 μl filtrate was analysed for amino acid concentration using an Alpha Plus Amino Acid Analyzer (Pharmacia-LKB Biochrom Ltd, Cambridge, Cambs) using lithium citrate buffers appropriate for analysis of physiological fluids. All values were corrected to the initial gravimetric addition of L-norleucine. Results are not presented for glycine as the high concentration in blood (600 μM) saturated the ninhydrin reaction at loadings preferred for the other amino acids. The arginine values also need to be treated with caution as arginase (*EC* 3.5.3.1) activity released from within the erythrocyte by haemolysis causes rapid reduction in the concentration of that amino acid.

Determinations of enrichment (as molar % excess; mpe) of blood $^{13}CO_2$ by isotope-ratio-mass-spectrometry and leucine and MOP by GCMS were as reported previously (Harris *et al.* 1992). From the same blood sample used for leucine analysis the enrichments of urea, aspartate, alanine, glutamate, serine and glycine were analysed as the M-57 fragments from tertiary butyldimethylsilyl derivatives on a Hewlett Packard 5989A MS Engine (Hewlett Packard, Avondale, PA, USA). Enriched standards were prepared from both [^{13}C] and [$^{15}N_2$]urea (99 atoms %; MSD Isotopes, Montreal, Canada) and appropriate correction factors determined for the additional contribution to m+2 m/z ions expected from increases in the m+1 ion. Calibrations for the amino acids were based on standards prepared from [^{15}N]glutamic acid (Sigma Chemical Co.).

Calculations and statistics

Blood flow (BF) was calculated as described by Katz & Bergman (1969*b*), corrected where necessary to plasma flow (PF) based on packed cell volume. Blood O_2 content was calculated from haemoglobin content and saturation, the latter derived from equations based on the data of Bartels & Harms (1959) for sheep. Plasma bicarbonate concentration was calculated from pH and pCO_2 data (Siggaard-Anderson, 1974).

Mass transfers of metabolites, M, across the PDV blood circulation (or plasma by use of PF rather than BF) were calculated from

$$(M_p - M_a) \times BF_{pv},$$

where the subscripts *a* and *p* represent concentrations related to the artery or portal vein respectively and *pv* is portal-vein flow. Positive values indicate net appearance into the portal circulation and negative values represent removal by the PDV tissues.

Similarly hepatic mass transfers were calculated from

$$(M_h \times BF_{hv}) - (M_p \times BF_{pv}) - (M_a \times BF_{ha}),$$

where *h* represents hepatic vein concentration and *hv* and *ha* are the flows in the hepatic vein and artery respectively.

Net isotope movements were determined across the PDV and liver by respectively

$$(M_p \times E_p - M_a \times E_a) \times BF_{pv}$$

and

$$(M_h \times E_h \times BF_{hv}) - (M_p \times E_p \times BF_{pv}) - (M_a \times E_a \times BF_{ha}),$$

where E is the enrichment of the metabolite (as mpe above the background natural abundance sample) in the appropriate blood sample.

Protein synthesis calculations based on leucine kinetics followed the principles used by Harris *et al.* (1992) for whole-body irreversible loss rate (ILR; flux) and tissue transfers. Because the isotope infusions were conducted separately from the respiration chamber measurements there was no direct determination of whole-body leucine oxidation (Harris *et al.* 1992). Instead the increase in arterial blood bicarbonate mpe during the infusion was multiplied by whole-body CO_2 production to give an estimate of the fraction of the infused leucine that was oxidized and this was then used to partition ILR into that due to amino acid oxidation and, by difference, that for protein synthesis (Harris *et al.* 1992). Across the PDV and liver the net isotope transfers from the blood for MOP and for plasma CO_2 were each subtracted from that for leucine for each tissue, i.e. for PDV tissues

$$= (L_p \times E_{lp} - L_a \times E_{la}) - (O_p \times E_{op} - O_a \times E_{oa}) \times BF - (C_p \times E_{cp} - C_{ca} \times E_{ca}) \times PF,$$

where $L_{(l)}$, $O_{(o)}$ and $C_{(c)}$ represent leucine, MOP and CO_2 concentrations (enrichments) respectively. Respiratory quotient values across the liver were below theoretical physiological limits due to loss of hepatic bicarbonate as urea C and in bile fluids so CO_2 exchange was calculated from $0{\cdot}9 \times O_2$ uptake. For liver net isotope transfers

$$= (L_h \times E_{lh} + O_h \times E_{oh}) \times BF_h - (L_p \times E_{lp} + O_p \times E_{op}) \times BF$$
$$- (L_a \times E_{la} + O_a \times E_{oa}) \times BF_a - (C_h \times E_{ch} \times PF_h - C_p \times E_{cp} \times PF_p - C_a \times E_{ca} \times PF_a).$$

The resultant figure was divided by the free leucine enrichment in either the arterial blood or that of free leucine or MOP in the appropriate venous drainage (portal or hepatic vein respectively) to provide estimates of tissue protein synthesis. The sections of the above equations involving CO_2 when divided by the enrichment of MOP in portal or hepatic venous blood provided estimates of leucine oxidation across the PDV and liver respectively.

Data were subjected to analysis of variance, with animals treated as blocks, for the effect of rate of NH_3 administration. All data were complete (eight values) for all the main measurements but only three residual degrees of freedom were available with the design adopted. This should be borne in mind when biological trends, rather than statistically significant data, are discussed.

RESULTS

Catheter patency

No problems were encountered with the health of the animals during the study and all completed the experiments on schedule. The most troublesome catheter was that in the more distal part of the mesenteric vein and which remained patent throughout for only one sheep. The other catheter in this vessel always remained operative, however, and was therefore used as necessary in the other animals for infusion of NH_4Cl and PAH. Because the data for mesenteric-drained viscera (MDV) are incomplete these are excluded from the current presentation. In one other animal the portal vein catheter caused occasional problems and this appeared to be related to posture because if the sheep was maintained standing, by means of a loose body sling, continuous sampling was possible. At post-mortem the tip of this catheter was discovered to have a thrombus alongside and in one other animal a similar, but smaller, reaction was noted. This may be related to the

Table 1. *Effect of 4 d chronic low (25 μmol/min) or high (235 μmol/min) infusion of ammonium chloride into the mesenteric vein of four lambs fed with 800 g lucerne pellets/d on arterial blood pH, pCO_2, bicarbonate concentration and on blood flow and oxygen consumption across the portal-drained viscera (PDV) and the liver**

	Low NH_3	High NH_3	SED	P†
Arterial blood variables				
ph	7·444	7·364	0·0114	0·006
pCO_2 (mm Hg)	33·4	33·2	2·80	0·937
$[HCO_3]$ (mM)	26·3	21·6	2·28	0·131
Blood flows (g/min)				
Portal vein	1648	1696	136·0	0·750
Hepatic vein	1681	1804	223·7	0·621
Hepatic artery	33	108	91·6	0·621
Oxygen consumption (mmol/min)‡				
PDV	1·67	1·69	0·157	0·920
Liver	1·54	1·84	0·422	0·535
% Whole-body O_2 consumption§				
PDV	20·4	23·0	1·53	0·238
Liver	19·3	26·1	4·31	0·255

SED, Standard error of the difference between means.
* For details of procedures, see pp. 668–670.
† Based on one-way analysis of variance, 3 residual degrees of freedom.
‡ Calculated from pO_2 and blood haemoglobin content (see p. 670).
§ Whole-body oxygen uptake from respiration chamber measurement.

positioning of the catheter against the flow because in all four sheep the hepatic vein catheter was impeccable and no signs of reaction were observed at post-mortem examination. One advantage of the method of portal catheter insertion was that it was positioned in the tributary of the sampled lobe and would reduce the possibility of streaming and consequent non-mixing effects of direct insertion into the common portal vein. The surgical procedure adopted also meant that portal and hepatic vein catheters were in the same lobe of the liver.

Blood variables and gaseous exchange

Blood flows were unaltered by the NH_4Cl loading (Table 1) and the mean portal vein flows were, at 49 g/min per kg LW and 118 g/min per kg $LW^{0·75}$, similar to those reported for sheep fed close to maintenance level (32–43 ml/min per kg LW, 80–118 ml/min per kg $LW^{0·75}$; Katz & Bergman, 1969*b*; Webster *et al.* 1975; Pell *et al.* 1986; Burrin *et al.* 1989; Ortigues *et al.* 1994). The contribution of the hepatic artery to hepatic venous flow was lower, at an average of 4% (range 0·5 to 12%), than observed in other studies (12–22%; Katz & Bergman, 1969*b*; Pell *et al.* 1986; Burrin *et al.* 1989; Ortigues *et al.* 1994). If the deacetylation step was omitted then portal flow was unaltered but hepatic venous flow increased and thus the arterial contribution was raised to a greater and more consistent value of 20% (results not shown). Arterial and portal venous blood samples showed a mean increase of 16% in chromaphore after deacetylation by hot acid treatment, but with a slightly larger elevation (19%) for the hepatic venous samples. This is greater than the 8 and 12% changes observed by Katz & Bergman (1969*b*) but consistent with their finding of substantial hepatic synthesis and release of *N*-acetyl-PAH, which is insensitive to the Bratton–Marshall reaction (Smith *et al.* 1945). It should be noted that initial analyses based

Table 2. *Effect of 4 d chronic low (25 μmol/min) or high (235 μmol/min) infusion of ammonium chloride in the mesenteric vein of four lambs fed with 800 g lucerne pellets/d on whole-body and splanchnic tissue leucine and protein metabolism**

	Low NH_3	High NH_3	SED	P†
Whole-body kinetics				
Arterial leucine (μmol/l)	155	149	3·7	0·178
Flux‡ (μmol/min)	99·3	100·5	4·02	0·895
	(103·3)	(103·7)	(4·14)	0·938
Leucine oxidation§ (μmol/min)	17·3	20·4	0·57	0·032
	82·1	80·2	3·27	0·971
Protein synthesis‖ (μmol/min)	82·1	80·2	3·27	0·971
(g/d)	(235)	(229)		
Splanchnic leucine kinetics (μmol/min)¶				
Net leucine transfers				
PDV	23·9	23·1	7·05	0·919
HEP	−11·3	−15·1	6·32	0·588
Protein synthesis				
$PDV_{a,l}$	25·8	21·5	4·72	0·432
$PDV_{v,l}$	32·2	27·1	6·08	0·465
$PDV_{m,v}$	25·3	21·9	4·84	0·530
$HEP_{a,l}$	9·3	8·5	3·41	0·840
$HEP_{v,l}$	13·0	12·1	4·67	0·856
$HEP_{m,v}$	9·3	8·6	3·28	0·839
Oxidation				
PDV	1·3	3·3	1·03	0·143
HEP	4·3	5·3	1·92	0·659

MOP, 4-methyl-2-oxo-pentanoate; PDV, portal-drained viscera; HEP, hepatic; SED, standard error of the difference between means.

* For details of procedures, see pp. 668–671.

† Based on one-way analysis of variance, 3 residual degrees of freedom.

‡ Based on enrichment of arterial MOP; values in parentheses based on blood free leucine enrichment.

§ Based on arterial $^{13}CO_2$ enrichment (adjusted to standard [1-^{13}C]leucine infusion rate) and CO_2 production determined by indirect calorimetry.

‖ Based on MOP (or leucine) flux − leucine oxidation and then corrected for leucine mass (131), content in body protein (66 g leucine/kg body protein) and time.

¶ For details of calculations see pp. 670–671. The subscripts $_a$ and $_v$ refer to arterial and venous blood while $_m$ and $_l$ detail whether the calculations are based on MOP or leucine enrichments.

on the volumetric approach pioneered by Katz & Bergman (1969*b*) tended to yield greater calculated arterial flows, due to a systematic bias associated with transfer and evaporative losses. To avoid such problems and generally to improve analytical precision gravimetric procedures were adopted for the present analysis and as a general approach throughout the study.

O_2 consumptions across both the PDV and liver each contributed between 19 and 26% of whole-body values and although these were not significantly different between the treatments there was a trend towards higher values, particularly for liver, at the increased NH_3 infusion (Table 1).

Initial tests had indicated that the NH_4Cl loading would cause only a transient alteration in blood pH but for all four experimental animals there was a chronic depression, yielding mild acidosis, with an average decrease of 0·08 pH units in arterial blood (Table 1). There was no consistent effect of the NH_4Cl load on pCO_2 but the calculated arterial plasma bicarbonate concentration was decreased substantially (> 5 mM) in three of the sheep (Table 1).

Leucine kinetics

Whole-body leucine flux was unaltered by the NH_3 loading, as was estimated protein synthesis (Table 2). There was a significant increase, however, in the absolute amount of leucine oxidized at the higher NH_3 infusion (Table 2). Net leucine PDV absorption, corrected for transfers between MOP and into CO_2, was not altered by infusion of extra NH_3 and slightly exceeded total leucine oxidation (Table 2), which would allow a small net anabolism. Net transfers of MOP across either the PDV or the liver did not exceed ± 1 μmol/min (results not shown). The contributions of the PDV and liver to total leucine oxidation were, on average, 12 and 25% respectively (Table 2). Approximately 50% of the apparent net absorbed leucine was removed across the liver and, of this, one-third was subsequently oxidized within the liver. Although none of these variables was significantly different between the two NH_3 infusions, almost all of the difference in whole-body leucine oxidation could be accounted for by the increased catabolism across the splanchnic bed (Table 2).

Absolute rates of tissue protein synthesis, calculated from net disappearance of leucine label, depended on which metabolite enrichment was selected as most representative of the precursor for protein synthesis. Similar values were obtained when either arterial blood free leucine or venous blood MOP was selected but these were lower than the estimates based on venous blood free leucine (Table 2). Regardless of which was selected there were no significant differences in either GIT or liver protein synthesis as a result of additional infusion of NH_3 (Table 2). Furthermore, hepatic protein synthesis was only 35–40% that of the PDV tissues.

Amino acid transfers (*Table 3*)

Arterial blood concentrations of circulating amino acids were unaltered by the two levels of NH_4Cl infusion (results only shown for leucine; Table 2). Similarly, net PDV appearances were not different for either the essential or non-essential amino acids, and all amino acids except glutamine showed a positive appearance (i.e. net absorption). At the lower NH_3 infusion, appearance of amino acid N in the portal vein was equivalent to 7·5 g N/d, approximately 42% N intake. Hepatic removal of total free amino acid-N was also not significantly different between the low and high NH_3 treatments and represented 50% of the net absorption into the portal vein. Amino acids varied considerably as to the proportion absorbed that was removed by the liver; of the essentials, low extractions were observed for the branched chains and threonine but high for lysine, phenylalanine, histidine and methionine. Of the non-essential residues absorbed, alanine was almost completely extracted by the liver while there was net hepatic release of glutamate and aspartate.

Urea and ammonia transfers (*Table 4*)

PDV NH_3-N flows, corrected for the amount of NH_4Cl infused, were 427 (low) and 424 (high) μmol/min, equivalent to 8·6 g N/d or 48% of apparent N intake. Urea was removed across the PDV at quantities of 56% and 50% of hepatic release at the low and high NH_3 infusions respectively. If allowance is made for this additional N input into the GIT, NH_3 appearance in the PDV would account for 10% of ingested N. The NH_3-N plus free amino acid-N appearance in the portal vein, at the lower NH_3 infusion, accounted for 790 μmol/min and when this is corrected for the 352 μmol/min urea-N extracted into the GIT the remainder yields an apparent N digestibility of 0·50. This compares with a value of 0·54 observed with a similar diet previously (Nash *et al.* 1994) but the current calculation does not include other absorbed sources of N, e.g. nucleic acids and amino sugars, which would increase the value. Arterial plasma concentrations of NH_3 were greater than those in the hepatic vein, indicative of both peripheral tissue NH_3 release and that hepatic removal of NH_3 always slightly exceeded PDV appearance.

Table 3. *Effect of 4 d chronic low (25 μmol/min) or high (235 μmol/min) infusion of ammonium chloride in the mesenteric vein of four lambs fed with 800 g lucerne pellets/d on apparent net free amino acid movements across the portal-drained viscera (PDV) and hepatic bed**

	Thr	Val	Met	Iso	Leu	Phe	Lys	His	Asp
Movement (μmol/min)									
PDV									
Low ammonia	22·2	23·8	2·2	16·1	23·9	16·8	22·5	7·9	20·3
High ammonia	19·9	20·0	4·3	18·2	23·1	13·6	21·5	6·9	8·9
SED†	8·7	9·4	2·3	4·5	7·1	2·3	5·1	2·5	5·9
Hepatic									
Low ammonia	−9·7	−11·3	−3·7	−4·2	−11·3	−11·9	−10·3	−5·8	18·4
High ammonia	−8·7	−7·7	−3·2	−5·4	−15·1	−13·0	−17·6	−11·0	9·1
SED†	6·4	2·63	1·5	2·5	6·3	2·4	6·8	3·6	4·4
Mean fractional removals§	0·44	0·43	1·21	0·28	0·44	0·83	0·64	1·16	‡

	Ser	Asp NH_2	Glu	Glu NH_2	Ala	Tyr	Arg	Pro
Movement (μmol/min)								
PDV								
Low ammonia	19·3	14·5	13·3	−27·7	40·8	11·6	6·0	36·8
High ammonia	23·4	12·6	31·0	−14·7	50·3	8·3	5·1	67·9
SED†	4·5	3·8	23·8	7·7	10·8	4·4	6·1	52·6
Hepatic								
Low ammonia	−13·6	−10·7	20·6	−16·0	−43·2	−10·2	−6·0	−27·0
High ammonia	−15·7	−5·2	16·1	−6·1	−44·2	−6·8	−2·4	−14·3
SED†	6·5	3·1	3·2	7·8	14·0	3·3	6·8	8·4
Mean fractional removals§	0·69	0·58	‡	0·44	0·97	0·85	0·74	0·47

SED, Standard error of the difference between means.
* For details of procedures, see pp. 668–671.
† No significant effect for any treatments.
‡ Positive release of glutamate and aspartate across the hepatic bed.
§ (Net hepatic removal)/(net PDV appearance) ratio.

Table 4. *Effect of 4 d chronic low (25 μmol/min) or high (235 μmol/min) infusion of ammonium chloride in the mesenteric vein of four lambs fed with 800 g lucerne pellets/d on ammonia and urea transfers across the portal-drained viscera (PDV) and hepatic bed**

Blood concentrations	Low NH_3	High NH_3	SED	P†
Ammonia (μmol/l)				
Arterial	99	127	10·6	0·079
Portal vein	379	505	15·0	0·003
Hepatic vein	93	110	23·8	0·524
Urea (mmol/)				
Arterial	3·60	4·63	0·197	0·014
Ammonia flows (μmol/min)				
PDV	452	659	4·7	< 0·001
Hepatic	−450	−664	25·4	0·003
Urea flows (μmol/min)				
PDV	−176	−258	123·3	0·544
Hepatic	314	516	73·4	0·071
Apparent hepatic N transfers flows (μmol/min)				
NH_3N+AA-N	694	920	122·1	0·161
Urea-N	628	1032	146·7	0·071
Balance	65	−134	96·4	0·130

AA-N, amino acid nitrogen; SED, standard error of the difference between means.
* For details of procedures, see pp. 668–671.
† By one-way analysis of variance, 3 residual degrees of freedom.

Arterial blood concentrations of urea increased by 29% ($P < 0·02$) during the high NH_3 infusion. Concomitantly, hepatic release of urea-N was also increased (+404 μmol/min, $P = 0·071$) by nearly twice the additional NH_3-N extracted by the liver.

The sum of NH_3-N and apparent free amino acid-N removal across the liver exceeded urea-N production during low NH_3 infusion but there was an 11% deficiency when the NH_3 load was increased.

^{15}N transfers

During the course of the $^{15}NH_3$ infusion urea became progressively more enriched and by 10 h had still not achieved a 'plateau' value (Fig. 1). From the change in enrichment over the past 4 h a value for the biological half-life of just over 6 h was derived. Of the [^{15}N]urea molecules synthesized at least 97% were as the single-labelled, i.e. [$^{14}N^{15}N$], form and the amounts of double-label [$^{15}N^{15}N$]urea present were close to the detection limits of the GCMS. The majority of ^{15}N recovered across the liver was as urea (93·5 (SE 0·8)%) and over the last 4 h this exceeded (by 12%) the amount of label infused as NH_3 during the same period. This suggests that ^{15}N recycling was occurring, possibly as labelled urea entering the GIT which is then reabsorbed as $^{15}NH_3$ or as [^{15}N]glutamine released from either the liver or muscle.

Some ^{15}N was incorporated into free amino acids, but the enrichments for alanine, glycine, serine, aspartate and glutamate was less than 1 mpe (Table 5). Isotopic activity was elevated ($P < 0·05$) for alanine and glutamate under high NH_3 administration. Glutamine showed the most substantial enrichments at both rates of NH_3 administration but with > 95% as the single-label form.

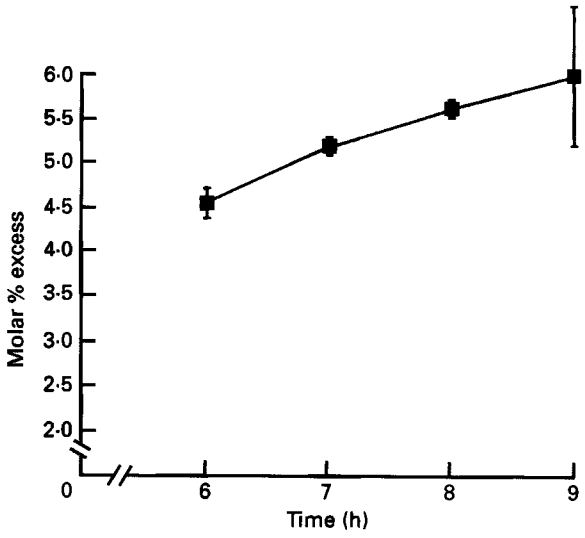

Fig. 1. Temporal changes in arterial urea molar % excess during infusion of $^{15}NH_3$ into the mesenteric vein of four sheep. Values are means for high and low ammonia infusions combined (n 8), with standard deviations indicated by vertical bars. For the first three time points data are normalized to the final time point values. For details of procedures, see pp. 668–671.

Table 5. *Arterial amino acid enrichments (mpe) and net ^{15}N-transfers (μmol ^{15}N/min) across the portal-drained viscera (PDV) and liver of four sheep infused with either low (25 μmol/min) or high (235 μmol/min) ammonium chloride into the mesenteric vein for 4 d and offered 800 g lucerne pellets/d**

	Alanine	Glycine	Serine	Aspartate	Glutamate	Glutamine
Enrichments (mpe)						
Low ammonia	0·24	0·26	0·22	0·15	0·38	1·44
High ammonia	0·34	0·34	0·42	0·22	0·64	3·05
SED	0·029	0·078	0·045	0·079	0·081	0·468
P†	0·042	NS	0·067	NS	0·052	0·041
^{15}N-transfers (μmol ^{15}N/min)						
PDV						
Low ammonia	0·040	−0·156	−0·042	−0·017	0·148	−0·788
High ammonia	0·122	−0·123	−0·022	0·008	−0·194	−0·325
SED	0·032	0·941	0·020	0·028	0·067	0·142
P†	0·014	NS	NS	NS	0·053	0·047
Liver						
Low ammonia	−0·025	0·284	0·068	0·024	0·415	0·104
High ammonia	0·028	0·582	0·139	0·112	0·863	1·887
SED	0·015	0·195	0·054	0·052	0·298	0·968
P†	NS	NS	NS	NS	NS	NS

SED, Standard error of the difference between means; NS, not significant.
* For details of procedures, see pp. 668–671.
† By one-way analysis of variance, 3 residual degrees of freedom.

Removal of [^{15}N]glutamine occurred across the PDV, in common with the net movements, but, in contrast, there was net output of labelled glutamine across the liver

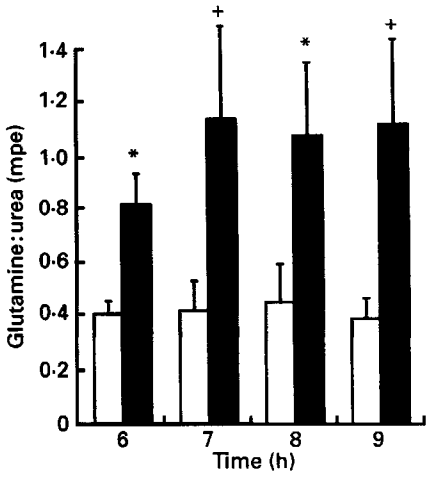

Fig. 2. Ratios of atom % excess (mpe) enrichments of glutamine and urea from hepatic venous blood with time, during low (25 μmol/min, □) or high (235 μmol/min, ■) infusions of $^{15}NH_4Cl$ into the mesenteric vein of four sheep. Values are means (n 4) with their standard errors indicated by vertical bars. * $P < 0{\cdot}05$; † $P < 0{\cdot}1$. For details of procedures, see pp. 668–671.

despite the net extraction (Table 5). The [^{15}N]glutamine hepatic release tended to be greater at the higher NH_3 infusion (Table 5) but was not statistically significant. This was due primarily to low glutamine concentrations in the hepatic venous samples of one sheep on the lower NH_3 infusion; this yielded a higher extraction for both net and labelled glutamine but may be due to breakdown of the amino acid on storage. A clearer picture of the increased flux through hepatic glutamine synthetase (*EC* 6.3.1.2) is given by comparison of the enrichments of glutamine:urea in the hepatic vein where for each sheep at every time point the ratio was greater for the higher NH_3 administration (average 2·5 (SE 0·2); Fig. 2). Although neither urea nor glutamine enrichments attained plateau within the infusion period the ratios of their enrichments had stabilized by the seventh hour of infusion.

DISCUSSION

Although hepatic detoxification of NH_3 is often considered a problem peculiar to ruminants with their substantial foregut fermentation it is almost certainly a universal requirement because even in the laboratory rat NH_3 concentrations in the portal vein are similar to those observed for ruminants (e.g. Cooper *et al.* 1987) and extensive extraction by the liver occurs in the human (e.g. Summerskill & Wolpert, 1970). This detoxification will certainly have an effect on hepatic bioenergetics but whether there is an additional penalty on protein metabolism is open to debate. The current study attempts to address the consequences for both energy expenditure and amino acid availability to the periphery under circumstances where only NH_3 supply is altered. One possible deficiency of the design adopted is that during chronic (4 d) infusion of NH_3 there may be recycling of the additional products, particularly urea and glutamine, to the digestive tract which may alter protein and (or) energy supply. This is considered unlikely as amino acid absorption across the PDV was unaltered by NH_3 infusion. The alternative approach was to monitor acute effects over a few hours but because these conditions may not establish a stable homeostatic or homeorhetic system this was rejected.

Whole-body and splanchnic tissue protein and energy metabolism

In both sheep and cattle, energy expenditure, either measured directly or estimated from gaseous exchange, across the splanchnic tissues represents a substantial proportion of whole-body heat production. For example, for the ovine PDV estimates from 22 to 33% have been reported (Webster *et al.* 1975; Burrin *et al.* 1989; see Ortigues, 1991) and similar values are observed for cattle (e.g. Huntington, 1990; Reynolds *et al.* 1991*a*). Although the proportion of whole-body energy expenditure attributable to the PDV does vary with intake (Webster *et al.* 1975; Burrin *et al.* 1989; see Huntington, 1990; Ortigues, 1991) it was unaffected by the additional NH_3 infusion. The contribution of the liver to total O_2 consumption matched that of the PDV, again in agreement with other observations (Huntington & Reynolds, 1987; Burrin *et al.* 1989; Reynolds *et al.* 1991 *a*), but there was a trend for increased energy expenditure with the supplemental NH_3. In cattle ureagenesis has been estimated to contribute 13–16% to liver energetics, based on the stoichiometric requirement of four high-energy phosphate bonds per mole urea synthesized and assuming six such bonds per mole O_2 consumed (Huntington, 1989; Reynolds *et al.* 1991*b*). Based on similar assumptions, under the conditions of low NH_3 infusion ureagenesis represented a minimum of 13% of the ovine hepatic O_2 demand; this value increased to 19% at the greater NH_3 load. In terms of the incremental responses between the two rates of infusion, however, urea synthesis accounted for 45% of extra liver energy expenditure. When considering the effects of NH_3 detoxification on hepatic bioenergetics these values must be considered as a minimum because additional costs will be associated with energy-requiring linked amination reactions involving, for example, GLDH and glutamine synthetase.

Even though the liver and PDV make substantial and similar contributions to whole-body energy expenditure differences exist with respect to protein metabolism. The arterio–venous leucine kinetics theoretically allow estimates to be made of protein synthesis and gain, and hence, by difference, protein breakdown. Although net isotope transfers can be quantified with reasonable accuracy, conversion into amino flows requires knowledge of the isotopic activity of the precursor pool for protein synthesis based on the enrichments of the intracellular leucyl-tRNA or nascent polypeptides from polysomes, measurements outwith the scope of the current project. The adopted use of the more-easily defined free amino acid pools involves an over-simplification because PDV and hepatic tissues produce both constitutive and export proteins synthesized in different compartments of the cell (e.g. Fern & Garlick, 1974). Furthermore, protein synthetic activities vary considerably between the various components of the PDV tissues, from reticulo–rumen to colon, and between the structurally distinct serosal and mucosal layers (Attaix *et al.* 1987; Southorn *et al.* 1992; Lobley *et al.* 1994). Animals in the fed state can utilize amino acids for PDV tissue protein synthesis from both systemic (high enrichment) and luminal (low enrichment) sources and, thus, systemic infusion of labelled leucine will tend to underestimate protein synthesis rates. To unravel such complexities would require either use of terminal procedures (Attaix *et al.* 1987, 1992; Southorn *et al.* 1992; Lobley *et al.* 1994) or combined luminal and systemic infusion of isotope (e.g. Alpers, 1972; MacRae *et al.* 1993).

Nonetheless, despite these above considerations, the arterio–venous approach can offer a good comparative base. For example, the hepatic rates for protein synthesis (8·6–9·3 μmol/min), based on venous MOP enrichment, are slightly greater than weight-adjusted values calculated from sheep studied using the large-dose procedure (7·3 μmol/min; Lobley *et al.* 1994). This latter technique will yield an underestimate equivalent to the amount of synthesized protein exported within the measurement period. Thus, in practice, the two procedures may give close agreement. The observed contribution of hepatic synthesis to whole-body protein synthesis (11%) also agrees well with other ruminant studies (Lobley *et al.* 1980; Attaix *et al.* 1987), although it is somewhat lower than the value

derived from studies with mature ewes (Pell *et al.* 1986).

In contrast, the rates of protein synthesis determined across the PDV by the arterio–venous procedure are less than those observed in both younger and older lambs (Attaix *et al.* 1987; Lobley *et al.* 1994), probably due to the considerations discussed above. Despite this, the PDV protein synthesis rate exceeded that of the liver by approximately 2·5-fold, similar to the near 3-fold difference observed by Attaix *et al.* (1987), again supporting the comparative strength of the approach.

What is clear, however, is that there was no significant change in whole-body protein synthesis nor in the absolute or proportional contribution from the PDV and liver as a consequence of increasing the NH_3 load. In some respects this is not unexpected because even with considerable increases in intake both the liver and GIT sections show little or no alteration in fractional synthesis rate, in contrast to the situation with peripheral tissues (Lobley *et al.* 1992, 1994) and differences in absolute rate are associated more with changes in tissue protein mass (Burrin *et al.* 1990). GIT size is unlikely to be altered under the current experimental conditions.

Amino acid transfers across the splanchnic tissues

The rate of NH_3 infusion did not affect net free amino acid absorption across the PDV and the values were similar to those observed previously for sheep at maintenance intake (Wolff *et al.* 1972; Heitmann & Bergman, 1981). In these earlier reports, glutamine, probably produced by peripheral tissues, was removed by the PDV tissues, an effect noted in the current study. The enrichment of [^{15}N]glutamine produced was not lowered during passage across the PDV, indicating that net absorption from dietary sources or GIT metabolism was probably small.

In general the proportional extraction of the absorbed amino acids from blood by the liver followed the pattern reported by other workers for plasma removals (e.g. Wolff *et al.* 1972) with lower fractional rates of extraction 0·28–0·43 for the branched-chain amino acids compared with methionine and the aromatic and basic residues. The major discrepancy relates to threonine of which, in the current study, less than 0·5 of that absorbed was removed by the liver compared with complete extraction in the earlier study (Wolff *et al.* 1972). Whether this relates to differences in diet, use of blood rather than plasma or technical difficulties is unclear.

Mass movements of amino acids across a tissue do not describe their fates which might involve either catabolism or anabolism and, for this reason, the alternative approach of measuring oxidation of an amino acid directly was adopted. While catabolism of most amino acids is confined primarily to the liver that of leucine, valine and isoleucine can occur within peripheral tissues, muscle and fat (Pell *et al.* 1986; Goodwin *et al.* 1987; Papet *et al.* 1988), as well as in the splanchnic region which accounted for 33–42% of the total in the current study. Changes in the latter appeared, however, to account for the increase in whole-body oxidation of leucine as a consequence of the higher NH_3 infusion. Although it is tempting to ascribe such increases in oxidation to a direct provision of amino-N for the ornithine cycle to aid removal of the extra NH_3, there is an alternative explanation based on the observation that in fasted humans, with similar chronic mild acidosis, leucine oxidation was stimulated (Reaich *et al.* 1992). They also observed, however, increases in both whole-body synthesis and breakdown which were not features of the current study. Furthermore, the response in humans was compared with observations in rats where acidosis was linked to increased muscle leucine catabolism, based on changes in the activity of the rate-limiting branched-chain oxoacid dehydrogenase (*EC* 1.2.4.4; May *et al.* 1987), whereas the current ovine data favour splanchnic tissue leucine oxidation. The contrary findings leave in doubt, therefore, whether the increased catabolism of the amino acid in

the sheep is a response to acidosis or a means of providing additional substrate for NH_3 removal. This can be tested by supplying NH_3 in a form which does not change acid–base status.

Hepatic ammonia and urea metabolism

Based on the assumption that liver mass was 1·6% of live body weight at the intake offered (Lobley *et al.* 1994), the rates of hepatic NH_3 clearance were 46–64 μmol/h per g wet tissue, within the reported maximal values for ruminants of 70–90 μmol/h per g wet tissue (Linzell *et al.* 1971; Symonds *et al.* 1981; Orzechowski *et al.* 1987) and thus peripheral hyperammonaemia was avoided. The maximum proportion of urea-N which could be derived from NH_3 was 0·66 and 0·54 at the low and high infusions respectively, requiring in both instances major inputs from other N sources. This shortfall in substrate for urea-N could, at the lower NH_3 supplementation, be more than accommodated by the apparent hepatic extraction of free amino acid N. Indeed, extracted amino acid would be in theoretical excess sufficient to provide 66 μmol N/min for synthesis of secreted proteins, equivalent to 35% of liver synthesis or 240 mg export protein/kg body weight per d, approximately double the amount required for albumin turnover in healthy adult human males (Ballmer *et al.* 1990).

With the greater NH_3 administration, however, the increase in urea synthesis exceeded apparent uptake of NH_3-N and free amino acid-N combined. Clearly, sufficient amino acid must be available to maintain net (export) protein synthesis by the liver and possibly alternative substrates, such as peptide material, may be utilized with, presumably, a net penalty to the N economy of the animal.

In other studies, the response of ruminants to direct NH_3 addition has proved equivocal. Norton *et al.* (1982), for example, infused 0·47 mol NH_4Cl/d into the rumen of sheep and observed a non-significant decrease in N retention. In contrast, for cattle with acute mesenteric vein infusion of NH_4Cl the additional urea-N produced exceeded the increment in hepatic NH_3 removal by nearly 3-fold (Wilton *et al.* 1988). Although incomplete recovery of infused material was obtained in the latter study, the value is comparable with the incremental ratio of 1·9 obtained in the present experiment, and where reduced N retention would be predicted.

In certain dietary comparisons the hepatic uptake of NH_3 can apparently account for 77–86% of urea-N appearance (e.g. Huntington, 1989; Maltby *et al.* 1991). When the hepatic NH_3 removal: urea-N appearance ratio does exceed 0·5 then the possibility exists that both N atoms of urea may arise from NH_3, probably through the actions of CPS and GLDH. It would therefore be predicted that following $^{15}NH_3$ administration [$^{15}N^{15}N$] and [$^{15}N^{14}N$]urea molecules will be formed. Nolan & Leng (1972) infused [^{15}N]ammonium sulphate into the rumen of sheep and observed that only 40–50% of the urea formed arose from ruminal NH_3, similar to the proportion of total NH_3 production: urea synthesis. In the current study only 55–65% of urea-N synthesis could be equated with hepatic NH_3 extraction and the labelled urea molecules formed were predominantly [$^{14}N^{15}N$]species, which suggests that channelling of NH_3-N simultaneously through the CPS and GLDH pathways has not occurred. In theory, GLDH can operate in both the forward and reverse directions but under controlled nutritional and physiological conditions one would be favoured, and if it is accepted that the action of mitochondrial enzyme is towards oxaloacetate formation, with the provision of NH_4^+ for carbamoyl phosphate synthesis (Lowenstein, 1972; Nissim *et al.* 1992), then transfer of ^{15}N from NH_3 to aspartate via glutamate would be limited, and [$^{15}N^{15}N$] urea would not be synthesized. Thus, rat hepatocytes incubated with [5-^{15}N]glutamine, to generate NH_3 via mitochondrial glutaminase (*EC* 3.5.1.2) action, produced only [$^{15}N^{14}N$]urea while both [2-^{15}N]glutamine

and [2-^{15}N]glutamate produced both [^{14}N^{15}N] and [^{15}N^{15}N]urea (Nissim *et al.* 1992). The incubation conditions employed *in vitro* do not necessarily stimulate the normal physiological and nutritional state of the rodent *in vivo*, and therefore the direction of GLDH action, because in fed, but anaesthetized, rats there was extremely rapid equilibration of labelled N from $^{15}NH_3$ to glutamate and aspartate (Cooper *et al.* 1987), conditions which should favour formation of doubly-labelled urea.

In the sheep enrichments of glutamate in the hepatic vein exceeded those of both the portal vein and aortal blood, indicative of formation of labelled glutamate either from NH_3 and oxaloacetate and (or) by transamination reactions in the liver. That this did not lead to formation of [^{15}N^{15}N]urea may be a consequence of liver architecture. Periportal hepatocytes are the predominant cell type and are the site of both gluconeogenesis and ureagenesis. These cells also contain glutaminase and release, but do not extract, glutamate. In rodents, perivenous cells, which surround the hepatic vein, do not synthesize urea or glucose but have present the appropriate Na^+-dependent X–AG transporter, required for both aspartate and glutamate uptake (Gebhardt & Mecke, 1983; Haussinger & Gerok, 1983). In addition, the perivenous hepatocyte can synthesize glutamate (Sies & Haussinger, 1984) from oxoglutarate and other tricarboxylate intermediates removed from the blood (Stoll & Haussinger, 1989, 1991). If a similar situation pertains in sheep then the site of glutamate uptake and synthesis is downstream from the ureagenic cells and this would inhibit the formation of [^{15}N^{15}N]urea.

Hepatic glutamine metabolism

There was net removal of glutamine during passage across the liver, and this probably contributed to the hepatic net appearance of glutamate. The substantial increase in enrichment of glutamine across the liver clearly demonstrates the existence of the hepatic intercellular glutamine cycle, in which amino acid is first removed from the blood and hydrolysed by mitochondrial glutaminase in the periportal cells followed by reformation in the perivenous region through the action of cytosolic glutamine synthetase (Haussinger, 1983; Haussinger *et al.* 1992). The magnitude of the cycle could not be assessed from the current study as only net movements were monitored and there are uncertainties concerning both the quantity of portal-vein-derived glutamine removed and the enrichments of NH_3 within the periportal and perivenous cells.

Although the nature of the predominant single-labelled glutamine produced by the liver could not be defined under the mass spectrometry conditions employed because the enrichment exceeded that of glutamate by two to four times it is presumed that the majority is as the [5-N]- rather than [2-N]-species, i.e. the amido group is labelled through the action of glutamine synthetase. This interpretation does depend on establishment of an equilibrium for free glutamate between blood and the efflux from intrahepatocyte sources (Haussinger & Gerok, 1984). The role that the glutamine synthetase pathway plays in NH_3 detoxification is exemplified by the doubling in the ratio of isotopic activities of glutamine:urea between the two rates of NH_3 infusion. The urea cycle is a high-capacity, low-affinity system and removes the majority of NH_3 entering from the portal vein, the periportal reaction being aided by the high initial NH_3 concentration (Haussinger *et al.* 1992). In rodents glutamine synthetase has a tenfold lower K_m for NH_3 than does CPS (Meijer *et al.* 1985) but is restricted to the perivenous cells, which comprise less than 10% of total hepatocytes (see Jungermann & Katz, 1989; Haussinger *et al.* 1992), and thus provides a high-affinity, low-capacity scavenging system and ensures NH_3 in hepatic venous blood entering the posterior vena cava is maintained at low concentrations. Under the current experimental conditions less than 4% of net ^{15}N transfer across the ovine liver

was as glutamine, a value similar to that observed for rat liver *in vivo* (7%; Cooper *et al.* 1987). The additional NH_3 was provided at a uniform rate and under circumstances where transient but higher rates of NH_3 absorption may occur, e.g. fresh forage, silage, the action of hepatic glutamine synthetase may become more important.

Conclusion

The current study has shown that in response to an increased NH_3 load ovine hepatic ureagenesis is stimulated with a requirement for additional N substrates. No evidence of increased free amino acid removal across the liver was observed although catabolism of leucine appeared to be stimulated. At least 50% of urea production was related to NH_3 detoxification and the effects on both the energy and N metabolism of the sheep are substantial. Nutritional circumstances which lead to considerable absorption of NH_3 from GIT fermentations may then inflict a double penalty in that, first, a proportion of feed-N is not made available to the animal as an anabolic form and, second, hepatic removal of the NH_3 may require net utilization of ingested amino acids.

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REFERENCES

Alpers, D. H. (1972). Protein synthesis in intestinal mucosa: the effect of route of administration of precursor amino acids. *Journal of Clinical Investigation* **51**, 167–173.

Atkinson, D. E. & Bourke, E. (1984). The role of ureagenesis in pH homeostasis. *Trends in Biochemical Sciences* **9**, 297–300.

Attaix, D., Aurousseau, E., Bayle, G., Manghebati, A. & Arnal, M. (1987). Protein synthesis and degradation in growing lambs. In *5th International Symposium on Protein Metabolism and Nutrition. European Association of Animal Production Publication*, no. 35, pp. 24–27. Rostock: Wilhelm-Pieck University.

Attaix, D., Aurousseau, E., Rosolowska-Huszez, D., Bayle, G. & Arnal, M. (1992). *In vivo* longitudinal variations in protein synthesis in developing ovine intestines. *American Journal of Physiology* **263**, R1318–R1323.

Ballmer, P. E., McNurlan, M. A., Milne, E., Heys, S. D., Buchan, V., Calder, A. G. & Garlick, P. J. (1990). Measurement of albumin synthesis in humans: a new approach employing stable isotopes. *American Journal of Physiology* **259**, E797–E803.

Bartels, H. & Harms, H. (1959). Saverstoffdissoziationskurven des Blutes von Säugetieren (Mensch, Karinchen, Meerschweinchen, Hund, Katz, Schwein, Rind und Schaf) (Oxygen dissociation curves for blood of mammals (man, rabbit, guinea pig, dog, cat, pig, cattle and sheep). *Pflügers Archivs* **268**, 334–365.

Blaxter, K. L., Brockway, J. M. & Boyne, A. W. (1972). A new method for estimating the heat production of animals. *Quarterly Journal of Experimental Physiology* **57**, 60–72.

Burrin, D. G., Ferrell, C. L., Britton, R. A. & Bauer, M. (1990). Level of nutrition and visceral organ size and metabolic activity in sheep. *British Journal of Nutrition* **64**, 439–448.

Burrin, D. G., Ferrell, C. L., Eisemann, J. H., Britton, R. A. & Nienaber, J. A. (1989). Effect of level of nutrition on splanchnic blood flow and oxygen consumption in sheep. *British Journal of Nutrition* **62**, 23–34.

Cooper, A. J. L., Nieves, E., Coleman, A. E., Filc-De Ricco, S. & Gelbard, A. S. (1987). Short-term metabolic fate of [^{13}N]ammonia in rat liver *in vivo*. *Journal of Biological Chemistry* **262**, 1073–1080.

Fern, E. B. & Garlick, P. J. (1974). The specific radioactivity of the tissue free amino acid pool as a basis for measuring the rate of protein synthesis in the rat *in vivo*. *Biochemical Journal* **142**, 413–419.

Fitch, N. A., Gill, M., Lomax, M. A. & Beever, D. E. (1989). Nitrogen and glucose metabolism by the liver of forage- and forage-concentrate-fed cattle. *Proceedings of the Nutrition Society* **48**, 76A.

Fleet, I. R. & Mepham, T. B. (1983). Physiological methods used in the study of mammary substrate utilisation in ruminants. In *Biochemistry of Lactation*, pp. 469–491 [T. B. Mepham, editor]. Amsterdam: Elsevier Science Publishers.

Gebhardt, R. & Mecke, D. (1983). Glutamate uptake by cultured rat hepatocytes is mediated by hormonally inducible sodium-dependent transport systems. *FEBS Letters* **161**, 275–278.

Goodwin, G. W., Gibboney, W., Paxton, R., Harris, R. A. & Lemons, J. A. (1987). Activities of branch-chain amino acid aminotransferase and branch-chain 2-oxo dehydrogenase complex in tissues of maternal and fed sheep. *Biochemical Journal* **242**, 305–308.

Harris, P. M., Skene, P. A., Buchan, V., Calder, A. G., Anderson, S. E., Connell, A. & Lobley, G. E. (1992). Effect

of food intake on hind-limb and whole-body protein metabolism in young growing sheep: chronic studies based on arterio–venous techniques. *British Journal of Nutrition* **68**, 389–407.

Häussinger, D. (1983). Hepatocyte heterogeneity in glutamine and ammonia metabolism and the role of an intracellular glutamine cycle during ureogenesis in perfused rat liver. *European Journal of Biochemistry* **133**, 269–274.

Häussinger, D. & Gerok, W. (1983). Hepatocyte heterogeneity in glutamate uptake in isolated perfused rat liver. *European Journal of Biochemistry* **136**, 421–425.

Häussinger, D. & Gerok, W. (1984). Regulation of hepatic glutamate metabolism. Role of 2-oxoacids in glutamate release from isolated perfused rat liver. *European Journal of Biochemistry* **143**, 491–497.

Häussinger, D., Lamers, W. H. & Moorman, A. F. M. (1992). Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. *Enzyme* **46**, 72–93.

Heitmann, R. N. & Bergman, E. N. (1981). Glutamate interconversions and glucogenicity in the sheep. *American Journal of Physiology* **241**, E465–E472.

Herd, J. A. & Barger, A. C. (1964). Simplified technique for chronic catheterisation of blood vessels. *Journal of Applied Physiology* **19**, 791–792.

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

Huntington, G. B. (1990). Energy metabolism in the digestive tract and liver of cattle: influence of physiological state and nutrition. *Reproduction, Nutrition, Developpement* **30**, 35–47.

Huntington, G. B. & Reynolds, C. K. (1987). Oxygen consumption and metabolite flux of bovine portal-drained viscera and liver. *Journal of Nutrition* **117**, 1167–1173.

Jungerman, K. & Katz, N. (1989). Functional specialization of different hepatocyte populations. *Physiological Reviews* **69**, 708–764.

Katz, M. L. & Bergman, E. N. (1969*a*). A method for simultaneous cannulation of the major splanchnic blood vessels of the sheep. *American Journal of Veterinary Research* **30**, 655–661.

Katz, M. L. & Bergman, E. N. (1969*b*). Simultaneous measurements of hepatic and portal venous blood flow in the sheep and dog. *American Journal of Physiology* **216**, 946–952.

Linzell, J. L., Setchell, B. P. & Lindsay, D. B. (1971). The isolated perfused liver of sheep: assessment of its metabolic, synthetic and secretory functions. *Quarterly Journal of Experimental Physiology* **56**, 53–71.

Lobley, G. E. (1992). Control of the metabolic fate of amino acids in ruminants: a review. *Journal of Animal Science* **70**, 3264–3275.

Lobley, G. E., Connell, A., Milne, E., Newman, A. M. & Ewing, T. A. (1994). Protein synthesis in splanchnic tissue of sheep offered two levels of intake. *British Journal of Nutrition* **71**, 3–12.

Lobley, G. E., Harris, P. M., Skene, P. A., Brown, D., Milne, E., Calder, A. G., Anderson, S. E., Garlick, P. J., Nevison, I. & Connell, A. (1992). Responses in tissue protein synthesis to sub- and supra-maintenance intake in young growing sheep: comparison of large-dose, and continuous-infusion techniques. *British Journal of Nutrition* **68**, 373–388.

Lobley, G. E., Milne, V., Lovie, J. M., Reeds, P. J. & Pennie, K. (1980). Whole body and tissue protein synthesis in cattle. *British Journal of Nutrition* **43**, 491–502.

Lowenstein, J. M. (1972). Ammonia production in muscle and other tissues: the purine nucleotide cycle. *Physiological Reviews* **52**, 382–414.

MacRae, J. C., Lobley, G. E., Bruce, L. A., Luo, Q. J., Calder, A. G. & Farningham, D. A. H. (1993). Leucine kinetics across the portal drained viscera of sheep. *Journal of Animal Science*, Suppl. **71** 1, 279 (Abstr.).

Maltby, S. A., Lomax, M. A., Beever, D. E. & Pippard, C. J. (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 *Energy Metabolism of Farm Animals, European Association for Animal Production Publication*, no. 58, pp. 20–33 [C. Wenk and M. Boessinger, editors]. Zurich: Schriftenreche aus dem Institut für Nutztierwissenschaften Gruppe Ernährung.

Marsh, W. H., Fingerhut, B. & Miller, H. (1965). Automated and manual direct methods for the determination of blood urea. *Clinical Chemistry* **2**, 624–627.

May, R. C., Hara, Y., Kelly, A., Block, K. P., Buse, M. G. & Mitch, W. E. (1987). Branched chain amino acid metabolism in rat muscle: abnormal regulation in acidosis. *American Journal of Physiology* **252**, E712–E718.

Meijer, A. J., Lamers, W. H. & Chamuleau, R. A. F. M. (1990. Nitrogen metabolism and ornithine cycle function. *Physiological Reviews* **70**, 701–748.

Meijer, A. J., Lof, C., Ramos, I. C. & Verhoeven, A. J. (1985). Control of ureogenesis. *European Journal of Biochemistry* **148**, 189–196.

Mondzac, A., Erlich, G. E. & Seegmiller, J. E. (1965). An enzymatic determination of ammonia in biological fluids. *Journal of Laboratory and Clinical Medicine* **66**, 526–531.

Nash, J. E., Rocha, H. J. G., Buchan, V., Calder, A. G., Milne, E., Quirke, J. F. & Lobley, G. E. (1994). The effect of acute and chronic administration of the β-agonist, cimaterol, on protein synthesis in ovine skin and muscle. *British Journal of Nutrition* **71**, 501–513.

Nissim, I., Cattano, C., Nissim, I. & Yudkoff, M. (1992). Relative role of the glutaminase, glutamate dehydrogenase, and AMP-deaminase pathways in hepatic ureagenesis: studies with ^{15}N. *Archives of Biochemistry and Biophysics* **292**, 393–401.

Nissim, T., Yudkoff, M. & Segal, S. (1985). Metabolism of glutamine and glutamate by rat renal tubules. *Journal of Biological Chemistry* **260**, 13955–13967.

Nolan, J. V. & Leng, R. A. (1972). Dynamic aspects of ammonia and urea metabolism in sheep. *British Journal of Nutrition* **27**, 177–194.

Norton, B. W., Janes, A. N. & Armstrong, D. G. (1982). The effects of intraruminal infusions of sodium bicarbonate, ammonium chloride and sodium butyrate on urea metabolism in sheep. *British Journal of Nutrition* **48**, 265–274.

Ortigues, I. (1991). Adaptation of energy metabolism to under-nutrition in ruminants. Quantification in whole animal and individual body tissues. *Reproduction, Nutrition, Developpement* **31**, 593–616.

Ortigues, I., Durand, D. & Lefaivre, J. (1994). Use of para-amino hippuric acid to measure blood flow through portal-drained-viscera, liver and hindquarters in sheep. *Journal of Agricultural Science, Cambridge* **122**, 299–308.

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

Papet, I., Lezebot, N., Barre, F. & Arnal, M. (1988). Influence of dietary leucine content on the activities of branched-chain amino acid transferase (*EC* 2.6.2.42) and branched-chain α-keto acid dehydrogenase (*EC* 1.2.4.4) complex in tissues of pre-ruminant lambs. *British Journal of Nutrition* **59**, 475–483.

Pell, J. M., Calderone, E. M. & Bergman, E. N. (1986). Leucine and α-ketoisocaproate metabolism and interconversions in fed and fasted sheep. *Metabolism* **35**, 1005–1016.

Reaich, D., Channon, S. M., Scrimgeour, C. M. & Goodship, T. H. J. (1992). Ammonium chloride-induced acidosis increases protein breakdown and amino acid oxidation in humans. *American Journal of Physiology* **263**, E735–E739.

Reynolds, C. K. & Tyrrell, H. F. (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, C. K., Tyrrell, H. F. & Reynolds, P. J. (1991*a*). Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef steers: whole body energy and nitrogen balance and visceral heat production. *Journal of Nutrition* **121**, 994–1003.

Reynolds, C. K., Tyrrell, H. F. & Reynolds, P. J. (1991*b*). Effects 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.

Sies, H. & Häussinger, D. (1984). Hepatic glutamine and ammonia metabolism. Nitrogen balance and intercellular glutamine cycle. In *Glutamine Metabolism in Mammalian Tissues*, pp. 78–97 [D. Häussinger and H. Sies, editors]. Berlin: Springer.

Siggaard-Anderson, O. (1974). *The Acid-Base Status of Blood*, 4th ed. Copenhagen: Munksgaard.

Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B. & Graber, M. (1945). The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dogs and man. *Journal of Clinical Investigation* **24**, 388–404.

Southorn, B. G., Kelly, J. M. & McBride, B. W. (1992). Phenylalanine flooding dose procedure is effective in measuring intestinal and liver protein synthesis in sheep. *Journal of Nutrition* **122**, 2398–2407.

Stoll, B. & Häussinger, D. (1989). Functional hepatocyte heterogeneity: vascular 2-oxoglutarate is almost exclusively taken up by perivenous glutamine-synthetase-containing hepatocytes. *European Journal of Biochemistry* **181**, 709–716.

Stoll, B. & Häussinger, D. (1991). Hepatocyte heterogeneity in uptake and metabolism of malate and related dicarboxylates in perfused rat liver. *European Journal of Biochemistry* **195**, 121–129.

Summerskill, W. H. J. & Wolpert, E. (1970). Ammonia metabolism in the gut. *American Journal of Clinical Nutrition* **23**, 633–639.

Symonds, H. W., Mather, D. L. & Collis, K. A. (1981). The maximum capacity of the bovine liver to metabolize ammonia. *Proceedings of the Nutrition Society* **40**, 63A.

Turnell, D. C. & Cooper, J. D. H. (1982). Rapid assay for amino acids in serum or urine by pre-column derivatization and reversed-phase liquid chromatography. *Clinical Chemistry* **28**, 527–531.

Webster, A. J. F., Osuji, P. O., White, F. & Ingram, J. F. (1975). The influence of food intake on portal blood flow and heat production in the digestive tract of sheep. *British Journal of Nutrition* **34**, 125–139.

Wilton, J. C., Gill, M. & Lomax, M. A. (1988). Uptake of ammonia across the liver of forage-fed cattle. *Proceedings of the Nutrition Society* **47**, 153A.

Wolff, J. E., Bergmann, E. N. & Williams, H. H. (1972). Net metabolism of plasma amino acids by liver and portal-drained viscera of fed sheep. *American Journal of Physiology* **223**, 438–446.

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