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The fate of absorbed and exogenous ammonia as influenced by forage or forage–concentrate diets in growing sheep

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Changes in splanchnic energy and N metabolism were studied in sheep, prepared with vascular catheters across the portal-drained viscera (PDV) and the liver, and maintained on supramaintenance intakes of either grass or grass + barley pellets. The animals were challenged, on both diets, with 4 d intra-mesenteric vein infusions of NH_4Cl (25 μmol/min) plus NH_4HCO_3 (at either 0 or 125 μmol/min). On the final day of each treatment the natural abundance NH_4Cl was replaced with $^{15}NH_4Cl$ over a 10 h infusion while over the same period [1-^{13}C]leucine was infused via a jugular vein. Measurements were made of blood flow plus mass transfers of NH_3, urea, free amino acids and O_2 across the PDV and liver. Enrichments of [^{14}N^{15}N]urea and [^{15}N^{15}N]urea plus [^{15}N]glutamine, aspartate and glutamate were also monitored. Whole-body urea flux was determined by infusion of [^{14}C]urea. At the end of the study the animals were infused for 3 h with $^{15}NH_4Cl$, killed and liver samples assayed for intracellular free amino acid enrichments and concentrations. Blood flows across the splanchnic region were unaffected by either diet or level of ammonium salt infusion. At the lower ammonium salt infusion there was a trend for greater absorption of NH_3 across the PDV ($P < 0{\cdot}10$) with grass + barley than with the grass diet, while removal of urea was unaltered. At the higher ammonium salt infusions there was a significantly greater appearance of NH_3 across the PDV and this exceeded the extra infused. Urea-N removal, however, was also elevated and by more than that required to account for the additional NH_3. The PDV contributed 19–28 % to whole-body O_2 consumption and the liver 23–32 %. Hepatic extraction of absorbed NH_3 was complete on all treatments and systemic pH remained constant. The fractions of urea-N apparently derived from NH_3 were similar on the grass (0·59–0·64) and grass + barley (0·64–0·67) diets. Hepatic production of urea agreed well with urea flux measurements. Between the two levels of ammonium salt infusion and within diets the additional NH_3 removed across the PDV was accounted for by the increased urea-N production. The [^{14}N^{15}N]:[^{15}N^{15}N] ratio of the urea produced was 97:3, while the enrichment of hepatic intracellular free aspartate was lower than that of [^{14}N^{15}N]urea. Glutamine enrichments were 0·23–0·37 those of [^{14}N^{15}N]urea, indicating a minor role for those hepatocytes (probably perivenous) which contain glutamine synthetase (*EC* 6.3.1.2). Leucine kinetics, either for the whole body or splanchnic tissues, were not different between diets or level of ammonium salt infusion, except for oxidation which was less on the grass + barley ration. Amino acid concentrations were lower on the grass + barley diet but net PDV absorptions were similar. The pattern of essential amino acids absorbed into the PDV showed good agreement with the published composition of mixed rumen microbial protein. Fractional disappearances of absorbed free essential amino acids across the liver varied from 0·4 (branched chains) to near unity (histidine, phenylalanine).

Nitrogen metabolism: Amino acid metabolism: Stable isotopes: Leucine

Absorbed N substrates are partitioned between anabolic and catabolic fates, with the end-product of the latter usually as urea. Considerable interest has therefore centred on factors which regulate and influence ureagenesis in the farm species, with the main aims to divert more absorbed N to productive routes and reduce excreta pollution. In ruminants, animal

performance is usually superior on rations containing more concentrates (Reynolds *et al.* 1991*a*); the reasons for this are unclear but may relate to the relative absorption of NH_3:amino acid-N. This ratio is greater for cattle fed on high-forage as opposed to high-concentrate diets (e.g. Huntington, 1989; Reynolds *et al.* 1991*b*) and, furthermore, hepatic removal of absorbed NH_3-N has been linked to an apparent over-production of urea-N (Huntington, 1989; Maltby *et al.* 1991; Reynolds & Tyrrell, 1991; Reynolds *et al.* 1991*b*). From these findings has evolved the concept that there may be a net requirement of amino acid-N to support hepatic NH_3 detoxification (Reynolds, 1992).

Hitherto, much of the information has been of a correlative nature and extracted from studies which often involved changes in diet quantity or formulation and, therefore, factors other than NH_3 absorption were probably also altered. Furthermore, the relationship between hepatic NH_3-N removal:urea-N production has varied considerably, on both an absolute and incremental basis, between the various studies (0·48–1·00). This may be due to the nature of the diets offered because Fitch *et al.* (1989), in a preliminary report, observed that although absolute hepatic urea synthesis was greater with steers offered a forage as opposed to a forage–concentrate ration, the fraction of urea-N apparently derived from NH_3 was much less (0·35 *v.* 0·79). Thus, on the forage diet, substantial quantities of non-NH_3-N entered the ornithine cycle, with amino acids as prime candidates to supply the additional N. The lower rate of ureagenesis with the forage–concentrate diet may relate to greater absorption of propionate (e.g. Reynolds *et al.* 1991*b*), which has a putative role as an inhibitor of N-acetylglutamate synthesis (Stewart & Walser, 1980), an activator of carbamoyl phosphate synthetase I (CPS; *EC* 6.3.4.16), the rate-limiting enzyme of the ornithine cycle (Meijer *et al.* 1990).

The hypothesis that the efficiency with which NH_3 can be converted to urea is related to diet type has been examined in the current study with sheep offered pellets of either roughage or roughage plus barley, at quantities sufficient to promote moderate growth. The specific conversion of NH_3 was monitored by chronic intravenous infusion of exogenous NH_4HCO_3 in an approach similar to that described previously (Lobley *et al.* 1995). The supplemental use of [^{15}N]ammonium salt and [1-^{13}C]leucine infusions allowed greater interpretation through comparison of mass and kinetic transfers.

METHODS

Animals

Four Suffolk cross-bred wether lambs (mean initial live weight 31 kg, 6–9 months old) were prepared with silastic catheters in the aorta, mesenteric (2), hepatic portal and hepatic veins (of the ventral lobe) as described previously (Lobley *et al.* 1995). Animals were allowed 3 weeks recovery from surgery before allocation to the trial (at mean start weight of 33·5 kg) and temporary catheters were inserted in the jugular veins (Lobley *et al.* 1995).

Diets

Animals were supplied with either 1200 g (as fed) of grass pellets (G; 10·5 MJ metabolizable energy (ME)/kg DM; 31·8 g N/kg DM; DM 910 g/kg) or 600 g of these plus 600 g (as fed) barley pellets (B; 12·8 MJ ME/kg DM, 24·8 g N/kg DM; DM 890 g/kg). Rations were provided as twenty-four similar portions daily by means of automated feeders.

Experimental design

Each sheep received each of the two diets (G and the mixture GB). During each diet period animals were infused (20 g/h) on two occasions via a mesenteric vein catheter with different

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sterile solutions which contained either low (L; 75 mM-NH_4Cl; 25 μmol NH_4^+/min) or high (H; 75 mM-NH_4Cl in 375 mM-NH_4HCO_3; 150 μmol NH_4^+/min) amounts of ammonium salt dissolved in physiological saline (0·15 M-NaCl). Infusions covered 4 d and on the last day mass and isotope transfers across the splanchnic tissues were quantified. Within-diet comparisons were made 7 d apart. Two weeks were then allowed to change diets. Each animal was therefore studied on four occasions, GL, GH, GBL, GBH, with the design balanced in such a way that two animals started on each diet and with the order of NH_3 infusions either LHHL or HLLH. At the end of each dietary period animals spent 4 d in automated confinement respiration chambers (Blaxter *et al.* 1972) for measurement of whole-body gaseous exchange, but without any ammonium salt infusion; this provided a comparative base for the contribution of splanchnic tissue metabolism to whole-body O_2 uptake.

Isotope infusions

[^{14}C]urea. In order to provide a comparison with urea production across the liver determined by the arterio-venous approach, urea entry rate was also quantified during a 54 h continuous infusion, into a jugular vein, of a [^{14}C]urea solution (0·0037 MBq/g; 5 g/h) prepared in sterile saline and containing 1 mM-unlabelled urea as carrier. Infusions were started 24 h after commencement of the ammonium salt administration with prior samples of urine, collected over 3 h, and blood (5 ml) taken to provide a 'background' sample. Over the last 4·5 h of [^{14}C]urea infusion urine samples were collected over 1·5 h intervals while two 1 h integrated blood samples (each 5 ml) were taken from the aorta, hepatic portal and hepatic veins (Lobley *et al.* 1995) between 50 and 52 h. These samples were also used as natural abundance references for the stable-isotope infusions performed the next day. Both urine and blood were immediately processed as described later.

[1-^{13}C]leucine and [^{15}N]ammonium salt. A sterile solution containing 0·5 g [1-^{13}C]leucine (99 molar %; Tracer Technology Ltd, Somerville, MA, USA) and 0·4 g sodium heparin in 200 g physiological saline was infused over 10 h (20 g/h) into the jugular vein on day 4 of the ammonium salt infusion. Over the same 10 h the ammonium salt infusate into the mesenteric vein was replaced with 200 g of a solution in which the NH_4Cl was replaced with $^{15}NH_4Cl$ (98·4 molar %; Europa Scientific, Crewe, Ches.) and which also contained 0·1 M-sodium p-amino hippurate (pAH) and 0·05 M-sodium phosphate buffer, final pH 7·4. This was also infused at a rate of 20 g/h, so that the same rate of ammonium ion supply was maintained as for the previous 3 d but now 25 μmol/min of which was as $^{15}NH_4^+$.

Between hours 5 and 9 of stable isotope infusion, four integrated hourly blood samples, each of 12 ml, were collected from the aorta, hepatic portal and hepatic veins as described previously (Lobley *et al.* 1995). Samples were immediately analysed for pH, pCO_2, pO_2 and haemoglobin; two 1 g portions were then stored for bicarbonate enrichment. From the remainder both plasma and haemolysed blood, prepared with an equivolume of a solution containing 2-oxohexanoate and L-norleucine, were obtained as described previously (Lobley *et al.* 1995). Three urine samples were also collected at 1·5 h intervals between hours 5 and 10 of this infusion.

At the end of the experiment animals were rested for at least 2 weeks and then infused via the mesenteric vein for 3 h with 70 mM-$^{15}NH_4Cl$ (25 μmol $^{15}NH_4^+$/min) and two 0·5 h integrated samples, taken from the same blood vessels as above, were collected over the last hour of infusion. Immediately following the last blood sample the animals were killed by an overdose of liquid anaesthetic and sections of liver rapidly excised, frozen in liquid N_2 and stored at −70°.

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Analyses

Preparation and measurement of blood pH, pCO_2, pO_2, haemoglobin (corrected for the spectral difference at 505 nm between human and sheep haemoglobin), bicarbonate enrichment, blood and plasma enrichments and concentrations of leucine and 4-methyl-2-oxopentanoate (MOP) were as described previously (Lobley *et al.* 1995) as was the determination of blood flow from pAH analyses. Plasma flow was calculated from blood flow and packed cell volume (PCV) determination. For enrichments (molar % excess; mpe) of [$^{15}N^{15}N$]- and [$^{14}N^{15}N$]urea and ^{15}N-labelled amino acids by gas-chromatography–mass-spectrometry (GCMS) the modification of the *t*-butyldimethylsilyl derivative preparation recommended by Patterson *et al.* (1993) was adopted. This allowed resolution of glutamine into [5-N], total ([5-N] plus [2-N]) and bis ([2,5-N_2]) labelled species. Liver free amino acid enrichments were determined in supernatant fractions from tissue homogenates prepared in 70 g/l sulphosalicylic acid based on the procedure described by Calder & Smith (1988).

Urea concentrations were determined on plasma and haemolysed blood by the diacetyloxime procedure while plasma NH_3 was determined immediately by a linked glutamate dehydrogenase (*EC* 1.4.1.2; GLDH) automated procedure as described previously (Lobley *et al.* 1995). Companion studies (G. D. Milano and G. E. Lobley, unpublished results) revealed that NH_3 concentrations in blood and plasma were equal and, because of technical problems associated with determination of the former, blood NH_3 transfers were calculated from the products of plasma concentrations and blood flows.

Amino acid concentrations in blood and plasma were determined on a pooled mixture prepared from the four samples obtained from each blood vessel within an infusion period. For hepatic portal and hepatic venous blood the samples were mixed in proportion to the respective blood (plasma) flows within each hour. Arterial blood was mixed on an equivolume basis; this was because the hepatic arterial flow was obtained by difference between hepatic portal and hepatic venous flow and was small (less than 0·1 of hepatic venous flow) with considerable variance. Mixing by derived flow might therefore tend to introduce errors and/or sample bias. Amino acid analysis was by ion-exchange chromatography and ninhydrin detection as used previously (Lobley *et al.* 1995). Monomethyllysine and 1-methylhistidine co-chromatographed and the peak was quantified based on the ninhydrin colour yield of the former as this was the expected major contributor.

For [^{14}C]urea, blood and urine samples were deproteinized with 1·0 volume 100 g/l TCA and duplicate 1 g portions of the supernatant fractions mixed in 10 ml Optima Gold (Canberra Packard, Pangbourne, Berks.). Radioactivity in all samples was determined using a Packard 1900 Tri-Carb liquid scintillation counter. Urea concentrations were determined in the TCA supernatant fractions by the automated diacetyloxime procedure. Four different preparative procedures were used for analysis of urine for [$^{14}N^{15}N$]- and [$^{15}N^{15}N$]urea. Urine was treated in one of the following ways: (1) reacted directly for GCMS analysis, (2) acidified to 100 g/l TCA and the supernatant fraction analysed, (3) the acidified supernatant fraction adjusted to pH > 9 with NaOH and freeze-dried to remove NH_3 or (4) the urea isolated by ion-exchange chromatography (Jackson *et al.* 1980). The last three preparations were then treated with alkaline hypobromite and the amounts of N_2 gas produced with m/z of 28, 29 and 30 quantified by isotope-ratio mass spectrometry (IRMS).

Calculations and statistics

Mass and isotope transfers were as detailed previously (Lobley *et al.* 1995) and the mean values of the four 1 h collections used for data analyses. Urea entry rate (μmol/min) was calculated from I/SRA_u, where I is the rate of infusion of [^{14}C]urea (μCi/min) and SRA_u the specific radioactivity (μCi/μmol) of urea in either blood or urine.

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The split-plot design of the experiment (with diet assigned to main plots) allowed standard ANOVA (Genstat 5; Rothamsted Experimental Station, Harpenden, Herts.) with separation of animal, diet, treatment and diet × treatment effects. Significances between treatments were assessed by use of the *t* statistic. For comparison of isotopic urea flux and hepatic release of urea, values were fitted to a linear regression equation where the latter was the independent variable and the constant set to zero.

RESULTS

All four sheep completed the trial but, as in the previous study (Lobley *et al.* 1995), most of the distal mesenteric catheters eventually failed, in that blood could not be withdrawn (the index used for vessel patency) and, where necessary, experiments were finished with the proximal mesenteric catheter used for infusion. In one sheep this catheter was found to have entered the portal vein and this proved fortuitous as it provided a substitute when the normal portal vein catheter failed after two measurements. All other catheters remained patent throughout. Average live-weight gain over the 6 weeks of experiment was 1·4 kg/week.

Blood (plasma) flow and gaseous exchange (Table 1)

At the higher ME intake used in the present study blood flow was greater than observed previously (Lobley *et al.* 1995) but was not significantly different between either diet or level of ammonium salt infusion. The contribution of hepatic artery to hepatic venous flow averaged 0·08 (range 0·05–0·10), a similar fraction to earlier observed values after the contribution of hepatic N-acetylation of pAH had been accounted (Lobley *et al.* 1995). PCV values were not significantly different between treatments and, therefore, plasma flows showed similar trends to blood. The PCV ranged 0·23–0·32 between sheep.

Whole-body O_2 consumption was slightly greater for the GB diet which may reflect the increased energy intake (predicted as G 11·5 MJ ME/d, GB 12·6 MJ ME/d). There were no significant differences with treatment for either portal-drained viscera (PDV) or hepatic O_2 consumption although there was a tendency for elevated values during the H infusions, as observed previously (Lobley *et al.* 1995). Overall, the splanchnic tissues contributed 0·43–0·58 of total O_2 consumption, with a slightly higher proportion (0·52–0·58) from the liver.

Blood pH, urea and ammonia concentrations (Table 2)

Arterial blood pH was unaffected by treatments and remained above pH 7·4; thus one criterion, avoidance of possible clinical acidosis, was attained. Similar pH values were observed between hepatic portal and hepatic venous blood; these were also unaltered by treatment and were 0·08–0·09 lower than the respective arterial pH (SED 0·006, $P < 0·001$, 24 residual df).

Plasma NH_3 concentrations were greater ($P < 0·001$) in the hepatic portal vein than either the aorta or hepatic vein, which themselves were not significantly different. Only for hepatic portal plasma was there a significant increase in NH_3 concentration as a result of the H infusions.

Urea concentrations were identical between plasma and blood water (results not shown) for all samples. Treatment had no statistically significant effect on blood urea concentrations within sample sites, although there was a trend for values to be greater during the H infusions. There were significant differences, however, between all three sites (arterial 5·66 mM, hepatic portal 5·51 mM, hepatic 5·84 mM, SED 0·014, $P < 0·001$, 2 df).

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Table 1. *Blood and plasma flows and oxygen exchanges across the portal drained viscera (PDV) and liver of sheep offered either 1200 g grass pellets (G) or 600 g grass pellets plus 600 g barley pellets (GB) and infused with either 25 μmol/min (L) or 150 μmol/min (H) of ammonium salt into the mesenteric vein**

(Mean values for four animals)

					Significance effects†			
Treatment...	GL	GH	GBL	GBH	SED‡	Diet	NH_3 level	Diet × level
Blood or plasma flow (g/min)								
Hepatic portal vein								
Blood	1881	2246	1969	1934	200·9	0·499	0·275	0·197
Plasma	1418	1670	1465	1464	140·8	0·495	0·240	0·237
Hepatic vein								
Blood	2058	2398	2176	2110	260·0	0·174	0·485	0·311
Plasma	1553	1787	1623	1597	188·7	0·121	0·469	0·237
Oxygen consumption (mmol/min)								
Whole body	10·92		11·87		0·648	0·240	—	—
PDV	2·46	3·11	2·22	2·46	0·434	0·287	0·149	0·467
Liver	2·52	3·24	2·89	3·14	0·533	0·675	0·252	0·550
Splanchnic:total	0·46	0·58	0·43	0·47	0·070	0·282	0·142	0·441

* For details of procedures, see pp. 232–235.

† By ANOVA; for complete data set 6 residual df except for whole-body oxygen consumption (3 residual df). Each treatment value per sheep is the mean of four integrated hourly measurements.

‡ Largest SED value presented for information.

Table 2. *Blood pH and concentrations of plasma ammonia and blood urea across the portal-drained viscera and liver of sheep offered either 1200 g grass pellets (G) or 600 g grass pellets plus 600 g barley pellets (GB) and infused with either 25 μmol/min (L) or 150 μmol/min (H) of ammonium salt into the mesenteric vein**

(Mean values for four animals)

					Significance effects†		
Treatment...	GL	GH	GBL	GBH	SED‡	NH_3 level	Diet × level
pH							
Aorta	7·439	7·433	7·451	7·437	0·0146	0·365	0·737
Hepatic portal vein	7·354	7·349	7·355	7·340	0·0192	0·469	0·726
Hepatic vein	7·351	7·349	7·362	7·346	0·0206	0·580	0·659
Plasma ammonia concentration (μmol/l)							
Aorta	69	96	107	65	17·8	0·556	0·035
Hepatic portal vein	357	494	456	561	20·3	0·003	0·124
Hepatic vein	44	44	76	62	23·3	0·246	0·272
Blood urea concentration (mmol/l)							
Aorta	5·01	5·86	5·54	6·22	0·740	0·074	0·805
Hepatic portal vein	4·88	5·70	5·43	6·05	0·743	0·089	0·776
Hepatic vein	5·17	6·08	5·73	6·40	0·771	0·076	0·758

* For details of procedures, see pp. 232–235.

† By ANOVA; for complete data set 6 residual df. Each treatment value per sheep is the mean of four integrated hourly measurements. There were no significant effects for diet.

‡ SED value presented for NH_3 level only.

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Table 3. *Blood mass transfers of ammonia* and urea-nitrogen across the portal-drained viscera (PDV) and liver of sheep offered either 1200 g grass pellets (G) or 600 g grass pellets plus 600 g barley pellets (GB) and infused with either 25 μmol/min (L) or 150 μmol/min (H) of ammonium salt into the mesenteric vein*†

(Mean values for four animals)

Treatment...	GL	GH	GBL	GBH	Significance effects‡ SED§	Significance effects‡ NH_3 level (*P*)
Ammonia (μmol/min)‖						
PDV	618	796	710	962	36·6	< 0·001
Liver	−676	−911	−757	−965	28·5	< 0·001
Urea-N (μmol/min)						
PDV	−496	−732	−444	−658	77·0	0·026
Liver	1150	1415	1186	1430	30·7	< 0·001
Urea-N entry rate (μmol/min)						
Blood	1214	1454	1266	1436	100·6	0·028
Urine	1157	1335	1238	1474	30·0	< 0·001

* Calculated from blood flows × ammonia concentration in plasma.

† For details of procedures, see pp. 232–235.

‡ By ANOVA; for complete data set 6 residual df. Each treatment value per sheep is the mean of four integrated hourly measurements.

§ SED value presented for NH_3 level. There were no significant effects of diet nor diet × level interactions.

‖ Positive signs indicate net appearance across the tissue and negative signs net removal.

Ammonia and urea splanchnic bed transfers (Table 3)

Both NH_3 appearance in the PDV ($P < 0·001$) and extraction by the liver ($P < 0·001$) were greater during the H infusions, but the absolute differences exceeded the extra NH_3 supplied.

In absolute terms the fraction of urea-N released into the blood from the liver which could be apparently accounted by NH_3 removal was similar for the GB diet (0·64–0·67) and G (0·59–0·64) diet. The incremental changes in blood urea-N output:NH_3 uptake between L and H infusions were 1·13–1·17 for the two diets. Thus, only small amounts of non-NH_3 N were apparently required to support the hepatic detoxification of the exogenous NH_3 administration.

There was good agreement between urea fluxes measured in urine and blood, with the former preferred because the amount of radioactivity monitored was at least one order of magnitude greater, with appropriate gain in precision. Similarly, the relationship between urinary [^{14}C]urea flux and hepatic release into blood averaged 1·02 (SE 0·028; *n* 16).

Amino acid transfers (Tables 4 and 5)

Arterial amino acid concentrations were not significantly affected by level of ammonium salt infusion but were by diet type, with elevated glycine and lowered valine, isoleucine, leucine, tyrosine, phenylalanine and monomethyllysine when animals were fed on ration GB compared with G. Similar differences were also observed for plasma and the two other sample sites monitored (results not shown). There were no significant differences between diets in the amount of any individual amino acid absorbed into the hepatic portal vein although there was a trend for increased net PDV appearance of non-essential, essential

Table 4. *Arterial blood† free amino acid concentrations (μmol/kg) of sheep offered either 1200 g grass pellets (G) or 600 g grass pellets plus 600 g barley pellets (GB) and infused with either 25 μmol/min (L) or 150 μmol/min (H) of ammonium salt into the mesenteric vein‡*

(Mean values for four animals)

Treatment	Asp	Thr	Ser	Asn	Glu	Gln	Gly	Ala	Cit	Val	Met
Arterial blood											
GL	74	145	70	54	340	133	627	222	197	549	32
GH	70	127	67	54	323	107	560	205	217	558	30
GBL	71	102	72	42	343	77	683	196	166	385	24
GBH	53	120	77	46	265	155	583	179	183	433	29
SED§	14·1	34·4	9·3	5·1	43·9	22·7	42·1	18·3	17·3	53·9	2·5
Arterial blood *P*§	—	—	—	—	—	—	—	—	—	*	—
Portal blood *P*§	—	—	—	—	—	—	*	—	—	**	—
Hepatic blood *P*§	—	—	—	—	—	—	*	—	—	*	—

Treatment	Ile	Leu	Tyr	Phe	Orn	Lys	Melys‖	His	Arg¶	Pro
Arterial blood										
GL	131	250	94	75	310	199	170	89	79	178
GH	156	280	85	80	287	201	173	93	79	148
GBL	96	172	66	57	272	194	133	84	79	136
GBH	127	206	77	62	293	207	135	80	91	176
SED§	13·9	19·5	4·8	5·6	16·8	13·4	7·2	6·1	9·3	13·5
Arterial blood *P*§	*	**	**	**	—	—	***	—	—	—
Portal blood *P*§	**	***	—	*	—	—	***	—	—	**
Hepatic blood *P*§	*	**	*	*	—	—	***	—	—	—

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For simplicity, values are given for arterial blood only. Comparable sets of data for portal blood and hepatic blood are not shown, but probabilities for diet effect are included.

‡ For details of diets and procedures, see pp. 232–235.

§ Values are for diet effect. There were no significant effects for NH_3 level or diet × level interactions.

‖ Also includes 1-methylhistidine.

¶ Values for plasma.

and branched-chain amino acids (BCAA) during H. Neither ornithine nor citrulline were fully resolved from other compounds during ion-exchange chromatography, while arginine in haemolysed blood was diminished due to arginase (*EC* 3.5.3.1) action. Therefore, net supply was estimated as the sum of essential and non-essential amino acids, with ornithine and citrulline omitted and arginine content assumed to be that in plasma. For the G diet PDV amino acid-N supply amounted to 0·40 and 0·59 of the N entry into the gastrointestinal tract (diet plus PDV urea extraction) at L and H with respective values for GB of 0·55 and 0·54.

The pattern of absorbed amino acids showed good agreement with that reported for rumen microbial protein (RMP; Storm & Ørskov, 1983) for the essential amino acids, based on leucine as reference (Fig. 1). The data shown are based on transfers through plasma which has been shown to be the major site of carriage of absorbed amino acids (Heitmann & Bergman, 1980; Lobley *et al.* 1996), but similar patterns were observed for blood (results not shown). Of the essential amino acids only lysine (K) showed any substantial deviation between the patterns of net PDV appearance and RMP. Whether the lower-than-theoretical appearance across the PDV represents a reduced availability from the gastrointestinal tract due to formation of ϵ-aminolysyl complexes with other nutrients in

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Table 5. *Blood transfers of amino acids (µmol/min) across the portal drained viscera (PDV) and liver of sheep offered either 1200 g grass pellets (G) or 600 g grass pellets plus 600 g barley pellets (GB) and infused with either 25 µmol/min (L) or 150 µmol/min (H) of ammonium salt into the mesenteric vein**

(Mean values for four animals)

Treatment...		GL	GH	GBL	GBH	SED†
Blood						
PDV	TAAN	888	1454	1081	1197	388·6
	NEAAN	501	902	655	701	268·6
	EAAN	346	433	378	429	92·5
	BCAAN	128	196	111	184	64·0
Liver	TAAN	−611	−1181	−856	−754	445·4
	NEAAN	−405	−788	−573	−594	268·3
	EAAN	−220	−351	−262	−257	104·7
	BCAAN	−93	−103	−59	−138	61·5

TAAN, total amino acid-N, includes all amino acids tabulated in Table 4; NEAAN, non-essential amino acid-N excluding citrulline, ornithine and monomethyllysine; EAAN, essential amino acid-N; BCAAN, branched-chain amino acid-N.

* For details of procedures, see pp. 232–235.

† SED values given for effect of NH_3 infusion. There were no significant effects of NH_3 level, diet or diet × level interactions.

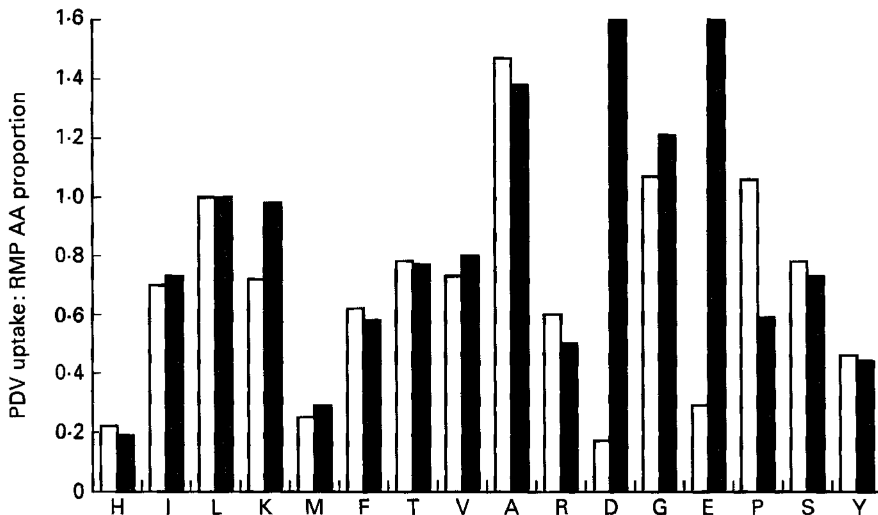

Fig. 1. Ratios of amino acids (AA) absorbed across the portal-drained viscera (PDV) (□) of sheep given 1200 g of either grass or grass plus barley pellets, in comparison with the amino acid composition of rumen microbial protein (RMP) (■). Values are normalized relative to leucine for both comparisons. The single-letter international symbols for amino acids are used.

the digesta remains to be elucidated. For the non-essential amino acids there was a marked deficit in apparent uptake of aspartate (D) and glutamate (E). These findings indicate that the net absorption of amino acids on both diets arises primarily from digestion in the small intestine of RMP and/or that dietary material which escapes rumen degradation is of similar composition. The net PDV appearances include material secreted from the pancreas

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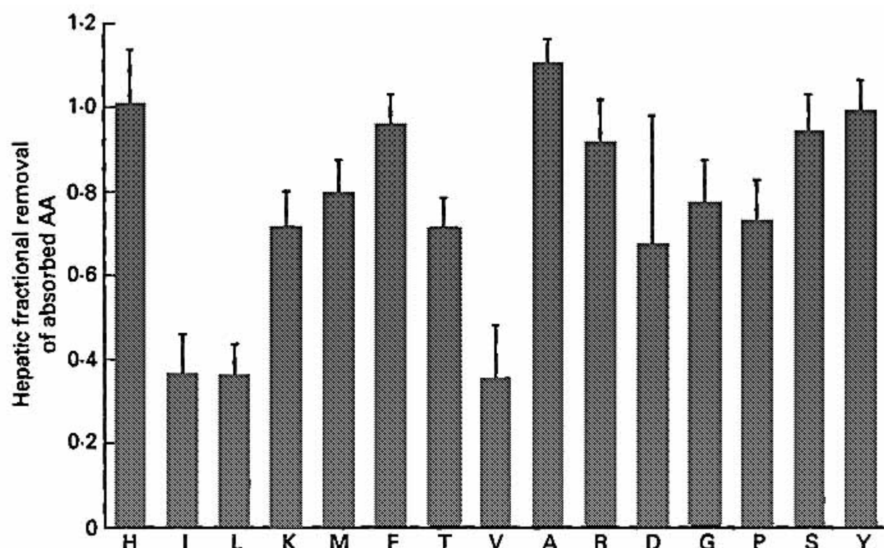

Fig. 2. The fraction of amino acids (AA) absorbed across the portal-drained viscera which were removed by the liver for sheep given 1200 g of either grass or grass plus barley pellets. The single-letter international symbols for amino acids are used.

and small intestine but subsequently reabsorbed, with the only main source not 'eliminated' through the arterio-venous measurements being pre-forestomach inflows (mouth, oesophagus etc.).

Removal of specific amino acids across the liver was unaffected by either diet or ammonium salt infusion, although the trend for increased net PDV appearance on diet GH was counterbalanced by a greater hepatic uptake. The mean fractional removal of net absorbed amino acids was 0·73 for both blood and plasma (results not shown). For some amino acids, however, notably the BCAA, the fractional extraction of that absorbed was low ($< 0·4$) while others were removed almost entirely (e.g. histidine, phenylalanine, tyrosine, alanine, serine), as shown for plasma (Fig. 2); blood mean values were similar, but with greater variance. The fractional extractions of threonine, methionine and lysine were intermediate, but still considerable (0·7–0·8), while there was net output of glutamate, probably related to the uptake and hydrolysis of glutamine (results not shown).

Leucine kinetics (*Table 6*)

Whole-body leucine flux was unaltered either by diet type or ammonium salt infusion (results not shown). Leucine movements to whole-body protein synthesis, calculated based on enrichments of either plasma leucine or MOP, were also uninfluenced by treatments. Whole-body leucine oxidation was significantly lower on GB compared with G ($P = 0·024$) although fractional oxidations of the MOP-based flux were similar across treatments (range 0·23–0·27). The values for calculated protein synthesis across the splanchnic tissues varied depending on the choice of precursor but, based on similar considerations to the whole-body protein flux estimations, i.e. the enrichment of plasma leucine for the inflowing vascular pool, the PDV contributed 0·22–0·28 of total protein synthesis, with neither the absolute nor fractional proportion altered by treatment. Protein synthesis was lower in the liver and represented 0·13–0·19 of the whole-body values, with again no significant differences for diet or level of ammonium salt infusion. Leucine oxidation was divided approximately equally between the PDV and liver with a tendency for both the PDV and

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Table 6. *Whole-body and splanchnic tissue leucine kinetics of sheep offered either 1200 g grass pellets (G) or 600 g grass pellets plus 600 g barley pellets (GB) and infused with either 25 μmol/min (L) or 150 μmol/min (H) of ammonium salt into the mesenteric vein**

(Mean values for four animals)

Treatment...	GL	GH	GBL	GBH	SED†	*P*
Whole-body kinetics (μmol/min)‡						
PS						
Leu_p	124	132	122	130	7·3	—
MOP_p	110	114	98	103	9·7	—
Oxidation	39·1	41·4	30·8	34·7	2·80	0·024
Splanchnic metabolism (μmol/min)						
PDV						
PS leu_p	31·5	36·4	26·3	32·4	4·40	—
PS leu_b	30·6	35·4	33·4	27·8	4·53	—
Oxidation§	8·1	13·8	6·5	5·1	2·56	0·075
Liver						
PS leu_p	17·5	24·7	15·6	17·0	3·54	—
PS leu_b	16·8	22·6	20·3	17·3	4·63	—
Oxidation§	7·4	9·8	6·9	7·2	1·89	—
Total splanchnic oxidation	15·5	23·7	13·3	12·3	3·68	0·100
Whole-body oxidation (%)	39	57	39	33	7·2	—

PDV, portal-drained viscera; PS, protein synthesis.

* For details of procedures, see pp. 232–235.

† By ANOVA, see p. 235. Values for SED are given for diet only. There were no significant effects of NH_3 level or diet × level interactions.

‡ Subscripts refer to values based either on enrichments of leucine (leu) or 4-methyl-2-oxopentanoate (MOP) in either blood (b) or plasma (p) of inflowing vascular pool to the tissue.

§ Oxidation values calculated based on MOP enrichments either for artery (whole-body kinetics) or outflowing vein (splanchnic tissues).

total splanchnic catabolism to be greater during the GH treatment. Overall, splanchnic tissues accounted for 0·33–0·57 of total leucine oxidation, with presumably much of the remainder due to peripheral tissue metabolism, notably muscle and fat (Harris *et al.* 1992).

^{15}N kinetics (Table 7)

Urea enrichments were identical in corresponding blood and plasma samples (results not shown). Enrichments of urea tended to be slightly greater in hepatic venous blood (plasma) than in either hepatic portal or arterial fluids, as observed previously (Lobley *et al.* 1995), indicative of synthesis within the liver from ^{15}N sources (results not shown). The differentials were less than in the earlier study, due to the lower enrichments of $^{15}NH_3$ obtained, and, in consequence, it was not possible to determine accurately the transfer of ^{15}N into urea by use of arterio-venous calculations. Instead an indirect approach was adopted. Based on the assumptions that body water is 0·65 of live weight and that urea is distributed evenly throughout the aqueous pool then, for a 36 kg sheep (mean live weight at experimental mid-point), the body urea pool was 155 mmol and after 7 h of infusion this achieved a mean [$^{14}N^{15}N$]urea mpe of 3·0. Therefore, the amount of label incorporated into urea would be 4·7 mmol ^{15}N while, in the same period, 10·5 mmol $^{15}NH_3$ was infused. When allowance is made for urinary elimination and gut recycling, approximately 0·8–0·9 of the administered ^{15}N had entered ureagenesis.

Enrichments of plasma NH_3 were not determined directly but, instead, were estimated from the mass flows in the hepatic portal vein and the rate of infusion of $^{15}NH_4Cl$, but

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Table 7. *Relative enrichments* of ^{15}N metabolites in sheep offered either 1200 g grass pellets (G) or 600 g grass pellets plus 600 g barley pellets (GB) and infused with either 25 μmol/min (L) or 150 μmol/min (H) of ammonium salt into the mesenteric vein*†

(Mean values for four animals)

Treatment...	GL	GH	GBL	GBH	SED‡
[$^{15}N^{15}N$]:[$^{14}N^{15}N$]urea					
GCMS	0·03	0·02	0·03	0·02	0·008
IRMS	0·03	0·02	0·03	0·03	0·003
[^{15}N]glu:[^{15}N]gln	0·21	0·18	0·20	0·17	0·028
[^{15}N]gln:[$^{14}N^{15}N$]urea	0·28	0·44	0·42	0·40	0·084
[5-^{15}N]gln:[2-^{15}N]gln	2·42	4·35	1·93	2·36	0·767

Glu, glutamate; gln, glutamine; GCMS, gas chromatography–mass spectrometry; IRMS, isotope-ratio mass spectrometry.

* Based on molar comparisons.

† For details of procedures, see pp. 232–235.

‡ By ANOVA, see p. 235, with 6 residual df. SED values presented for level of NH_3. There were no significant effects for diet, NH_3 level or diet × level interactions.

excluding any label recycled back to NH_3 from, for example, glutamine. The derived values approximated to 3·5–6·4 mpe across the four treatments. Because of the rapid turnover of the NH_3 pool in the hepatic portal vein, due to the high rate of extraction by the liver, these can be assumed to be 'plateau' values during the infusions. For the last sample on each treatment the corresponding [$^{14}N^{15}N$]urea mpe were 0·76–1·05 of the calculated $^{15}NH_3$ mpe in hepatic portal venous plasma. Of course the enrichment of N atoms in the [$^{14}N^{15}N$]urea would be approximately half that derived from the urea mpe calculations and so were considerably less than for $^{15}NH_3$. From the rate of increase of urea enrichment the half-life of the urea pool was predicted as 5 h. A corresponding calculation based on the [^{14}C]urea flux (Table 3) and estimated size of the body urea pool, as described previously, yielded an average half-life of 4 h.

The proportion of [$^{15}N^{15}N$]:[$^{15}N^{14}N$]urea molecules, obtained by GCMS analysis of blood, averaged only 0·03 after 9 h of infusion, although the relative amounts of ^{15}N would be twice this value. Similar relative distributions were also observed in urine samples analysed by IRMS procedures although the absolute enrichments were slightly lower (results not shown). These differences may relate to the time delay between appearance in blood and kidney filtration followed by bladder emptying. Alternatively, [$^{14}N^{14}N$]urea may be produced within the kidney or urine by, for example, hydrolysis of arginine. Values obtained from the column-purified urea and alkali freeze-dried urine tended to be slightly lower than from acidified urine.

The amount of label found in [$^{15}N_2$]glutamine was below the limits of GCMS detection ($<$ 0·05 mpe). The combined enrichments of mono-labelled glutamine species, i.e. [2-^{15}N]glutamine plus [5-^{15}N]glutamine, were less than 0·4 those for the corresponding [$^{15}N^{14}N$]ureas across the four treatments, with the predominant species the 5-^{15}N form. Blood glutamate enrichments were lower than observed for [2-^{15}N]glutamine. All the glutamine and glutamate species appeared to have attained a pseudo-plateau within the first 5 h of infusion of [^{15}N]ammonium salts (results not shown).

Of the free amino acids monitored from liver homogenates prepared after the terminal procedure, all exhibited lower enrichments than hepatic [$^{14}N^{15}N$]urea, which also exceeded by 0·1 plasma urea mpe (Fig. 3). Enrichments of homogenate aspartate and glutamate were

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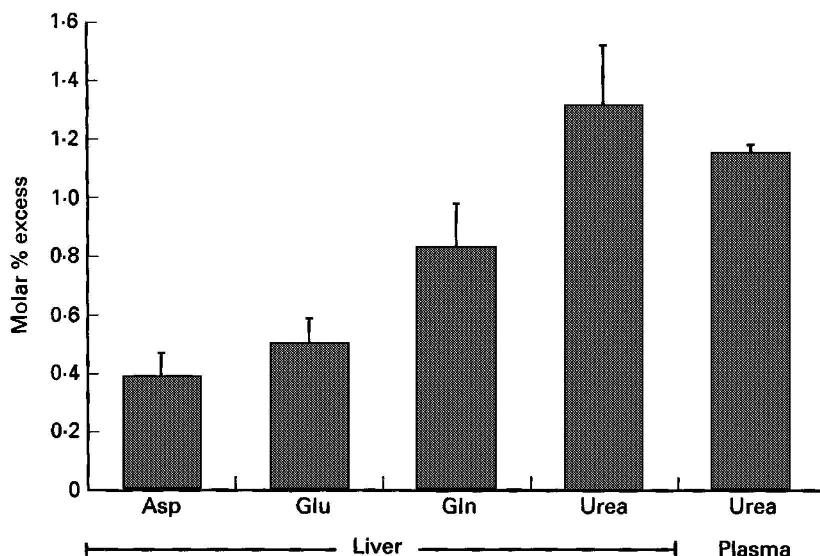

Fig. 3. Enrichments (molar % excess) of liver homogenate free aspartate (Asp), glutamate (Glu), glutamine (Gln) and urea plus plasma urea following a 3 h infusion at 70 μmol/min of [^{15}N]ammonium chloride into the mesenteric vein.

greater than the corresponding amino acid in plasma but the situation with glutamine was less clear. This may be due to the relative amounts of periportal and perivenous material in the tissue samples analysed. Homogenate concentrations (mM) were: glutamate 14·1 (SE 2·7), aspartate 7·9 (SE 1·6), glycine 11·3 (SE 2·4) and serine 5·0 (SE 2·0), and these exceeded plasma concentrations in the hepatic portal vein by 20–100-fold.

DISCUSSION

Mass N transfers

In general, urea production is lower, and whole-body N retention greater, on diets containing more concentrates (e.g. Reynolds *et al.* 1991*a*). Several explanations have been offered for this phenomenon, including: sparing of amino acids as substrates for gluconeogenesis when propionate supply is enhanced; greater microbial protein synthesis due to more efficient capture of rumen NH_3; suppression of hepatic ureagenesis through the inhibitory action of propionate on N-acetylglutamate synthesis and, thus, CPS activity (Stewart & Walser, 1980; Derr & Zieve, 1982; Garwacki *et al.* 1990); or that the lower demands on hepatic detoxification of NH_3 require less aspartate-N for the ornithine cycle to be provided from amino acids (Reynolds, 1992). These last two considerations formed the conceptual basis of the current study. That extra NH_3 is usually absorbed with forage rations has been reported in several studies (e.g. Fitch *et al.* 1989; Reynolds *et al.* 1991*b*). Fitch *et al.* (1989) also observed that the ratio of urea-N production:NH_3 removal by the liver was 2·8 and 1·3 for grass *v.* grass+barley rations respectively, although this was not supported in two other cattle trials where similar ratios were observed between high forage and high concentrate rations (1·2, Huntington, 1989; 1·6–1·8, Reynolds *et al.* 1991*b*). Overall, however, any reduced input, either in absolute or relative terms, to urea synthesis from non-NH_3 sources would potentially spare such nitrogenous metabolites for anabolic processes.

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Direct links between hepatic NH_3 uptake and urea synthesis cannot be established from these studies, however, because the experimental designs involved alterations in either diet quality or quantity and thus more than one metabolite inflow was changed. To overcome the interpretative difficulties which ensue, recourse has been made to intravenous infusion of ammonium salts, and with both acute (Wilton *et al.* 1988) and chronic (Lobley *et al.* 1995) administrations of NH_4Cl the extra-hepatic urea-N synthesis was double that of the N infused. This was also accompanied by an increase in leucine oxidation across the splanchnic bed.

The current study with sheep does not support several of the previous findings, with neither NH_3 absorption across the PDV, nor hepatic ureagenesis nor whole-body urea flux different between the GL and GBL diets. Such disparities between the various studies are difficult to reconcile but, setting aside species effects, may relate to differences in ruminal fermentation and/or N intake. Linear relationships have been fitted between PDV NH_3 flux and N intake (see Seal & Reynolds, 1993), but with up to a 5-fold range of values at any specific intake. Similar large variance is observed also for the correlation between PDV α-amino-N flux and N intake (Seal & Reynolds, 1993), indicative of the considerable variation that can occur in rumen fermentation and thus the amounts of the major N-products formed. For example, in the current study, NH_3 absorption was only 0·63–0·67 of amino acid-N PDV uptake, for both GL and GBL, whereas for the cattle studies this ratio was much greater (1·3–1·9, Reynolds *et al.* 1991*b*; 1·4–3·0, Huntington, 1989). Comparatively, therefore, amino acids were the dominant absorbed N source in the sheep while NH_3 was the major N product of digestive metabolism in the cattle. In consequence, the metabolic demands between the various studies might be expected to differ considerably, as would the relative contribution of NH_3-N to urea-N.

These differences in the pattern of absorbed N metabolites, when extended to liver metabolism, provide one explanation for the different responses between the current sheep data and both the cattle studies and the ovine data reported previously (Lobley *et al.* 1995). The earlier sheep study offered lowered intakes, close to N equilibrium, where amino acid oxidation would link to maintenance of body N reserves and form part of the fundamental protein homeostasis. Furthermore, under those dietary conditions the absorbed NH_3-N:amino acid-N ratio was 1·4, more similar to the cattle studies whereby NH_3 was the predominant N substrate, and this was raised to 2·1 with the exogenous NH_3 infusion. Under such conditions, NH_3-N must be transferred to aspartate in order to provide the required equal inputs of N for arginosuccinate synthesis but if the actions of GLDH and aspartate aminotransferase (*EC* 2.6.1.1) are limited (by enzyme amount, oxo-acid provision or mitochondrial redox status) then alternative N sources for aspartate must be used, with amino acid-N as the favoured candidate. These would provide the circumstances in which incremental urea-N output would considerably exceed NH_3 input and lead to stimulation of leucine catabolism. In the current study the NH_3-N:amino acid-N ratio available in the hepatic portal vein was much lower and, even when NH_3 was infused, did not rise above 0·8 and, at worst, hepatic extraction of amino acid-N was at least 0·88 of NH_3-N removal. Thus, amino acids could usually have provided sufficient N for eventual transamination to aspartate, if required, and reduced the quantity of NH_3 required to react with GLDH. Under such circumstances it was not surprising to observe that hepatic free amino acid extraction and leucine oxidation were unchanged, both between diets G and GB and when the extra NH_3 was infused. These relationships between hepatic extraction of NH_3-N:amino-N can be contrasted with the two cattle studies (Huntington, 1989; Reynolds *et al.* 1991*b*) where the ratio ranged from 2·0 to 5·2, a clear imbalance in N inputs into the ornithine cycle compared with the current study. Thus, in the bovine trials, free (α) amino-N removal by the liver was larger on the rations with greater roughage (Huntington,

1989; Reynolds *et al.* 1991*b*) and this may have been catabolized to maintain aspartate inputs for arginosuccinate synthesis with, in consequence, less free amino acid available for peripheral tissue protein gain (Reynolds *et al.* 1991*a*).

The majority (0·85–0·90) of the additional urea synthesized between L and H infusions was extracted across the digestive tract and a fraction of this (0·22–0·59) was returned to the circulation as NH_3 due to urease (*EC* 3.5.1.5) action of gut micro-organisms. Thus, NH_3 appearance across the PDV was augmented on the H infusions by more than the amount infused. For this reason the additional hepatic urea-N synthesis was 2·0–2·1 that of the exogenous NH_3-N administered but less than 1·2 that actually removed by the liver. These calculations depend on accurate measurement of both blood flow and urea concentration differences but the excellent concordance between urea fluxes, monitored from either urine or blood samples and release across the liver, does support the reliability of the arterio-venous approach. Such comparisons also allay concerns about acute metabolic effects in response to the continuous blood-sampling procedure because the urea fluxes relate to integrated values over a prior 2 d period. These conclusions do presuppose, however, that urea flux is dominated by liver output and that production from other sites in the body, e.g. the kidney and erythrocyte (Covolo & West, 1947), are minor, as is recycling of the labelled urea-C (Long *et al.* 1978).

An alternative explanation for the differences between the current and earlier ovine studies relate to acid–base balance because, previously, NH_4Cl was infused and this lowered arterial pH by 0·08 units, whereas in the present study, with NH_4HCO_3 as the predominant salt, pH was unaltered. In fasted humans, slightly greater pH decreases, following NH_4Cl administration, caused an increase in whole-body leucine oxidation (Reaich *et al.* 1992) and, presumably, in catabolism of other amino acids. Such oxidation would provide extra bicarbonate and raise pH (Bean & Atkinson, 1984).

[^{15}N] transfers

Overall, approximately 0·8–0·9 of the infused [^{15}N] was estimated to appear in urea, which agrees well with the relative mass transfers observed. This also fits with the observation of Luo *et al.* (1995) with ovine hepatocytes, in which the majority of administered $^{15}NH_3$ appeared as urea under near-physiological incubation conditions.

If NH_3-N can enter both carbamoyl phosphate and aspartate the infusion of $^{15}NH_3$ should result in synthesis of [$^{15}N^{15}N$]urea. Exactly how much is formed depends on the relative enrichments of mitochondrial carbamoyl phosphate and the cytosolic aspartate pool, neither of which could be measured directly in the current study. If, however, it is assumed that these are in complete equilibrium and that, in both precursors, the proportions of substrate ^{14}N and ^{15}N are then p and q respectively (where $p = 1-q$), the probabilities of the products [$^{14}N^{14}N$]-, [$^{14}N^{15}N$]- and [$^{15}N^{15}N$]urea being formed are p^2, $2pq$ and q^2 respectively (see Walser *et al.* 1954). Under such equilibrium conditions the enrichment (a) of carbamoyl phosphate and aspartate can be obtained from the molar ratios of singly and doubly-labelled urea by,

$$a = 2/(([^{14}N^{15}N]:[^{15}N^{15}N])+2) \quad \text{(Milano } et\ al. \text{ 1996).}$$

Thus, for [$^{14}N^{15}N$]:[$^{15}N^{15}N$] values of 33–50 (from Table 6), a would be predicted at 5·7–3·8 %, which encompasses the calculated NH_3 enrichments presented to the liver. The fact that [$^{15}N^{15}N$]urea is produced indicates that NH_3-N does enter both carbamoyl phosphate and aspartate but the extent of inflow into each metabolite cannot be determined without direct measurement of the enrichment of one of these ornithine cycle precursors. The combination, however, of the good agreement between the incremental mass transfers of NH_3

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and urea-N, plus the concordance in the predicted portal vein NH_3 enrichment and the predicted value of *a* at complete equilibration, suggests near equal flows of NH_3 into the CPS and GLDH reactions.

This would agree with data from ovine hepatocytes *in vitro* where, in the absence of amino acids, urea output is closely quantified with NH_3 uptake and with [$^{15}N^{15}N$]urea as the predominant product (Luo *et al.* 1995). Furthermore, in the hepatocytes the enrichments of intracellular aspartate and glutamate matched that of urea (Luo *et al.* 1995), indicative of a rapid isotopic exchange between NH_3 and the dibasic amino acids through the actions of GLDH and aspartate aminotransferase, as has also been shown for rodent liver *in vivo* (Cooper *et al.* 1987). That the intracellular enrichments of either aspartate or glutamate obtained at the end of the current study did not apparently confirm such an equilibrium can be accounted by intracellular compartmentalization *in vivo*. Cytosolic free aspartate and glutamate concentrations in ovine liver cells are 20–100-fold greater than in plasma (Demigné *et al.* 1991; current study), and 12–25-fold the net uptakes of NH_3. The [^{15}N]glutamate formed by GLDH action is restricted, however, to the small mitochondrion pool (Meijer *et al.* 1990) and does not mix readily with the large cytosolic pool, which consequently has a much lower enrichment. The aspartate formed within the mitochondrion by transamination of glutamate and oxaloacetate does enter the cytosol but spatial organization of enzymes close to the mitochondrial outer membrane may direct this to formation of arginosuccinate and again prevent equilibration with cytosolic aspartate. Compartmentalization is a feature of the ornithine cycle, e.g. preferential use is made of ornithine from extra-mitochondrial sources which does not mix with the intra-mitochondrial pool (Cohen *et al.* 1987), while the arginine formed during ureagenesis remains separate from that transported into the cell or arising from protein degradation (Cheung *et al.* 1989). To monitor true precursor enrichments would require, therefore, measurement of either carbamoyl phosphate or citrulline (for NH_3 inflows) and, by difference with arginosuccinate or urea isotopic activities, data obtained for aspartate. These approaches were outwith the current study *in vivo*.

Further differences between the current and the previous study (Lobley *et al.* 1995) were apparent from the rate of labelling of glutamine. Under both low and high NH_3 loading the enrichments of [5-^{15}N]glutamine were always less than 0·45 that in [$^{15}N^{15}N$]urea whereas previously they were similar and, thus, less $^{15}NH_3$ was extracted by the perivenous hepatocytes. This may be due to the NH_3 load remaining within the extraction capacity of the periportal hepatocytes (which synthesize urea) or to a lesser demand on glutamine production within the perivenous cells to help balance acid–base status (see Häussinger *et al.* 1992). Because glutamine reached a plateau within 4 h, the enrichment must reflect that of the precursor NH_3 pool(s) in the sites of *de novo* synthesis, which include the liver (Lobley *et al.* 1995), lung, kidney and skeletal muscle (Heitmann & Bergman, 1978). Recent observations under similar conditions have suggested that removal of $^{15}NH_3$ from the portal vein can be almost complete across the ovine liver (Nieto *et al.* 1996), with low enrichments of NH_3 ($< 0·5$ ape) in the hepatic vein and aorta, and thus the probable source of the labelled glutamine is the perivenous hepatocytes. Furthermore, the difference in isotopic activity between [$^{15}N^{15}N$]urea and [5-^{15}N]glutamine supports the concept of a zonal gradient of NH_3 enrichment between their respective sites of synthesis, the periportal and perivenous hepatocytes (Häussinger *et al.* 1992).

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