Influence of hepatic ammonia removal on ureagenesis, amino acid utilization and energy metabolism in the ovine liver

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The mass transfers of O_2, glucose, NH_3, urea and amino acids across the portal-drained viscera (PDV) and the liver were quantified, by arterio–venous techniques, during the last 4 h of a 100 h infusion of 0 (basal), 150 or 400 μmol NH_4HCO_3/min into the mesenteric vein of three sheep given 800 g grass pellets/d and arranged in a 3 × 3 Latin-square design. Urea irreversible loss rate (ILR) was also determined by continuous infusion of [^{14}C]urea over the last 52 h of each experimental period. PDV and liver movements of glucose, O_2 and amino acids were unaltered by NH_4HCO_3 administration, although there was an increase in PDV absorption of non-essential amino acids ($P = 0{\cdot}037$) and a trend for higher liver O_2 consumption and portal appearance of total amino acid-N, glucogenic and non-essential amino acids at the highest level of infusion. PDV extraction of urea-N ($P = 0{\cdot}015$) and liver removal of NH_3 ($P < 0{\cdot}001$), release of urea-N ($P = 0{\cdot}002$) and urea ILR ($P = 0{\cdot}001$) were all increased by NH_4HCO_3 infusion. Hepatic urea-N release (y) and NH_3 extraction (x) were linearly related (R^2 0·89), with the slope of the regression not different from unity, both for estimations based on liver mass transfers (1·16; SE 0·144; $P_{b \neq 1} = 0{\cdot}31$) and [^{14}C]urea (0·97; SE 0·123; $P_{b \neq 1} = 0{\cdot}84$). The study indicates that a sustained 1·5 or 2·4-fold increase in the basal NH_3 supply to the liver did not impair glucose or amino acid supply to non-splanchnic tissues; nor were additional N inputs to the ornithine cycle necessary to convert excess NH_3 to urea. Half of the extra NH_3 removed by the liver was, apparently, utilized by periportal glutamate dehydrogenase and aspartate aminotransferase for sequential glutamate and aspartate synthesis and converted to urea as the 2-amino moiety of aspartate.

Sheep: Liver: Ammonia: Ureagenesis

NH_3 of both endogenous and gastrointestinal origin is normally removed completely by the liver and converted to urea. Hepatic ureagenesis depends, however, on the coordinated supply of N to the ornithine cycle from two different precursors, mitochondrial NH_3 and cytosolic aspartate. Blood free amino acids are, together with NH_3, the only N-substrates extracted by the liver in amounts sufficient to mantain the rates of ureagenesis observed in ruminants *in vivo* (Huntington, 1989; Reynolds *et al*. 1991; Lobley *et al*. 1995). If amino acids were the predominant N-donors to aspartate via transamination reactions with glutamate, the immediate aspartate-N precursor, then the ratio NH_3 removal : urea-N production across the liver should be 0·5 or even lower because NH_3 derived from the 5-amido group of glutamine can also contribute N to the mitochondrial synthesis of carbamoyl phosphate (Nissim *et al*. 1992). Such ratios, on both an absolute and incremental basis, have been observed in several studies with ruminants *in vivo* (see reviews by Reynolds, 1992; Parker *et al*. 1995; Lescoat *et al*. 1996), leading to the hypothesis that, to detoxify NH_3, the liver would require an equal N input from amino acids. This would penalize net protein availability to the animal. Other data, however, yield values greater than 0·5 (see Huntington, 1986; Seal & Reynolds, 1993; Parker *et al*. 1995), suggesting that blood NH_3 can provide N to both urea-N precursors. Indeed, the equimolar conversion of NH_3 to urea-N has been firmly established by isotopic studies *in vitro* (Luo *et al*. 1995; Brosnan *et al*. 1996). Although this efficient conversion has not always been observed in response to increased hepatic NH_3 extraction *in vivo* (Huntington, 1986; Reynolds *et al*. 1991; Goetsch *et al*. 1996), this may relate to experimental conditions in which both diet

Abbreviations: GDH, glutamate dehydrogenase; GIT, gastrointestinal tract; ILR, irreversible loss rate; PDV, portal-drained viscera; RMS, residual mean square.

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quality and/or quantity were altered. Thus, factors other than portal NH_3 flow that might also stimulate ureagenesis from amino acids, were changed simultaneously.

Ureagenesis can influence both liver energy expenditure and gluconeogenesis. The theoretical energy costs of urea synthesis have been exceeded in many studies *in vivo* (e.g. Reynolds *et al*. 1991; Lobley *et al*. 1995), but the data are confounded by the number of factors altered by the nutritional treatments imposed. Similarly, evidence for interaction between NH_3-stimulated ureagenesis and hepatic glucose production comes primarily from studies *in vitro* (Weekes *et al*. 1978; Martinrequero *et al*. 1993), where non-physiological concentrations and balances of substrates are often employed.

The objective of the present experiment was to investigate the response of hepatic urea-N production and amino acid removal to increasing rates of NH_3 supply in growing wethers. This was examined by use of chronic (4 d) infusions of NH_4HCO_3 into the mesenteric vein. Additionally, the consequences for energy metabolism, based on changes in liver O_2 demand and glucose output, were quantified.

Materials and methods

Animals

Three Suffolk cross-bred wethers (35–40 kg body weight), surgically prepared with indwelling catheters in the posterior aorta, portal, hepatic and mesenteric veins (Lobley *et al*. 1995), were placed in metabolism cages under continuous lighting conditions and adjusted to receiving 71 g grass pellets (850 g/d; 920 g DM/kg; 10 MJ metabolizable energy/kg DM; 22 g N/kg DM) every 2 h using automatic feeders. Water was offered *ad libitum*. A temporary jugular catheter was inserted 24 h before the start of each experimental period.

Design

The experiment was arranged as a 3×3 Latin square with three experimental periods each of 100 h separated by a 15 d interval. Throughout each experimental period the sheep were infused into the mesenteric vein catheter with one of: physiological saline (0·15 M-NaCl), 0·45 M-NH_4HCO_3 or 1·2 M-NH_4HCO_3 (both in 0·15 M-NaCl) at a rate of 20 g/h, to provide 0 (C0), 150 (C150) or 400 (C400) μmol NH_4HCO_3/min plus a constant input of 50 μmol NaCl/min. At 48 h after the start of each experimental period, a solution containing 1 mM-[^{14}C]urea (9·25 kBq/g) in physiological saline was infused for 52 h into the right jugular vein catheter at a rate of 4 g/h. At 94 h after the start of the experimental period, a 0·1 M-sodium *p*-amino hippuric acid, 0·05 M-sodium phosphate buffer (pH 7·4) solution containing 400 IU heparin/g was infused into the mesenteric vein catheter at a rate of 20 g/h for 6 h. All the solutions were sterilized and the infusions performed by means of peristaltic pumps.

Samples

Four simultaneous blood samples were continuously withdrawn from the aorta, portal and hepatic veins at hourly intervals (10 ml/h per catheter) during the last 4 h (96–100 h) of each experimental period, using a peristaltic pump. The collection lines were allowed to pass through ice-cold water to reduce both risk of blood clotting and enzyme activities in the blood samples. The blood samples were collected directly into 10 ml syringes stored in ice-cold water. An additional mixed blood sample (10–15 ml) was collected during each experimental period for determination of blood and plasma DM. Three urine samples were also collected during the last 4 h of the experimental period. An additional urine sample was collected 18 h before the [^{14}C]urea infusion for determination of background radioactivity.

Blood analysis

Blood samples in each syringe were carefully mixed and analysed for blood $p(O_2)$, $p(CO_2)$, pH, bicarbonate and haemoglobin concentration immediately after collection using a Blood Gas Analyser (Acid Base Laboratory ABL3, Radiometer, Copenhagen, Denmark). The packed cell volume was determined by the microhaematocrit method.

One portion (0·5 g) of blood was deproteinized with 5 g 120 g/l TCA and processed for gravimetric determination of *p*-amino hippuric acid as previously described (Lobley *et al*. 1995). Then 4 ml blood was centrifuged at 1200 ***g*** for 10 min at 4° and the plasma (approximately 2·5 ml) collected. Two portions (0·5 ml) of plasma were used for the enzymic determination of NH_3 (Mondzac *et al*. 1965) and glucose concentration (Bergmeyer, 1985) by automated procedures (Kone Autoanalyzer, Espoo, Finland). Two additional portions of 1·2 and 0·9 ml plasma were stored at –20° until analysis for urea and amino acid concentration respectively.

The four arterial, portal vein and hepatic vein plasma samples from each experimental period were pooled in proportion to the plasma flow (pooled sample weight, approximately 0·5 g), and processed for amino acid analysis of physiological fluids with an Alpha Plus Amino Acid Analyser (Pharmacia-LKB Biochrom Ltd, Cambridge, Cambs, UK) as described by Lobley *et al*. (1995).

One portion of 0·3 g plasma was enriched with 0·3 g of a solution of 5 mM-[^{15}N^{15}N]urea (99·7 atom %), carefully mixed, deproteinized with 0·1 ml sulfosalicyclic acid (480 g/l) and centrifuged at 7000 ***g*** for 5 min. From the supernatant fraction, the *N*-methyl-*N*-(tertiary butyldimethylsilyl) trifluoroacetamide derivative of urea was prepared as described by Calder & Smith (1980). Plasma urea enrichment was determined by electron impact GC–mass spectrometry analysis on a VG Trio-1 mass spectrometer (VG Masslab, Manchester, UK) coupled to a Hewlett Packard 5890 GC (VG Organic, Manchester, UK; Calder & Smith, 1980). The fragment ions at *m/z* 231 and 233 were monitored under selective ion recording conditions.

Urine analysis

The specific radioactivity of urea was determined on 4 g urine acidified with 1 g 250 g/l TCA. Scintillation liquid (10 ml) (Ultima Gold, Camberra Packard Ltd, Pangbourne, Berkshire, UK) was added to 0·2 g acidified urine and the ^{14}C radioactivity was measured by liquid scintillation

counting (Tri-carb 1900 TR, Camberra Packard Ltd) using an external standard correction (Lobley *et al*. 1996*b*). A portion (0·5 g) of the acidified urine was diluted for determination of urea concentration by a Technicon automated procedure (Marsh *et al*. 1965).

Calculations

O_2 concentration in blood (mmol/l) was calculated as

$$H \times 1{\cdot}34 \times S/22{\cdot}4,$$

where H is the haemoglobin content of blood (g/l), 1·34 is the maximum O_2 transport capacity of the haemoglobin (ml O_2/g haemoglobin), 22·4 is the gas constant (ml O_2/mmol O_2) and S is the O_2 saturation of the haemoglobin estimated from the equation developed by Margaria (1963):

$$S = \{[(1 + k_1 \times cp(O_2))/k_1 \times cp(O_2)]^3 + k_2 - 1\}/\{[(1 + k_1 \times cp(O_2))/k_1 \times cp(O_2)]^4 + k_2 - 1\}.$$

In this equation, the value of $p(O_2)$ has been corrected ($cp(O_2)$) to allow for the effects of $p(CO_2)$ and blood pH on S according to the formula proposed by Kelman (1966):

$$cp(O_2) = p(O_2) \times 10^{[0{\cdot}4 \times (pH - 7{\cdot}4) + 0{\cdot}06 \times (\log 40 - \log p(CO_2))]},$$

and k_1 (0·005491) and k_2 (1042) were estimated by fitting the equation to the data obtained for the O_2 saturation of sheep haemoglobin at different O_2 tensions reported by Bartels & Harms (1959).

Blood flows (F, g/min) were calculated as:

$$F_p = I/(C_p - C_a) \quad \text{and} \quad F_h = I/(C_h - C_a),$$

where F_p and F_h are the blood flows in the portal and hepatic veins respectively, I is the infusion rate of *p*-amino hippuric acid (μmol/min) and C_a, C_p and C_h are the concentrations of *p*-amino hippuric acid (μmol/g) in posterior aorta, portal vein and hepatic vein respectively.

Plasma flows and whole-blood water flows (g/min) were calculated as:

$$F \times (1 - PCV) \quad \text{and} \quad F \times (1 - BDM),$$

respectively, where F is the relevant blood flow and PCV and BDM are the corresponding packed cell volume and blood DM content of the sample respectively.

Mass transfers of metabolites and O_2 across the portal-drained viscera (PDV) and the liver (μmol/min or mmol/min) were calculated as:

$$F_p \times (C_p - C_a) \quad \text{and} \quad (F_hC_h) - (F_pC_p) - (F_h - F_p) \times C_a,$$

where F_p and F_h are the blood (for O_2 and NH_3), plasma (for amino acids and glucose) or whole-blood water (for urea) flows in the portal vein and hepatic vein (g/min) respectively, and C_a, C_p and C_h are the concentrations of O_2 in blood (μmol/g) and metabolites in plasma (amino acids, NH_3 and glucose; nmol/g) or plasma water fraction (urea, estimated as plasma urea concentration : plasma water fraction; μmol/g) in posterior aorta, portal vein and hepatic vein respectively. Urea transfers were calculated as whole-blood water transfers under the assumption that plasma and blood water fractions have equal urea concentrations (Milano, 1997). NH_3 concentrations in blood and plasma were assumed to be equal (Milano, 1997) and thus plasma concentration and blood flows quantified to yield transfers across the PDV and the liver. Virtually no glucose is transported within the erythrocytes in sheep (Arai *et al*. 1995) and only for glycine and threonine is plasma transfer across the PDV significantly smaller than blood movements (Lobley *et al*. 1996*a*). Therefore, glucose and amino acid movements across the PDV and the liver were estimated as plasma transfers.

The irreversible loss rate (ILR, μmol/min) of urea was calculated as:

$$I_r/Sr,$$

where I_r is the infusion rate (kBq/min) of [^{14}C]urea and Sr is the specific radioactivity of [^{14}C]urea (kBq/μmol) in urine.

Statistical analysis

The data were initially analysed by ANOVA for the effects of treatment (rate of infusion of NH_4HCO_3), block (animals) and period, with two residual degrees of freedom. In the case of portal vein NH_3 concentration and urea and NH_3 transfers across the PDV and the liver, the mean squares for the effect of period and block were equal to, or lower than, that of the residual and never significant. For these data, the period and block sum of squares were therefore included in the residual sum of squares and the data re-analysed for the effect of treatment alone, with 6 d.f. for the residual mean square (RMS). The relationships between hepatic NH_3 removal, hepatic urea-N release and urea-N IRL were studied by regression analysis.

Results

Animals and catheter patency

All animals completed the experiment and the catheters maintained their full patency (i.e. they allowed blood collection as well as infusion of solutions) during the experiment, with the exception of one mesenteric catheter which, after the first experimental period, was suitable only for infusion.

Blood variables (Table 1)

The 4 d infusion of NH_4HCO_3 had no measurable effect on blood pH, $p(CO_2)$, HCO_3^- or haemoglobin concentration and therefore the acid–base status of the sheep remained unaltered during the experiment.

Plasma ammonia, urea and amino acid concentrations (Table 1)

An apparent small reduction of 14 μmol/l was detected during treatment C150 in the average arterial concentration of NH_3 ($P < 0{\cdot}05$) which otherwise lay between 60 and 75 μmol/l, within the ranges normally reported for sheep (e.g. Orzechowski *et al*. 1988; Lobley *et al*. 1995, 1996*b*). No changes were observed in NH_3 concentration in the hepatic vein, while that in the portal vein increased ($P < 0{\cdot}003$) by 191 (C150) and 400 (C400) μmol/l during the NH_4HCO_3 administration. The increases in portal NH_3

Table 1. Arterial blood variables and plasma ammonia and urea concentrations in sheep infused with 0 (C0), 150 (C150) or 400 (C400) μmol ammonium hydrogen carbonate/min into the mesenteric vein for 4 d*

(Mean values for three sheep with the standard error of the difference between means)

	C0	C150	C400	SED	*P*†
Arterial blood variables					
pH	7·416	7·453	7·436	0·029	0·55
Blood $p(CO_2)$ (mmHg)	35·0	35·03	33·32	0·134	0·80
Blood HCO_3^- (mmol/l)	22·24	24·35	22·18	0·273	0·55
Haemoglobin (g/l)	103·6	101·6	102·6	1·62	0·57
NH_3 (μmol/l)					
Aorta	63	49	74	2·8	0·024
Portal vein	343	534	743	68·8	0·003
Hepatic vein	30	43	45	13·6	0·563
Urea (mmol/l)					
Aorta	4·36	5·99	8·01	0·406	0·024
Portal vein	4·23	5·80	7·84	0·406	0·025
Hepatic vein	4·50	6·19	8·32	0·423	0·024

*For details of procedures, see p. 308.
† The data were analysed by ANOVA, with 2 or 6 (portal vein) d.f. for the error term. t (0·05, 2) = 4·30; t (0·05, 6) = 2·45.

concentration were larger than would be expected (i.e. 125 and 290 μmol/l for C150 and C400 respectively) on the basis of the portal blood flow, basal portal NH_3 concentration and the infusion rates of NH_4HCO_3.

The arterial concentrations of urea increased by 1·63 and 3·65 mmol/l after the 4 d infusion of NH_4HCO_3 at 150 and 400 μmol/min ($P < 0·05$), with parallel responses in the portal and hepatic venous concentrations.

With the exception of leucine, which increased ($P < 0·05$) from 157 μmol/l in C0 to 186 μmol/l in C400, no changes were detected in the arterial concentration of amino acids as a result of the infusion of NH_4HCO_3 (results not shown). Regardless of the treatment, plasma concentrations of several amino acids (e.g. phenylalanine, $P < 0·06$; leucine, $P < 0·01$; isoleucine, $P < 0·08$; results not shown) in the first experimental period were lower than in subsequent periods.

Blood flow, gas exchange and glucose transfers (Table 2)

The blood flows in the portal and hepatic veins were not

Table 2. Blood flow and net mass transfer of oxygen, glucose, ammonia, urea and amino acid-nitrogen across the portal-drained viscera (PDV) and the liver and urea irreversible loss rate (ILR) in sheep infused with 0 (C0), 150 (C150) or 400 (C400) μmol ammonium hydrogen carbonate/min into the mesenteric vein for 4 d*†

(Mean values for three sheep with the standard error of the difference between means)

	C0	C150	C400	SED	*P*‡
Blood flow (g/min)					
Hepatic artery	104	66	82	52	0·78
Portal vein	1268	1218	1380	248	0·82
Hepatic vein	1372	1284	1463	197	0·71
PDV (μmol/min)					
O_2§	−1593	−1647	−1843	105·0	0·24
Glucose§	−32	−39	−88	37·0	0·43
NH_3	344	589	908	39·7	<0·001
Urea-N	−322	−430	−610	24·6	0·015
Amino acid-N§‖	316	346	522	46·4	0·080
Liver (μmol/min)					
O_2§	−1610	−1600	−2003	174·0	0·13
Glucose§	315	325	352	10·6	0·13
NH_3	−391	−600	−954	46·2	<0·001
Urea-N	710	912	1356	105·6	0·002
Amino acid-N§‖	−263	−331	−377	197·9	0·86
Urea IRL (μmol urea-N/min)	774	1012	1336	79·2	0·001

*For details of procedures, see pp. 308–309.
† Positive and negative values indicate net production and net extraction of the metabolite across the relevant organ respectively.
‡ The data were analysed by ANOVA with 2 or 6 d.f. for the error term; t (0·05, 2) = 4·30; t (0·05, 6) = 2·45.
§ 2 d.f.
‖ Does not include valine and proline.

altered by NH_4HCO_3 infusion. The small contribution of the hepatic artery to the liver blood flow (6%) was also unaltered. The liver and the PDV each accounted for 0·5 of the O_2 consumption measured across the splanchnic bed (3·20 mmol/min) in C0. Based on the traditional estimation of 0·66 mol O_2/mol urea (i.e. 4 ATP/mol urea), urea synthesis would account for 0·15 of liver energy expenditure under basal dietary conditions. The O_2 consumption by both tissues showed an upward trend during the infusion of 400 μmol NH_4HCO_3/min, but only 0·54 of the observed increase in liver O_2 consumption could be theoretically accounted for by the additional urea synthesised. Basal glucose uptake by the PDV was, at 32 μmol/min, 0·10 of that released by the liver, with neither altered by the administration of NH_4HCO_3.

Ammonia and urea transfers (Table 2)

Dietary N intake was 16·4 g/d^{-1}, equivalent to 815 μmol N/min under continuous feeding conditions. Although urea transfer to the gastrointestinal tract (GIT) remained constant at 0·45–0·47 of liver production across all three treatments, the absolute transfer increased with both levels of NH_4HCO_3 infusion ($P < 0·02$). This endogenous urea-N recycling to the GIT was equivalent to 0·40–0·75 of ingested N.

Basal NH_3 appearance in the portal vein (y, μmol/min; i.e. net NH_3 appearance corrected for the infusion rate of NH_4HCO_3) amounted to 0·42 of dietary N intake in C0, increased by 95 and 164 μmol/min in C150 and C400 respectively ($P < 0·02$), and showed a linear correlation with urea-N transfer to the GIT (x, μmol/min; $y = 188·5$ (SE 43·12) + 0·53 (SE 0·091) x; $P < 0·001$; RMS 1324·8; R^2 0·83).

Liver extraction of NH_3 was augmented ($P < 0·001$) in response to the NH_3 load and was 1·02–1·14 higher than NH_3 appearance across the PDV, indicating that the liver also removed NH_3 released from non-splanchnic tissues. Urea-N production by the liver was 1·81 times the basal NH_3 uptake for treatment C0 and rose significantly by 202 and 646 μmol/min following the infusion of 150 and 400 μmol NH_4HCO_3/min. Similar trends were observed for urea ILR (y, μmol/min), which showed good concordance with hepatic urea production (x, μmol/min; $y = 321·4$ (SE 160·38) + 0·734 (SE 0·155) x ; $P = 0·002$; $P_{b \neq 1} = 0·13$; RMS 18137·77; R^2 0·76). A strong linear relationship was observed between urea-N release (y, μmol/min) and NH_3 extraction (x, μmol/min) across the liver, with the slope of the regression equation not different from unity, regardless of whether estimations were based on GC–mass spectrometry analysis ($y = 241·3$ (SE 100·05) + 1·16 (SE 0·144) x; $P < 0·001$; $P_{b \neq 1} = 0·31$; RMS 10626·8; R^2 0·89; Fig. 1) or [^{14}C]urea IRL ($y = 409·5$ (SE 84·60) + 0·97 (SE 0·123) x; $P < 0·001$; $P_{b \neq 1} = 0·84$; RMS 7597·6; R^2 0·89).

Amino acid transfers (Table 3)

For all treatments there was net absorption of amino acids across the PDV and net removal by the liver, with the exception of glutamate, citrulline and ornithine, which exhibited net release by the liver (results not shown). Initial calculations also showed a net hepatic release of valine;

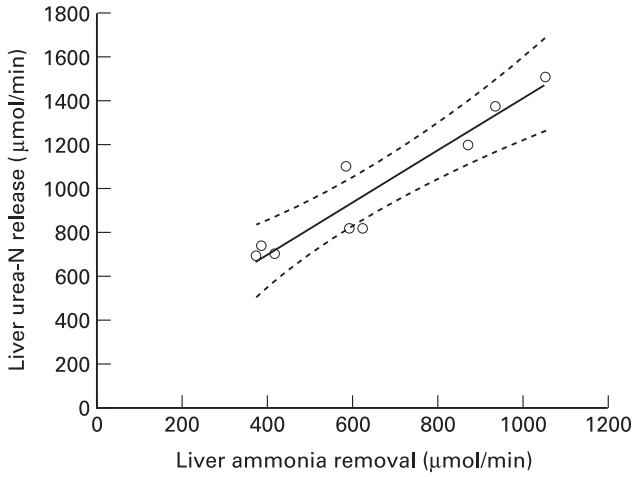

Fig. 1. Regression of urea-nitrogen release (estimates based on GC–mass spectrometry analysis) *v.* ammonia extraction across the liver in sheep infused with 0, 150 or 400 μmol ammonium hydrogen carbonate/min into the mesenteric vein for 4 d. For details of procedures, see pp. 308–309.

later studies revealed that a non-identified peak co-chromatographed with valine and interfered with the determination of plasma valine concentrations. Valine data were, therefore, not included in the estimation of net PDV, hepatic and total splanchnic transfers of total, essential and branched-chain amino acids.

Under basal dietary conditions, net amino acid-N absorption by the GIT (316 μmol/min) accounted for 0·38 of the dietary N intake (or 0·28 of dietary N plus urea-N inputs to the GIT). The movement of amino acids across the PDV remained unaltered by the infusion of either level of NH_4HCO_3 ($P > 0·05$), although portal appearances of arginine ($P < 0·03$) and non-essential amino acids ($P < 0·04$) increased while those of total amino acid-N (see Table 2), threonine, asparagine, glutamate, glutamine and glycine also tended to be greater ($P < 0·1$; results not shown) at the highest level of infusion. Net absorption of amino acid-N and urea-N removal by the GIT were highly correlated (R^2 0·84; Fig. 2).

Hepatic extraction of total, essential, non-essential, branched-chain, glucogenic or individual amino acids was unaltered by the treatments. Hepatic extraction of total amino acid-N accounted, on average, for 0·82 of the amino acid-N appearance across the PDV. The hepatic fractional removal (hepatic removal : PDV appearance) varied markedly among individual amino acids, however, being close to, or higher than, unity for tyrosine, phenylalanine, histidine, threonine and the glucogenic serine, alanine, glycine and glutamine (the latter removed at a rate seven times higher than the PDV appearance), intermediate (0·5–0·7) for methionine and lysine and lowest (< 0·2) for leucine and isoleucine (results not shown).

Liver nitrogen balance

The net balance of N across the liver (estimated as: NH_3 removal + free amino acid-N removal – urea-N release) was not affected by the NH_4HCO_3 infusion and averaged –21 (SEM 21·8) μmol/min.

Table 3. Net mass transfer of amino acids across the portal-drained viscera (PDV), the liver and the splanchnic bed (μmol/min) in sheep infused with 0 (C0), 150 (C150) or 400 (C400) μmol ammonium hydrogen carbonate/min into the mesenteric vein for 4 d*†

(Mean values for three sheep with the standard error of the difference between means)

	TA	EA	BA	NA	GA
PDV					
C0	219·1	90·1	38·8	117·2	80·4
C150	238·8	94·4	36·2	132·6	75·9
C400	361·7	148·0	61·4	200·7	123·3
SED	42·72	32·46	21·91	12·35	10·40
P‡	0·132	0·337	0·556	0·037	0·073
Liver					
C0	–173·1	–45·6	–2·5	–132·6	–118·9
C150	–224·5	–65·7	–9·2	–169·7	–136·4
C400	–257·5	–77·8	–17·4	–193·2	–156·7
SED	111·75	45·17	17·93	55·27	33·70
P‡	0·775	0·794	0·743	0·621	0·614
Splanchnic bed					
C0	46·0	44·5	36·4	–15·4	–38·5
C150	14·2	70·2	27·1	–37·1	–60·5
C400	104·2	70·2	44·1	7·4	–33·4
SED	131·08	50·25	18·99	63·42	42·97
P‡	0·805	0·742	0·714	0·802	0·817

TA, total amino acids (Asp, Asn, Glu, Gln, Ser, Gly, Ala, Tyr, Arg, Thr, Met, Ile, Leu, Phe, Lys, His, Cit, Orn); EA, essential amino acids (Thr, Met, Ile, Leu, Phe, Lys, His); BA, branched chain amino acids (Leu, Ile); NA, non-essential amino acids (Asp, Asn, Glu, Gln, Ser, Gly, Ala, Tyr, Arg); GA, glucogenic amino acids (Ser, Gln, Gly, Ala).

* For details of procedures, see pp. 308–309.

† Positive and negative values indicate net production and net extraction of the metabolite by the relevant organ respectively.

‡ The data were analysed by ANOVA, with 2 d.f. for the error term; $t(0{\cdot}05, 2) = 4{\cdot}30$.

Discussion

The present study was designed to address the question of whether increasing the portal supply of NH_3 to the liver could alter the availability of energy and amino acids to non-splanchnic tissues, thus compromising protein and energy deposition in growing male lambs. Data from previous studies *in vivo* and *in vitro* indicated that both phenomena might indeed arise under conditions of high input of NH_3 to the liver, but the information available was controversial on both a qualitative and a quantitative basis because NH_3 was not the only variable modified by the treatments (Orzechowski *et al*. 1988; Reynolds *et al*. 1991; Lobley *et al*. 1995; Luo *et al*. 1995). In most cases either nutrient supply or acid–base status had also been altered and, thus, the interpretation of the results was confounded. In order to minimize these problems an experimental design was adopted in which only one variable, the NH_3 supply to the liver, was modified by direct infusion of two levels of NH_4HCO_3 into the mesenteric vein.

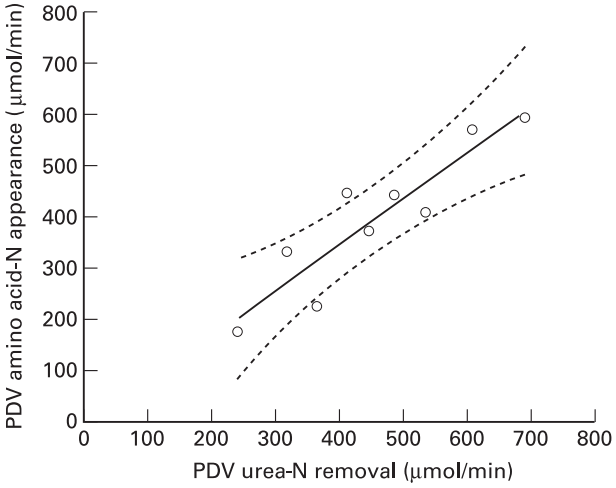

Fig. 2. Regression of portal-drained viscera (PDV) amino acid-nitrogen appearance (*y*, μmol/min) *v.* PDV urea-nitrogen removal (*x*, μmol/min; $y = -16{\cdot}2$ (SE 71·73) + 0·90 (SE 0·151) *x*; $P < 0{\cdot}001$; $P_{b \neq 1} = 0{\cdot}57$; residual mean square 3664·87; R^2 0·84) in sheep infused with 0, 150 or 400 μmol ammonium hydrogen carbonate/min into the mesenteric vein for 4 d. For details of procedures, see pp. 308–309.

Liver oxygen consumption and gluconeogenesis

There is little disagreement, at present, that stimulation of ureagenesis increases liver O_2 consumption (e.g. Reynolds *et al*. 1991); the resultant elevated energy expenditure will reduce whole-body energy deposition. Yet doubts still exist about the actual magnitude of this effect. The measured increase in liver O_2 consumption during the administration of NH_4^+ into the mesenteric vein has usually ranged from 1·5 to 5·5 mol O_2 per additional mol of urea synthesized (i.e. 2–8-fold greater than theoretical estimates based on 4 ATP or 0·66 mol O_2 per mol urea synthesized; Milano, 1994; Lobley *et al*. 1995, 1996*b*). With the exception of determinations carried out in fasted sheep infused with NH_4HCO_3 (Milano, 1994), the differences with pre-infusion levels never achieved statistical significance.

This experiment is, unfortunately, no exception in that the largest average increase in liver O_2 consumption (393 μmol/min or 1·2 μmol O_2 per additional μmol urea synthesized, for C400) was 1·85 times higher than the expected theoretical maximum but the variance was, again, large (SED 174 μmol O_2/min, $P = 0·13$). If energy expenditure elicited by NH_3-stimulated ureagenesis exceeds theoretical values then other reactions (e.g. transport of substrates across subcellular membranes, intrahepatic cycling of glutamine) must be also enhanced. Lately, however, it has been pointed out that if the traditional P : O of 3 : 1 were replaced by other less efficient ratios (e.g. 2 : 1 or even 1·5 : 1; see Lobley, 1994), then much of the discrepancy between theoretical considerations and empirical evidence would disappear.

Studies in isolated sheep hepatocytes have revealed that gluconeogenesis from propionate could be depressed by 20–40 % at NH_4Cl concentrations of 500–660 μmol/l (Weekes *et al*. 1978; Luo *et al*. 1995). When this was tested *in vivo*, however, the results were contradictory. Liver glucose output was either depressed by 48 % (Orzechowski *et al*. 1988) or unaltered (Barej *et al*. 1987) during short-term infusions (120 min) of NH_4Cl into the mesenteric vein of sheep at rates sufficient to achieve portal concentrations of 800–850 μmol/l and exceed the capacity of the liver for NH_3 removal (1·5 μmol/min per g wet tissue). In the present experiment, hepatic glucose production remained unaffected after a 4 d administration of NH_4HCO_3 and at similar portal NH_3 flows and concentrations (i.e. 740 μmol/l in C400) to the previous studies. This suggests that the high portal NH_3 load that may occur in ruminants, e.g. those given rations with a high non-protein-N content or fresh forage rich in rapidly degradable protein, does not impair liver glucose supply for extra-hepatic tissue metabolism.

Ammonia, urea and amino acid transfers

Experimental evidence obtained from studies of splanchnic transfer of N-compounds over the last decade (see reviews of Reynolds, 1992; Parker *et al*. 1995; Lescoat *et al*. 1996), indicated that, on an incremental basis, removal of NH_3 by the liver is associated with a larger than equimolar release of urea-N; the slope of the regression of urea-N release *v.* NH_3 uptake across studies ranged between 1·6 and 1·9. Free amino acids were the only N-compounds available to the liver in sufficient amounts to provide the additional N. This led to the hypothesis that amino acid availability for hepatic synthesis of export proteins, or for non-splanchnic tissue utilization, would be progressively reduced as the rate of NH_3 removal by the liver increased (Reynolds, 1992; Parker *et al*. 1995).

The hypothesis gained further support from long-term studies in sheep. When the basal rate of hepatic NH_3 removal was increased by continuous administration of NH_4Cl into the mesenteric vein for 5 d, not only was the additional urea-N released from the liver 2-fold greater than the additional NH_3 removed but whole-body leucine oxidation increased significantly by 18 % (Lobley *et al*. 1995). Slight decreases in plasma pH (0·08 units) and plasma HCO_3^- concentration (5 mmol/l) were also reported during the NH_4Cl infusions. Studies in human subjects (Reaich *et al.* 1992) and rodents (May *et al*. 1992) have demonstrated that acidosis elicited by the administration of NH_4Cl can stimulate protein breakdown and amino acid oxidation, leaving a surplus of amino acid-N available for urea synthesis. Thus, it was unclear whether the higher leucine oxidation and the additional N appearing in urea observed by Lobley *et al*. (1995) were consequences of the higher NH_3 removal or of the concomitant mild acidosis.

In a subsequent study, in which acidosis was avoided by a continuous 4 d infusion of NH_4HCO_3 into the mesenteric vein, the incremental values for urea-N release : NH_3 removal across the liver of growing lambs were 1·12 and 1·17 after hepatic NH_3 uptake was increased by 208 or 325 μmol/min respectively (Lobley *et al*. 1996*b*). The nutritional conditions involved were, however, substantially different, with a higher feed intake (2·0 *v.* 1·2 times energy maintenance) compared with the initial experiment of Lobley *et al*. (1995). This increased markedly the amino acid-N absorbed by the animals (1155 *v.* 372 μmol/min) and reduced the ratio NH_3 : amino acid-N appearing in the portal vein from 1·3–1·9 to 0·5–0·8, lower than expected for diets with high non-protein-N content (1·1–1·5; Maltby *et al*. 1991). In addition, it is well established that the activity of urea cycle enzymes is enhanced in response to high-protein diets, primarily as a consequence of changes in enzyme mass (Morris, 1992). There was concern, therefore, that these changes may have altered the hepatic capacity to handle excess NH_3, as shown in the perfused rat liver, where the ability to form urea from 600 μM-NH_4Cl increased with the amount of casein in the diet (Saheki, 1972).

Thus, the present experiment was designed to meet two fundamental criteria. First, the acid–base status of the animals remained unchanged during the administration of NH_4^+ and, in this respect, the choice of the bicarbonate salt seemed appropriate because it had been used without obvious alteration of the acid–base status in two experiments at a rate similar to that of C150 (Milano, 1994; Lobley *et al*. 1996*b*). At higher doses, however, $NaHCO_3$ had been shown to cause acute metabolic alkalosis in rats (Boon *et al*. 1994) and dogs (Rodriguez *et al*. 1989). This raised concerns about the possible long-term increase in plasma HCO_3^- concentration with the highest level of NH_4HCO_3 infusion (C400). Nevertheless, the acid–base status of the sheep remained within the normal range throughout the experiment.

Second, amino acid-N appearance across the PDV had to match that reported by Lobley *et al*. (1995) (i.e. 406 *v.* 372 μmol/min) but at comparatively higher rates of hepatic NH_3 removal (900 *v.* 600 μmol NH_3/min). It was, therefore, critical that the additional source of N would not alter the net amino acid supply to the lambs. It was expected that some of the urea synthesized by the liver, as a result of the infusion of NH_4HCO_3, would be transferred to the GIT. This could increase net microbial protein yield if the basal (C0) rumen degradable N supply was below that required to sustain optimum microbial protein yield *in vivo* (29–32 g N/kg organic matter truly digested in the rumen; Agricultural and Food Research Council, 1993). Any additional amino acid absorption could result in increased urea synthesis and tend to lower the apparent efficiency of conversion of NH_3 to urea. The marked trend towards higher values of PDV

amino acid-N absorption in C400 and the linear correlation between amino acid-N and urea-N transfers across the PDV suggest that the assumption of unchanged amino acid supply did not hold for the highest level of infusion.

Nevertheless, the slope of the regression of hepatic urea-N release (1·16; SE 0·14) or [^{14}C]urea-N IRL (0·97; SE 0·12) on hepatic NH_3 removal, together with the steady hepatic amino acid extraction observed under these experimental conditions support earlier observations *in vivo* in steers (Maltby *et al.* 1991) and sheep (Lobley *et al.* 1996*b*) and contradict the concept that equal inputs of N from sources exogenous or endogenous to the liver, namely amino acids, are necessary to maintain NH_3-stimulated ureagenesis. Studies *in vitro*, where [^{15}N^{15}N]urea was synthesized from $^{15}NH_4Cl$, the only exogenous N source (Luo *et al.* 1995; Brosnan *et al.* 1996), have provided evidence that NH_3-N can enter the ornithine cycle by two separate routes: synthesis of carbamoyl phosphate, through a reaction catalysed by carbamoyl phosphate synthase I, and reductive amination of 2-oxoglutarate followed by transamination to aspartate, a pathway controlled by glutamate dehydrogenase (GDH) and aspartate aminotransferase. If the additional NH_3 supply to the liver in the current experiment was converted to urea-N without extra amino acid-N inputs to the ornithine cycle, then the GDH–aspartate aminotransferase pathway must have had the capacity to utilize half the extra NH_3 removed. For C400, for example, this required an additional flux of 281 μmol N/min to glutamate and aspartate synthesis, which represents an 8-fold increase in the net flux through GDH (calculated as: liver NH_3 removal − (0·5 × urea-N release)), from an estimated basal rate (C0) of 36 μmol N/min. The capacity of liver GDH to utilize excess NH_3 to synthesize glutamate is, therefore, central to the question of detoxifying NH_3 with or without alteration in the partition of amino acid-N flow towards ureagenesis.

The current study indicates that the capacity of GDH to meet such demands is either inherent or can be adapted when portal NH_3 input to the liver is augmented and sustained for 4 d at nearly 3-fold that in basal conditions. Distinction between these mechanisms seems of less practical significance for long-term than for short-term (i.e. 2–3 h) increases in NH_3 supply to the liver. The former (i.e. intrinsically high liver GDH activity) would be of importance to allow removal of acute excesses of NH_3 without penalizing amino acid availability to the animal.

Confirmation of equal partition of the additional NH_3 inputs to the liver between the GDH–aspartate aminotransferase and carbamoyl phosphate synthase I pathways requires further experimentation, however. First, it is not certain that the conclusions of the current, and previous, experiments in growing lambs and steers can be extrapolated to other diets (e.g. fresh grass or legumes), feeding routines (e.g. discontinuous feeding) and physiological conditions (e.g. pregnancy, lactation) where different balances of hormones and nutrients (e.g. propionate) may occur. Second, the deviation from unity of the slope of the regression of urea-N release *v.* NH_3 removal across the liver (i.e. 0·16) might still represent a minor, but not negligible, stimulation of amino acid-N utilization for ureagenesis. With a 300 μmol/min increase in hepatic NH_3 extraction, for example, the deviation of the ratio from unity observed in the current study would amount to 0·96 g N/d. Moreover, enhancement of amino acid oxidation during sustained increase of NH_3 inputs to the liver could be entirely unrelated to limitations in N flux through GDH, because amino acids could also be used as energy substrates to meet the increased demands of hepatic ureagenesis. In C400, for example, with an increase in liver O_2 consumption of 393 μmol/min, an extra 84 μmol amino acid-N per min might have been oxidized to provide the necessary energy to sustain ureagenesis (i.e. 176 J/min, based on 448 kJ/mol O_2 and 24 MJ/kg protein or 2·1 kJ/mol protein N). The numerical agreement between this value and the additional amino acid-N flow to urea in C400 as measured by GC–mass spectrometry (83 μmol/min, estimated from additional urea-N release − additional NH_3 removed across the liver) is remarkable, but probably coincidental.

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