Urea recycling in sheep: effects of intake

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The effect of intake on urea production, entry into the digestive tract and return of N to the ornithine cycle was studied in four sheep. Each sheep received 0.6, 1.2 and 1.8 × estimated maintenance energy intake quantities of grass pellets for 9 d. After 4 d of adjustment, N balance measurements were conducted between days 5 and 8. From day 7 to day 9 animals were continuously infused, via the jugular vein, with [15N15N]urea and three urine samples were collected at approximately 2 h intervals 48–54 h after the start of infusion. Total urea and enrichments of [15N15N]- and [14N15N]urea in the urine samples were determined. Urea production was calculated from the isotopic dilution of [15N15N]urea and entry into the gastrointestinal tract (GIT) obtained from the difference between this and urinary urea elimination. Urea which enters the GIT undergoes hydrolysis to liberate NH3 which may be reabsorbed and enter the ornithine cycle, in which case the product is [14N15N]urea, based on the probabilities of labelled and unlabelled N providing ureagenic precursors. The quantity of urea-N which returns to the ornithine cycle from the GIT can thus be calculated. Existing models based on this approach yield overestimates of the fate of individual urea molecules due to a failure to allow for multiple recycling of [15N15N]urea species through the GIT. Refinements introduced to cover this resulted in a 33–48% reduction in calculated return of label for the current study. The present model also predicted that 95% of the label movements across the GIT could be accommodated by three or fewer entries and returns of urea-N and 99% by recycling for a maximum of six occasions. Urea-N production increased with intake (P < 0.001) and exceeded digestible N values at all intakes. Urea which entered the digestive tract, both in absolute terms (P < 0.001) and as a proportion of production (0.62, 0.69, 0.73; P =0.027), increased with intake. The proportion of entry into the digestive tract which was returned to the ornithine cycle remained reasonably constant (0.37-0.41) across all intakes but the absolute amount increased (5.6, 9.2 and 15.0 g N/d; P < 0.001) with intake. If allowance is included for losses of 15N in faeces then the approach offers a relatively simple means of estimating anabolic reuse of urea by digestive tract micro-organisms and can complement data obtained from the technically more demanding arterio-venous and multiple-isotope techniques used hitherto.

Urea: Gastrointestinal tract: [15N]kinetics: Sheep

Urea, the major mammalian end-product of NH3 and amino acid metabolism, is produced by the liver in greater amounts than are eliminated in the urine. This is because a proportion of the synthesized urea enters the digestive tract where it is hydrolysed to NH3, which can then be either reabsorbed or used as a N source for microbial protein synthesis. This latter process may provide a mechanism for salvage of urea-N into bacterial matter which can be digested and yield amino acids to the animal. This mechanism may be important in man (Jackson, 1995) and substantial amounts of NH3-N can be transferred to amino acids in pigs and rats with an active gut microflora

Abbreviations: ape, atoms percent excess; GCMS, gas chromatography–mass spectrometry; GER, gut entry rate; GIT, gastrointestinal tract; IRMS, isotope ratio mass spectrometry; ME, metabolizable energy; UER, urea-N entry rate; UUE, urinary urea-N elimination.

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The approach employed is based on systemic infusion of grass pellets, is altered from below to above maintenance. Under multiple entries into the digestive tract and returns to the body ornithine cycle as reabsorbed amino acids of bacterial protein, but derived from urea as a N source, may be absorbed and then catabolized within the liver to yield urea as an end-product.

Despite the many data which quantify the magnitude of 'gut hydrolysis' of urea under various nutritional conditions (see reviews by Harmeyer & Martens, 1980; Kennedy & Milligan, 1980; Egan et al. 1986) only limited information is available (Siddons et al. 1985) on the partition of this urea-N between a substrate source for microbial protein gain and return to the body ornithine cycle as reabsorbed NH$_3$, i.e. discrimination between potential 'anabolic' and 'catabolic' fates. The current study attempts to quantify such transfers as intake of a standard ration, based on grass pellets, is altered from below to above maintenance. The approach employed is based on systemic infusion of $^{15}$N$_2$ and formation of $^{[15N15N]}$urea from NH$_3$ following hydrolysis within the gut, as developed by Walser and colleagues (Walser et al. 1954; Walser, 1968), and used extensively by Jackson and co-workers (1984, 1993). During this work certain technical aspects were examined to reduce artefacts which would result in errors in the calculated extent of recycling. One was development of a mathematical model which allowed for urea-N atoms to undergo multiple entries into the digestive tract and returns to the ornithine cycle. Failure to accommodate such multiple recycling leads to overestimates of the proportion of 'gut hydrolysis' which is returned as NH$_3$. Under conditions of high recycling, as occurs in many situations with ruminants, the overestimate can be substantial.

Materials and methods

Animals and diet

Four Suffolk cross-bred wether sheep (12–15 months old; 40–50 kg live weight) were each prepared with a temporary polyvinyl chloride catheter inserted into an exterior jugular vein. The ration offered was grass pellets (estimated 10 MJ metabolizable energy (ME)/kg DM; 22 g N/kg DM; DM 960 g/kg) supplied from continuous belt feeders. All animals received the three intakes in a 3 x 3 Latin square design with one sequence repeated. The intakes were set at 0.6 (low), 1.2 (medium) and 1.8 (high) times estimated maintenance energy intake (M; set at 400 kJ ME/kg body weight$^{0.75}$ per d). This equated to 560, 1110 and 1670 mg N/kg$^{0.75}$ and 25, 51 and 76 g DM/kg$^{0.75}$ for the three daily intakes. Sheep were adjusted to the ration level for 4 d followed by 4 d of N balance determination. Daily excreta collection was by bag for faeces and by suction into 100 ml 17 M-acetic acid for urine. Fixed proportions of the excreta were pooled for chemical analysis. Samples of urine were collected on day 2 for determination of urea 'background' enrichment. From day 3 of N balance determination the animals were infused, via the jugular vein, with $^{[15N15N]}$urea (98.1 atom %; MassTrace Inc., Woburn, MA, USA) prepared in sterile 0.15 m NaCl. Infusion rates were maintained constant at 9 g/h and the concentration of the urea solution adjusted based on the expected entry rate to give a predicted enrichment at 'plateau' of 0.15 atom % excess (ape) above background for $^{[15N15N]}$urea. Three samples of urine were collected at 2 h intervals from 48–54 h of infusion for determination of $^{[15N]}$urea enrichments. The infusion was then stopped and animals adjusted to the next diet level for 4 d before the collection and infusion procedures were repeated. During the final period for all animals, samples of faeces were collected on day 2 of the N balance ('background') and 48–54 h after the start of the urea infusion.

Chemical analysis

N contents in feed, faeces and urine were determined by Dumas combustion using an automated procedure (Foss Heraeus Macro N, York, North Yorkshire, UK). Urinary urea was measured by the method of Marsh et al. (1965) using a Technicon Auto Analyzer (Technicon Instruments Corporation, Tarrytown, NY, USA). For measurement of $^{15}$N content the urea was separated from NH$_3$ and amino acids by passage of acidified urine, containing 250 pmol urea, through 2 ml cation exchange resin (AG-50, 100–200 mesh, ×8, H$^+$ form; Biorad, Richmond, CA, USA). The column was washed with 7 ml N-free water, which was discarded, and then the urea eluted with a further 20 ml N-free water. All samples were prepared in duplicate, i.e. six analyses per sheep per period. The samples were then freeze-dried before $^{15}$N analysis.

$^{15}$N analyses

Technique evaluations. Hypobromite treatment of urea leads to a Hoffman degradation which, under monomolecular conditions, eventually produces N$_2$ gas with both atoms arising from a specific urea molecule. Thus, under the electron impact conditions within the source of the mass spectrometer N$_2$ gas liberated from pure $^{[15N]}$-, $^{[15N15N]}$- and $^{[15N15N]}$urea molecules should yield ions with mass/charge (m/z) values of 28, 29 and 30 respectively. When the procedure was tested with standards prepared from $^{[15N]}$urea and natural abundance (0.364 atoms % $^{15}$N) urea to yield enrichments expected for single
dose studies (Bunting et al. 1987) the determined ratios for m/z ions 29:30 were always greater than expected theoretically. These errors remained even after correction for the small amount of [14N15N] species present in the standard [15N15N]urea. The reaction was found to be sensitive to the concentration of urea, with more m/z 29 occurring from a constant enrichment sample as the concentration increased (Fig. 1(a)). The assay was not sensitive to alterations in the enrichment of the sample at standard concentrations (Fig. 1(b)).

In practice, the Hoffman reaction (Schestakow, 1905) is only monomolecular at infinite dilutions (i.e. in the gaseous phase). In solution, the closer proximity of molecules means that amino groups from adjacent urea molecules can be used to produce the N₂ gas and thus in samples with low proportions of [15N15N]urea the m/z yield of 29 is increased at the expense of 30. It was necessary, therefore, to adopt standard conditions for concentrations of reactants so that a correction factor, appropriate to those conditions, could be applied.

![Graph](https://www.cambridge.org/core/terms).

Table: Dilution

<table>
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<tr>
<th>Dilution</th>
<th>Contribution of m/z 29 ions to total 15N excess (%)</th>
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<tr>
<td>0.108</td>
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<tr>
<td>0.144</td>
<td>2</td>
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<tr>
<td>0.202</td>
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</tr>
<tr>
<td>0.635</td>
<td>4</td>
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<td>1.080</td>
<td>5</td>
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<td>1.735</td>
<td>6</td>
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<td>4.500</td>
<td>7</td>
</tr>
<tr>
<td>9.448</td>
<td>8</td>
</tr>
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</table>

Enrichment (atom % excess)

![Graph](https://www.cambridge.org/core/terms).

Fig. 1. Effect of reaction conditions on production of 29N₂ gas from mixtures of [15N15N]urea diluted with natural abundance urea. Values represent the contribution of m/z 29 to total 15N above the contribution from natural abundance and contained within the stock [15N15N]urea. (a) Effect of diluting a urea-nitrogen sample at a constant enrichment (2.0 atom %) from 500 µg urea-nitrogen in 0.52 ml (x) to 113 µg in 4.52 ml (x38). Values are means of two to four measurements with standard deviations, which in some cases are too small to show. (b) Effect of enrichment changes from 0.421 to 9.813 atom % at a fixed concentration of 500 µg urea-nitrogen in 0.5 ml solution. Values are means of three measurements with standard deviations.
Assay conditions. The assay conditions were set at 18 \( \mu \)mol urea (500 \( \mu \)g N) dissolved in 2 ml N-free water. To avoid the problems associated with dissolved \( N_2 \) (from air) this mixture was degassed for 30 min at 0-1 mbar in a Rittenberg tube. Afterwards the mixture was carefully frozen, from the base up, in liquid \( N_2 \). The top was removed and 0.5 ml LiOBr (Hauck, 1982) previously bubbled with He gas, added. The top was then replaced and the bottle evacuated to 0-1 mbar for 10 min, while continuously immersed in liquid \( N_2 \). Reaction was then instituted by heating at 60° for 15 min.

Even under these rigorous conditions the assay did not produce a pure monomolecular reaction and, therefore, standards of comparable \([^{15}N]^{15}N\) enrichment (0-15 ape) and concentration (9 mM) to the urinary urea were measured alongside each set of analyses and corrections applied for the 'loss' of \( ^{15}N \) from the m/z 30 and ‘gain’ as m/z 29. Under the conditions employed this correction was 4-68 (SD 0.437) %, n 12. Furthermore a correction also needs to be accounted for the fraction of \([^{14}N]^{15}N\)urea (3-26 % on a molar basis; 1-66 % as \( ^{15}N \) form) present in the original infusate. This was determined by gas chromatography–mass-spectrometry (GCMS) of the tertiary butyldimethylsilyl derivative of urea following the procedures described by Calder & Smith (1988).

Liberated \( N_2 \) was then analysed as m/z species 28, 29 and 30 by use of a dual inlet isotope ratio mass spectrometer (IRMS; SIRA 12, VG Isogas, Middlewich, Cheshire, UK).

Calculations. Urea-N entry rate (UER; g N/d), as- sumed to be equal to total synthesis, was calculated from the dilution of infused \([^{15}N]^{15}N\)urea in the urine compared with the infusate, i.e.

\[
\frac{(96-45 \text{ ape}) \times \text{urea-N infused (mol N/d)}}{\text{corrected m/z 30 ape}} \times 14
\]

where 96-45 is the percentage of infusate N as \([^{15}N]^{15}N\)-urea.

The urea-N ‘lost’ as presumed entry into the gut (GER) was taken as the difference between UER and urinary urea-N elimination (UEL),

i.e. \( \text{GER} = \text{UER} - \text{UEL} \).

Recycling model

The concept behind the approach is based on the assumption that when urea enters the gut as a \([^{15}N]^{15}N\) molecule, and then undergoes hydrolysis due to bacterial urease (EC 3.5.1.5) action, this will yield two molecules of \( ^{15}NH_3 \). If these \( ^{15}NH_3 \) molecules are then reabsorbed and extracted by the liver then they may combine with \( ^{14}N \) atoms (from aspartate) within the hepatic ornithine cycle to yield two \([^{15}N]^{15}N\)urea molecules (Walser, 1968; Jackson et al. 1984, 1993), based on the laws of probability. The chances of \([^{15}N]^{15}N\)urea returning to the system after entry into the gut, whether directly (without any hydrolysis) or indirectly by combination of two \( ^{15}N \)-containing molecules within the ornithine cycle, are considered to be negligible.

Previous models, based either on continuous infusion or single dose approaches (Jackson et al. 1984, 1993), fail to accommodate the fate of \([^{14}N]^{15}N\)urea which, after gut entry and hydrolysis followed by reabsorption as NH3, may yield further \([^{14}N]^{15}N\] and \([^{14}N]^{14}N\) species after ornithine cycle activity. The parent and daughter single-labelled \([^{15}N]^{15}N\)urea molecules involved are chemically indistinguishable, so while entry of \([^{14}N]^{15}N\)urea into the gut leads eventually to a dissimilar product, \([^{14}N]^{15}N\)urea, entry of \([^{14}N]^{14}N\)urea may produce an identical species. The single-labelled urea can, therefore, theoretically recycle an infinite number of times, without resulting in a physical change detectable by mass spectrometry. Failure to allow for this within a model leads to the unrealistic scenario that \([^{15}N]^{15}N\)urea is free to move from the urea pool to the GIT but \([^{14}N]^{15}N\)urea is not, yet to the body they are identical.

Fig. 2 represents a compartmental model of the recycling. The fate of the dose (D) can be partitioned between that eliminated in the urine (u) and that which enters the gut (1 - u). Of this latter value, a proportion (r) is returned to the urea pool (via NH3 or other metabolic products produced in the gut); the proportion (f) of the original dose which is returned is thus equivalent to r(1 - u).

Two extremes for the model can be envisaged. The first assumes a maximum of only one entry and return across the GIT for urea-N and, further, that any \([^{14}N]^{15}N\)urea formed leaves the body urea pool by one exit route only, i.e. to urine. These concepts are inherent in current models (e.g. Jackson et al. 1984, 1993) and relate to the inability to distinguish experimentally between parent and daughter \([^{14}N]^{15}N\)urea molecules.

The other extreme accommodates infinite recycling and this concept allows an approach based on steady-state differential equations to be taken. From Fig. 2, this gives the equations:

\[
\text{rate of change of } [^{15}N]^{15}N\text{urea in the body}
\]

\[
= \text{rate of } [^{15}N]^{15}N\text{urea dose}
\]

\[
- \text{loss rate in urine}
\]

\[
- \text{rate of GIT transfer}
\]

\[
dh_{30}/dt = D_{30} - uh_{30} - (1 - u)h_{30},
\]

which simplifies to

\[
dh_{30}/dt = D_{30} - h_{30} = 0,
\]

and

\[
\text{rate of change of } [^{14}N]^{15}N\text{urea}
\]

\[
= \text{dose rate } [^{14}N]^{15}N\text{urea}
\]

\[
- \text{urine loss } - \text{GIT transfer}
\]

\[
+ [^{14}N]^{15}N\text{urea recycling}
\]

\[
+ [^{15}N]^{15}N\text{urea recycling}
\]

\[
dh_{29}/dt = D_{30} - uh_{29} - (1 - u)h_{29} + r(1 - u)h_{30}
\]

\[
+ r(1 - u)h_{30},
\]

which simplifies to

\[
dh_{29}/dt = D_{29} - (1 - r + ur)h_{29} + r(1 - u)h_{30} = 0,
\]
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Fig. 2. Model of urea transfers. Of the dose (D) which passes through the body urea pool a proportion (u) is eliminated in the urine while the remainder (1 - u) enters the gastrointestinal tract (GIT) where it undergoes hydrolysis. A proportion (r) of this nitrogen is returned to the body urea pool while the remainder is split between faecal losses (x) and transfer into body and microbial synthetic processes (s). Any nitrogen from [15N15N]urea which enters the GIT can only form [15N15N]urea on return to the ornithine cycle, while nitrogen from [14N15N]urea can reform chemically indistinguishable [14N15N]urea after recycling.

where \(D_{29}, D_{30}, h_{29}\) and \(h_{30}\) represent the quantities of \([^{14N15N}]\) and \([^{15N15N}]\)urea in the dose and body respectively. Solving these equations and using the substitutions

\[
x = \frac{D_{30}}{D_{29}}, \quad \beta = \frac{h_{30}}{h_{29}},
\]

we obtain

\[
r(1 - u) = f = \frac{x - \beta}{\alpha(1 + \beta)}.
\] (1)

This provides a solution for the proportion of the dose returned from the GIT based entirely on isotope ratios, i.e. no mass movements need to be quantified.

These two extremes represent constrained models, involving once-only or infinite recycling, and neither is likely to be correct in vivo. Instead, a more flexible approach, which remains algebraically simple, yet allows the magnitude of multiple recycling to be adjusted, is required. This can be obtained by model generations which consider the time-related fates following introduction of the dose into the system and appearance of labelled urea species in the urine.

First generation (i.e. sufficiently short timescale such that no re-entry and return of \([^{15N15N}]\)urea molecules across the gut occurs):

\[D_{30}u + D_{29}u;\]

second generation (one entry and return across the gut occurs) the additional movements of \([^{14N15N}]\)urea will be:

\[(D_{29} + D_{30})(1 - u)ru,
\]

(note that no additional \(D_{30}\) is returned to the system as the probability of \(^{15N15N}\) recombination has been taken as negligible).

At the \(n\)th generation (allowing for multiple entries and returns) the additional transfers of \([^{14N15N}]\)urea will be:

\[(D_{29} + D_{30})(1 - u)^{n-1}ru,
\]

which for both \([^{15N15N}]\)urea and \([^{14N15N}]\)urea the total (\(T\)) for the 1st, 2nd, ... \(n\)th generation is given by:

\[T = (D_{30} + D_{29})u \left(1 - r^n(1 - u)^n \right) \left(1 - r(1 - u)\right).\] (2)

For the case \(n = 2\), which corresponds to once-only recycling, the values obtained can be compared with those obtained by the approaches of Jackson et al. (1984, 1993) which are based on similar concepts. The case when \(n\) approaches infinity corresponds to the steady-state differential equation approach described earlier.

Equation 2 introduces flexibility, and by appropriate numerical solution it is possible to evaluate the contribution of each recycle. In practice, for large values of \(u\) and small values of \(r\), \(n\) can be taken as small, because contributions from additional recycling will be negligible. For the \([^{14N15N}]\)urea molecules the return of \(^1\)N label declines progressively, in a geometric fashion, with each entry into the GIT. After \(m\) entries and returns the proportion of label remaining (\(A\)) can be derived from:

\[A = r(1 - u)^m.\] (3)

Statistical analysis

Data were analysed by means of Genstat for Windows Release 3.2 (Lawes Agricultural Trust, Rothamsted, Herts., UK). Although effects of treatment and period are not orthogonal, checks showed no evidence of a period effect, which was therefore omitted. A randomized block analysis was then performed with animals as blocks and intake as
the treatment. This slightly conservative approach allowed six residual degrees of freedom for all analyses as there were no missing observations.

Results

Nitrogen balance (Table 1)

Each sheep completed all phases of the experiment. Between each level of intake there were significant differences (\( P < 0.01 \) or better) in faecal output, urinary elimination and retention of N. The data represent a 4-d measurement following only a 4-d adaptation and may, therefore, not represent the maximum value of N retention at each intake. There were no significant effects on N digestibility. Urea-N comprised 55–60% of total urine N but again with no significant effect of intake.

Urea kinetics (Table 2)

Production of urea-N (UER) changed significantly \(( P < 0.01 \) or better) between each intake and exceeded N intake for the below maintenance treatment but not above maintenance (1·19 v. 0·76 v. 0·77, for low, medium and high respectively, SED 0·091, \( P = 0·005 \)). In contrast, urea-N production always exceeded digestible N and this proportion was significantly larger at the lower intake (2·03 v. 1·19 v. 1·29, for low, medium and high respectively, SED 0·220, \( P = 0·017 \)). As intake was raised there were significant increases in the amount of urea entry into the digestive tract, both on an absolute basis: \( P = 0·012 \) or better) and as a proportion of UER \(( P < 0·027 \)

The method of calculation made a substantial difference to estimates of the amount of urea which had been returned to the body pool via hydrolysis mechanisms in the GIT. Based on the proportion of that entering the GIT which was returned to the urea pool, the ratio approach gave values 33, 42 and 48% higher at the three intakes than the model (equation 1) which allowed for multiple recycling of urea molecules (Fig. 3(a)). Good concordance with the ratio approach was obtained if urea molecules were constrained to enter the GIT only once (equation 2; Fig. 3(b)). In practice, by use of equation 3, 95% of the \([^{15}N]^{14}N\) urea molecules were recycled for three or fewer occasions while six entries and returns would account for 99% of the associated \( ^{15}N \) movements.

The absolute amount of UER which was derived from hydrolysis of urea within the GIT and returned to the ornithine cycle increased with intake, although the change was not significant between the lower intakes. Despite this, the proportion of GIT entry which returned to the urea pool was unaltered across the intakes and averaged 37–41% (range 30–47%). Thus within the time-scale of the infusion 60% or more of the \( ^{15}N \) which entered the GIT was not returned to the urea pool of the body. The amount of \( ^{15}N \) excreted in the faeces was monitored during the last period only when losses increased with intake at 0·8, 3·3 and 8·6% of urea production and 1·2, 4·6 and 11·7% of GIT entry.

Discussion

Methodological considerations

Few studies have examined urea recycling by tracer kinetic approaches in farm species, although related data can be obtained from arterio–venous measurements conducted across the portal drained viscera and liver (e.g. Huntington, 1989; Reynolds et al. 1991; Lobley et al. 1996). These latter observations rely, however, on precise measures of both blood flow and the small differences in urea concentration which occur across the splanchnic tissues. Also such observations relate to a small time window (a few hours only) and may be subjected to diurnal influences. Although good agreement can be obtained between such mass transfer determinations and entry rate techniques based on radio-labelled urea (Lobley et al. 1996), the latter do have advantages of simplicity. The usual approach to quantify urea entry rate in ruminants has involved a single injection of either one isotope (usually \([^{14}N]^{15}N\) urea) alone or in combination with \([^{15}N]^{15}N\) urea to allow for recycling (Nolan & Leng, 1972; Bunting et al. 1987). The latter is then determined as total \( ^{15}N \) and thus contains a proportion of \([^{15}N]^{15}N\) urea and \([^{15}N]^{15}N\) urea species. The \([^{15}N]^{15}N\) urea will have a slower decline (lower rate constant) than the
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Fig. 3. Percentage of gastrointestinal tract (GIT) entry which is recycled to the ornithine cycle at three different levels of dietary intake. For data obtained in the current study values are means for three consecutive samples of urine obtained for each sheep. (a) Comparison of new model (■■) described on pp. 82–83 with an earlier approach (●●●●●, Jackson et al. 1984, 1993) and which does not take account of multiple recycling through the GIT of [14N15N]urea. (b) Comparison of values based on previous approaches (●●●●●; Jackson et al. 1984, 1993) and equation 2 (○○○; where [14N15N]urea molecules are constrained to enter the GIT once only).

[14C]urea which, in practice, relates to the amount of [14N15N]urea formed.

The early work of Walser and colleagues (Walser et al. 1954; Walser, 1968) identified that the recycling could also be obtained by examination of the rate of production of [14N15N]urea following a [15N]urea injection or infusion and this approach has been used to follow the effect of diet and development on urea recycling in human subjects (e.g. Jackson et al. 1984, 1993; Jackson & Wootton, 1990). The advantages offered by such an approach include the requirement for only a single isotopic measurement based on ratio mass spectrometry, lower isotope costs, and less perturbation of pool sizes compared with GCMS approaches (e.g. Wolfe, 1981).

Although the technique based on IRMS has been used in human studies for several years, three separate practical problems need to be considered. The first involves the presence of [14N15N]urea in the infusate. This problem has been recognized in the earlier studies and was determined directly here by GCMS analysis. When such additional facilities are not available, however, a correction might be applied by diluting the infusate with natural abundance urea.
and determining the ‘contamination’ from the increase in the m/z 29 ion. This would not yield a correct value due to the second problem, i.e. under the normal aqueous conditions the reaction is not completely monomolecular. Presumably in solution urea molecules are sufficiently close to provide reactivity between N atoms on an inter-molecular rather than an intra-molecular basis. The probability of incorrect proportions of m/z 29 being formed are thus a function of concentration. For these reasons it was necessary to adopt strict adherence to the amount of urea analysed in the final 2.5 ml reaction mixture.

The third consideration relates to the model adopted, where the more correct estimate of the proportion of urea-N which is returned to the ornithine cycle from the GIT has important quantitative implications. The previous models yield the cumulative fate of urea-N atoms which may undergo several passages into the GIT lumen and return through the ornithine cycle. Because the models differ in the inclusion of a geometric series the effect on recycling will vary with the proportion of molecules which enter the GIT. For example, data from human subjects (Jackson et al. 1984) would yield a decrease in recycling from the reported 18 % to 15 % by use of the current model. In comparison, for the present study the values would change from 56 to 39 % between the two approaches. In practical terms for each sheep the maximum number of generations (i.e. urea-N entry and returns across the GIT) required to reach near constant values (greater than 0-99) was six or fewer. This number of generations is compatible with the observations that urea elimination in urine was 0-25–0-33 of UER. One consequence of the current model, and related to the number of generations required for each animal, was that the data exhibited lower coefficients of variation associated with multiple recycling compared with one entry only.

**Nutritional effects on urea metabolism**

Many studies with ruminants have demonstrated that urea production increases with intake (see Harmeyer & Martens, 1980; Kennedy & Milligan, 1980; Egan et al. 1986). Under conditions of low or zero intake, urea-N production exceeds N intake in both ruminants (e.g. Havassey et al. 1973; Amos et al. 1976; Whitelaw et al. 1990) and non-ruminants (Meakins & Jackson, 1996) as the body mobilizes protein stores and undergoes negative N retention. At supramaintenance intakes, however, urea-N production still exceeds apparent digestible N absorption in both human subjects (e.g. Meakins & Jackson, 1996) and ruminants (e.g. Bunting et al. 1987; current study). This can be due to two reasons. First, a substantial portion of faecal N may be synthesized from urea and thus be derived from digestible sources. The current study indicates that, although urea does provide N to faecal material, the contribution is relatively minor. For example the N enrichment in faeces at the highest intake was only 0-11 of that in urinary urea (presumed equal to plasma enrichment; Lobley et al. 1996) and this ratio was lower still at 1-2 and 0-6 x maintenance (0-052 and 0-016 respectively). The second explanation is that a proportion of the urea is returned, via derived metabolites (notably NH₃), to bolster production as a recycled component, as has been observed previously (e.g. Walser et al. 1968; Nolan & Leng 1972; Jackson et al. 1984, 1993; Bunting et al. 1987) and in the current study.

Both the absolute amount and the proportion of urea production which entered the GIT increased with intake. Many factors appear to influence such entry. For example, the concentration gradient of urea established between the plasma and the fluids of the GIT compartments is important (see Harmeyer & Martens, 1980; Egan et al. 1986), with the gradient dependent on the activity of the ureolytic bacteria associated with the lumen walls of the digestive tract (Cheng & Wallace, 1979; Cheng et al. 1979). Similarly, provision of fermentable carbohydrate sources increases urea entry, presumably by stimulation of the bacterial population, which may utilize urea as a source of N for protein gain (Engelhardt et al. 1978; Whitelaw & Milne, 1991). In the current study, plasma urea concentrations were not measured but many reports have shown a general increase in response to extra intake (see Harmeyer & Martens, 1980), which will also provide more fermentable carbohydrate sources to all regions of the GIT. Urea transfer is by diffusion (Houpit, 1970), plus inflows in digestive fluids including saliva and pancreatic juice, and thus occurs at all sections of the GIT. The relative magnitudes of removal by the reticulorumen, small and large intestines have been quantified under a variety of conditions, with the foregut tending to have a greater role (see Kennedy & Milligan, 1980; Egan et al. 1986; Whitelaw et al. 1990).

**Recycling from the gastrointestinal tract**

The [¹⁴N¹⁵N]urea formed can arise from several different routes, with ¹⁵NH₃ as the common precursor. These sources include hepatic extraction of NH₃ direct (Huntington, 1989; Reynolds et al. 1991; Lobley et al. 1996); removal of citrulline formed in the intestinal cells; from body amino acids (and then proteins) via either amidation (glutamine, asparagine; Lobley et al. 1995) or transamination (Cooper et al. 1987; Brosnan et al. 1996) products; and through bacterial protein and other N products synthesized within the GIT from urea-N (Nolan & Leng, 1972; Bunting et al. 1987) and which may be degraded back to urea within the animal. In isotopic terms, the probable end-product is [¹⁴N¹⁵N]urea as, even with ornithine cycle precursor enrichments as great as 5–10 ape (considerably in excess of the maximum 0-2 ape possible in the current study), this is the dominant molecular species (Brosnan et al. 1996; Lobley et al. 1996). Over long time scales, which depend on the half-lives of the various body and microbial N pools, the [¹⁵N¹⁵N]:[¹⁴N¹⁵N]urea ratio would increase. The decision to adopt a 54h measurement period was a compromise to allow ‘plateau’ conditions for UER to be determined yet reduce major recycling of ¹⁵N from tissue proteins (mean half-life in sheep 16–27 h; from Harris et al. 1992).

One interesting feature of this study was the constancy of the fraction of urea which entered the GIT that was recycled back to the urea pool (r 0-37–0-41) across intakes. Although this meant that the absolute quantity returned
increased with intake it suggests that 60% of the urea-N which entered the GIT could be retained by the microbes and/or the animal. A similar value (r 0.34) was obtained for chaffed lucerne (Medicago sativa) hay (Nolan & Leng, 1972) but these values for roughage rations are lower than the recycling values of 0.55–0.58 obtained by Bunting et al. (1987), with two maize-based diets which differed markedly in N content. Investigation of the reasons for this apparent ‘constancy’ within, but not between, ration types may provide important information on the regulation of the N economy of ruminants.

In studies such as these, it is important to distinguish between the anabolic use of urea-N and the simple exchange of $^{15}$N for $^{14}$N during transamination reactions within the body. Such concerns formed the basis of human studies (El-Khoury et al. 1987), with two maize-based diets which differed none of the recycled N is available to support anabolism. In pigs, rats and man $^{15}$N from NH$_4$Cl ingestion has led to increased enrichment in tissue or vascular proteins of all amino acids (Torrallardona et al. 1994, 1996), including lysine and threonine which are not considered to undergo transamination reactions and must be synthesized de novo. The latter probably arise from microbial synthesis within the gut (Torrallardona et al. 1996). In ruminants, the presence of a functional rumen increases the potential to utilize urea-N as anabolic end-products of microbial metabolism and studies have indicated that 7–77% of bacterial-N may arise from urea-N (Nolan & Leng, 1972; Bunting et al. 1987). This wide range of values easily encompasses the proportion of urea-N which entered the GIT and was not returned quickly to the body urea pool observed within the current study. Adaptation of the present approach to include sampling over a wider time-scale, allied to appropriate sampling of GIT microbial enrichments, should allow the quantities of urea-N which return to the animal as either NH$_3$ or constitutive products of micro-organisms to be distinguished.

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