The transfer of ^{15}N from urea to lysine in the human infant

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(Received 9 April 1999 – Revised 16 September 1999 – Accepted 19 October 1999)

To explore the nutritional significance of urea hydrolysis for human subjects, male infants being treated for severe undernutrition were given oral doses of 10 mg [$^{15}N^{15}N$]urea every 3 h for 36 h, on admission, during rapid growth and after repletion with either moderate or generous intakes of protein. Urea hydrolysis was calculated from the ^{15}N enrichment of urinary urea, and where possible, lysine, alanine, glycine and histidine were isolated from urine by preparative ion-exchange chromatography for measurement of ^{15}N enrichment. Sufficient N was obtained for ^{15}N enrichment of lysine to be measured on fifteen occasions from six children. Urea hydrolysis accounted for half of all urea production with 130 (SD 85) mg N/kg hydrolysed per d, most of which appeared to be utilized in synthetic pathways. Of the samples analysed successfully, nine samples of lysine were enriched with ^{15}N (mean atom percent excess 0·0102, range 0·0017–0·0208) with relative enrichment ratios with respect to lysine of 1·63 (range 0·18–3·15), 1·96 (range 0·7–3·73) and 0·9 (range 0·4–1·8) for glycine, alanine and histidine respectively. Enriched samples were identified at each treatment phase and 68 % of the variation in lysine enrichment was explained by the variation in urea enrichment with 54 % explained by the overall rate of delivery of ^{15}N to the lower gastrointestinal tract. The results indicate a minimum of 4·7 mg lysine per kg body weight made available by *de novo* synthesis with the more likely value an order of magnitude higher. Thus, urea hydrolysis can improve the quality of the dietary protein supply by enabling an increased supply of lysine and other indispensable amino acids.

Undernutrition: Urea: Amino acids: Stable isotopes

N metabolism within the gastrointestinal tract involves complex cycling between the systemic circulation, the mucosa and luminal pools of protein, amino acids and other nitrogenous material of both dietary and endogenous origin. In addition, N-cycling occurs between luminal N pools and microbial N pools which meet their own nutritional requirements through the degradation of both food-derived and endogenous substances (Fuller & Reeds, 1998). Substrates for bacterial growth include urea since the microbial population contains a bacterial urease (*EC* 3.5.1.5) allowing urea entering the lumen from the systemic pool to be degraded and to provide a source of N for microbial amino acid and protein synthesis. Since microbial N pools exhibit considerable turnover, the potential exists for the *de novo* synthesis of amino acids. We are concerned here with the fate of such amino acids after microbial death, namely, whether they can make a nutritionally significant contribution to the amino acid supply of the host organism.

Urea turnover studies in human subjects suggest considerable hydrolysis of urea N in a form which is not simply recycled to urea in both adults (Hibbert & Jackson, 1992) and infants (Steinbrecher *et al.* 1996). If the recycled N includes amino acids, then N-cycling through the gastrointestinal tract could influence the amino acid composition and hence the protein quality of dietary protein available to meet nutritional demands (Jackson *et al.* 1984; Jackson, 1998). Several studies with human subjects have in fact reported evidence that suggested that essential amino acids could also be recycled during this process (Giordano *et al.* 1968; Furst, 1972; Sheng *et al.* 1977; Tanaka *et al.* 1980; Rikimaru *et al.* 1984). We report here stable isotope studies designed to further explore urea hydrolysis in the human infant and in particular to identify the transfer of ^{15}N from urea to lysine and some other amino acids in the circulating free amino acid pool of malnourished infants in relation to the extent of urea hydrolysis. Preliminary reports

Abbreviations: APE, atom percent excess; HP, high protein; LP, low protein.

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of the ^{15}N-labelling of lysine have been published (Yeboah *et al*. 1996; Gibson *et al*. 1997) and the urea turnover data are described in full elsewhere (Badaloo *et al*. 1999).

Methods

Experimental design

The measurements reported here were made in children from a larger study of the influence of dietary protein on the growth and urea kinetics in twenty male infants and children aged 6 to 24 months admitted for treatment of severe undernutrition at the Tropical Metabolism Research Unit, Jamaica (Badaloo *et al*. 1999). The intention was to examine the extent of the transfer of ^{15}N from ^{15}N-labelled urea to lysine, and any relationship between the extent of the labelling of lysine and urea hydrolysis. All isotopic measurements were made on urinary metabolites because for ethical reasons blood sampling was avoided. However, variation in urinary lysine concentration limited the number of children where successful measurements of lysine enrichment could be made to a subset of six children in which satisfactory measurements were made on fifteen occasions studied on admission (five children), during rapid growth (five children) and after repletion (five children) (Table 1). Protein intakes were either adequate (nine occasions) or generous (six occasions), which ensured a wide range of urea hydrolysis rates. Urea hydrolysis was measured from the ^{15}N enrichment of urinary urea during orally administered frequent doses of di-labelled [^{15}N]urea and ^{15}N enrichment was measured in amino acids isolated from urine collected during the [^{15}N]urea administration. The assumption is made that the urinary amino acid pool is in isotopic equilibrium with renal arterial blood from which it derives (de Benoist *et al*. 1984).

Nutritional management

Measurements were made in infants on three separate occasions during the course of their hospitalization. Stage 1 was shortly after admission in subjects still severely malnourished, but after treatment of acute complications such as infections and electrolyte disturbances and after loss of oedema. Stage 2 was during a stable pattern of rapid weight gain, after repletion of 50 % of their weight deficit. Stage 3 was after the completion of nutritional rehabilitation. This was indicated by attainment of >95 % of the appropriate weight in relation to their height and/or after a reduction in the rate of weight gain and dietary intake for 3 d. They were fed according to the approach used for the nutritional rehabilitation of severely malnourished subjects admitted to the Tropical Metabolism Research Unit (Jackson & Golden, 1987). This involved a standard formula with energy fed at maintenance levels in the initial phase (418 kJ/kg per d), with increased energy during catch-up (711 kJ/kg per d), and at 459 kJ/kg per d on recovery. In order to induce variable levels of urea hydrolysis, protein was fed at either adequate (low protein, LP) or generous levels (high protein, HP) (0·6 and 3 g protein/kg stabilization phase, 3·1 and 4·9 g/kg catch-up phase and 0·6 and 3 g/kg recovered respectively). Generous supplements of minerals and vitamins were included at all times. The Ethical Committee of the University Hospital of the West Indies approved the study protocol that included informed consent given by the parent or guardian of each subject. The attending physicians took all clinical decisions regarding the care and treatment of each subject.

Urinary amino acid concentrations and ^{15}N enrichment

Intermittent oral doses of [^{15}N^{15}N]urea (10 mg tracer (99·8

Table 1. Urinary lysine enrichment with ^{15}N in male infants being treated for severe undernutrition*

Infant	Treatment stage†	Dietary protein level‡	Lysine enrichment (APE)		
			Value	Mean	SD
a1	1	LP	0·0188		
b1	1	LP	0·0208		
c1	1	LP	0·0117		
d1	1	HP	0·0042		
e1	1	HP	0·0000		
				0·011	0·009
a2	2	LP	0·0135		
b2	2	LP	0·0000		
c2	2	LP	0·0028		
d2	2	HP	0·0000		
e2	2	HP	0·0000		
				0·0033	0·0059
a3	3	LP	0·0131		
c3	3	LP	0·0000		
d3	3	HP	0·0050		
e3	3	HP	0·0000		
f3	3	HP	0·0017		
				0·0039	0·0055

APE, atom percent excess; LP, low protein; HP, high protein.
* For details of procedures see p. 507.
† For details of treatment stage see p. 506.
‡ Dietary protein levels: LP 0·6, 3·1 and 0·6 g/kg, HP 3, 4·9 and 3 g/kg in the stabilization, catch-up and recovered periods respectively.

atoms percent, Isotope Laboratories, Cambridge, MA, USA) were given every 3 h for 36 h. Rapid attainment of plateau was achieved with a priming dose of isotope (40 mg) given 6 h before the first three-hourly dose. A baseline specimen of urine was collected before isotope administration. Subsequently, urine was collected by continuous aspiration from a perineal urine bag into chilled containers for periods of approximately 6 h for a total of 36 h. The weight of each 6 h urine collection was determined and a portion was acidified to pH 2 with 6 M-HCl and stored at −20° for later analysis. Measurements of amino acid concentrations and ^{15}N enrichment were in all cases made on the baseline and final 6 h urine sample collected between 30 and 36 h after the first dose of [^{15}N]urea. However, in one case to follow the time course ^{15}N enrichment of lysine was measured in each urine sample. The laborious nature of the methodology precluded extensive measurement of enrichment in all amino acids so that the analysis was limited to lysine in all samples with alanine, glycine and histidine isolated from seven of the nine urine samples in which lysine was shown to be enriched.

Urinary amino acids were analysed using the Waters Pico-Tag system (Millipore, Mitford, MA, USA). Urine was first ultrafiltered using PLGC (Millipore) membrane filters. The phenyl isothiocyanate derivative was prepared and chromatographed following procedures for physiological amino acids. A set of standards designed to include most plasma amino acids (physiological amino acids) was used.

The enrichment of amino acid N with ^{15}N was measured in amino acids isolated from urine by preparative ion-exchange chromatography and mass spectrometry using a triple collector isotope ratio mass spectrometer (Europa 20/20, Europa Scientific Ltd, Crewe, UK) fitted with a combustion interface (Europa Roboprep). Urine (15 ml) was lyophilized, reconstituted in 1 ml 0·1 M-HCl, and 200 μl was loaded onto a 800 mm × 9 mm column packed with a strongly acidic cation ion-exchange resin (Locarte Co., London, UK). Acidic and neutral amino acids were eluted by lithium citrate (pH 2·78, 0·3 M-Li, 0·09 M-citrate), run for 420 min while the basic amino acids were eluted with lithium citrate (pH 4·07, 1·2 M-Li, 0·21 M-citrate), run for a further 600 min with a column temperature of 37° at a buffer flow rate of 0·5 ml/min, and with supernatant fraction collection intervals of 2·1 min for the acid/neutrals and 6·0 min for the basics. The supernatant fractions containing amino acids were identified by fluorescence after 100 μl of each buffer fraction was mixed with 100 μl *o*-phthaldialdehyde solution (8 mg *o*-phthaldialdehyde; Sigma, St Louis, MO, USA) in 25 ml pH 10·7 borate buffer, activated by 8 ml 2-mercaptoethanol (BDH, Poole, Dorset, UK) on a ninety-six well microtitre plate (Polysorb F96, Nunc, Life Technologies Ltd, Paisley, Strathclyde, UK) read after 4 min at 340 nm on a modified microtitre plate reader (Labsystems Multiskan® Bichromatic, Labsystems, Helsinki, Finland). The amino acid profile was then plotted and the corresponding peak fractions for alanine, glycine, histidine and lysine pooled separately. Care was taken to ensure the pooled samples included the entire peak to account for any isotope fractionation. Thus, where possible the pooled fractions included baseline fluorescence before and after the peak. This was always the case for lysine and histidine and for most but not all of the glycine and alanine samples. The amino acid peak fractions were prepared for isotope ratio mass spectrometry after desalting on Dowex-50W strongly acid cation exchanger (Sigma) and eluting with 6 M-HCl. The dried samples were reconstituted in 100 μl 0·1 M-HCl, applied to tinfoil containers, combusted on the Europa Roboprep (Europa Scientific Ltd) and ^{15}N, ^{14}N mole fraction measured in terms of the relative mass 29 and 28 peaks on the isotope ratio mass spectrometer.

[^{15}N]-urea kinetics

Urea kinetics were calculated from the urinary urea enrichment measured over the 36 h during which oral doses of 10 mg [$^{15}N^{15}N$]urea were given every 3 h (Jackson *et al.* 1984). The urea production rate is indicated by tracer dilution of [$^{15}N^{15}N$]urea at steady state where dose [$^{15}N^{15}N$]urea is expressed as mmol N/kg per d and the ratio tracer : tracee as moles % excess:

$$\text{urea production} = \text{dose } [^{15}N^{15}N]\text{urea/ratio tracer : tracee in urinary urea.}$$

A proportion of this urea is excreted in urine and a negligible amount is excreted in stool (Jackson *et al.* 1984). Therefore, the difference between urea production and urea excreted in urine, defined here as urea hydrolysis, represents urea presumed to have entered the gastrointestinal tract, and to have been hydrolysed by resident flora possessing the bacterial urease (mainly in the colon). The proportion of hydrolysed [$^{15}N^{15}N$]urea-N returned to the general metabolic pool and recycled via $^{15}NH_3$ into further urea synthesis is indicated by the relative plateau enrichment of [$^{15}N^{14}N$]- and [$^{15}N^{15}N$]urea: i.e. the chance that [$^{15}N^{15}N$]urea will be formed in this way will be low. The difference between urea hydrolysis and that recycled into further urea synthesis is that retained in the general metabolic pool for further metabolism in other pathways.

Urinary urea N was measured by the Berthelot method (Kaplan, 1965) and the enrichment of N_2 gas liberated from the urea with ^{15}N measured in terms of mass 28, 29 and 30 (Walser *et al.* 1954) using a triple collector isotope ratio mass spectrometer (SIRA 10, VG Isogas, Cheshire, UK) as previously described (Jackson *et al.* 1984).

Results

The clinical aspects, growth and N balance and [^{15}N]urea kinetic measurements for all the children from this study are described elsewhere (Badaloo *et al.* 1999). In the children we studied, the [^{15}N]urea kinetic measurements indicated that the extent of urea production was on average 32 % of the intake with somewhat lower values in group 1 (LP 29 %, HP 26 %) and in group 2 LP infants (27 %), and with the highest values in the group 3 HP infants (49 %; see Table 2). Urea hydrolysis accounted for on average just over half (53 %) of all urea production with the highest values in the group 1 infants (LP 63 %, HP 61 %) and group 2 LP infants (57 %). This meant that on average 130 mg N/kg were hydrolysed each day, a supply of N returned to the systemic circulation equivalent to 0·88 g protein/kg per d. The amount varied considerably

Table 2. Urea kinetics (mg N/kg) in malnourished male infants being treated for severe malnutrition*
(Values are means and standard deviations)

Treatment stage†	Dietary protein level‡	*n*	Body weight (kg)		N intake		Urea kinetics									
							Excretion		Production		Hydrolysis		Recycling		Other pathways	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	LP	3	6·00	0·90	85	1	24	9	65	15	40	8	2·7	0·7	37	7
1	HP	2	6·40	0·57	474	1	123	40	313	13	190	27	18·9	0·6	171	9
2	LP	3	6·97	1·04	477	44	94	48	212	45	117	9·6	21·4	13	96·4	40
2	HP	2	7·45	0·78	660	158	265	13	510	70	245	83	38·3	6	207	35
3	LP	2	8·65	1·06	90	5	28	21	49	0	22	21	1·8	0·1	20	2
3	HP	3	7·77	1·17	479	66	237	73	424	98	187	26	29·8	6·1	157	31
All		15	7·1	1·2	371	223	127	102	256	180	130	85	17·4	14	113	61

* Values shown are from a subset of a larger group of subjects in which the enrichment of urinary [^{15}N]lysine was measured. The values for the larger group are discussed elsewhere (Badaloo *et al.* 1999). For details of procedures see p. 507.
† For details of treatment stage see p. 506.
‡ Dietary protein levels: LP 0·6, 3·1 and 0·6 g/kg, HP 3, 4·9 and 3 g/kg in the stabilization, catch-up and recovered periods respectively.

between groups from only 20–40 mg N/kg per d in the LP infants of group 1 and 3 to 164–190 mg/kg per d in the HP infants and the LP infants in group 2. Furthermore most of this N (> 85 %) appeared to be utilized in synthetic pathways rather than being recycled to further urea synthesis as indicated by the low level of the [^{15}N^{14}N] N peak from the urea.

The concentrations of the amino acids in the urine were variable in a way which was unrelated to the stage of recovery or protein intake. The nonessential amino acids were generally more abundant than the essential amino acids. Lysine accounted for less than 2 % of the total amino acid pool at an average concentration of 0·04 mM, equivalent to about 1 μg N/ml (ranging from < 0·2 to 5 μg/ml). With recovery for mass spectrometry of lysine-N at about 50 % of that in the urine sample available for chromatography, and with 5 μg N required for an optimum measurement, low concentrations precluded satisfactory measurements of enrichment in several children. However, satisfactory measurements were made on fifteen occasions. Of these, nine samples were enriched with ^{15}N (Table 1).

To investigate the time course of the enrichment for one child with clear evidence of ^{15}N enrichment in the final urine sample, sequential measurements of enrichment of lysine were made for the 36 h period of the infusion. Significant increases in enrichment were observed after 24 h, after which time enrichment increased progressively (Fig. 1).

Infants demonstrating urinary lysine enriched with ^{15}N in the final urine sample included those from all three stages of

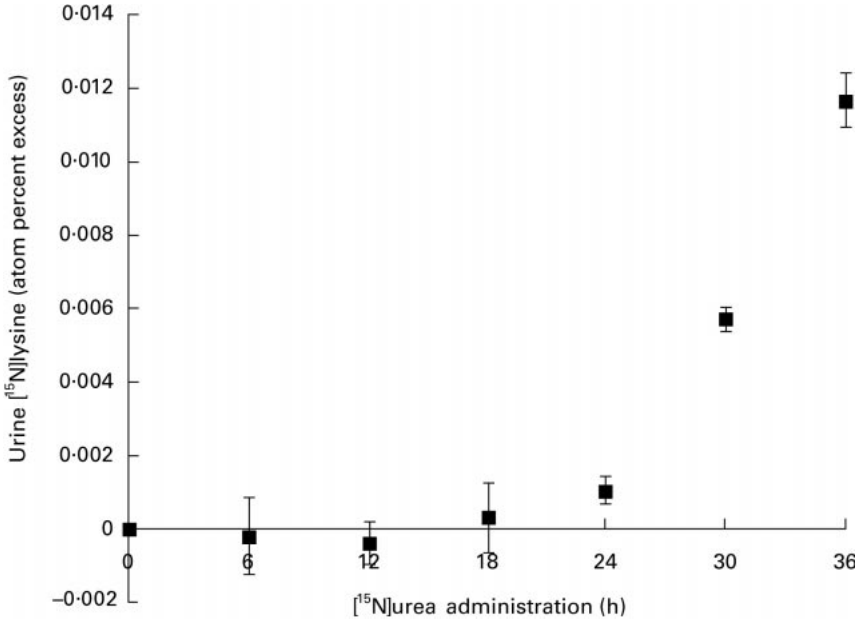

Fig. 1. Enrichment of urinary lysine with ^{15}N during [^{15}N]urea administration in a malnourished male infant. Values are the mean replicate (*n* 4) isotopic abundance measurements, with standard deviations shown by vertical bars, of single lysine samples isolated from urine collected during 6 h periods for a total of 36 h. For details of procedures see p. 507.

treatment and fed on both HP and LP diets suggesting that the phenomenon was general. A comparison was made of the extent of lysine ^{15}N-labelling in individual children with the extent of urea hydrolysis to examine whether the labelling varied in a predictable way. Thus, it would be expected that lysine ^{15}N-labelling would reflect the extent of enrichment of urea with ^{15}N and the extent of urea hydrolysis, i.e. the delivery of ^{15}N to intestinal bacteria, calculated from the urea hydrolysis rate and the plateau ^{15}N urea enrichment. This relationship is shown in Fig. 2: 68 % of the variation in lysine enrichment was explained by the variation in urea enrichment (Fig. 2(a)) while 54 % of the variation was explained by the overall rate of delivery of ^{15}N to the gastrointestinal tract (Fig. 2(b)). However, the inclusion of the five samples which were unlabelled reduced the overall significance of these relationships between the lysine enrichment and [^{15}N]urea hydrolysis (r^2 0·38) and urea enrichment (r^2 0·49).

As indicated above, the laborious nature of the methodology precluded isolation and measurement of ^{15}N enrichment in all amino acids but alanine, glycine and histidine were isolated from seven urine samples in addition to lysine. Glycine and alanine were generally more enriched than lysine with relative enrichment ratios of 1·63 (range 0·18–3·15) for glycine, and 1·96 (range 0·7–3·73) for alanine whilst for histidine the labelling was similar to lysine at 0·9 (range 0·4–1·8).

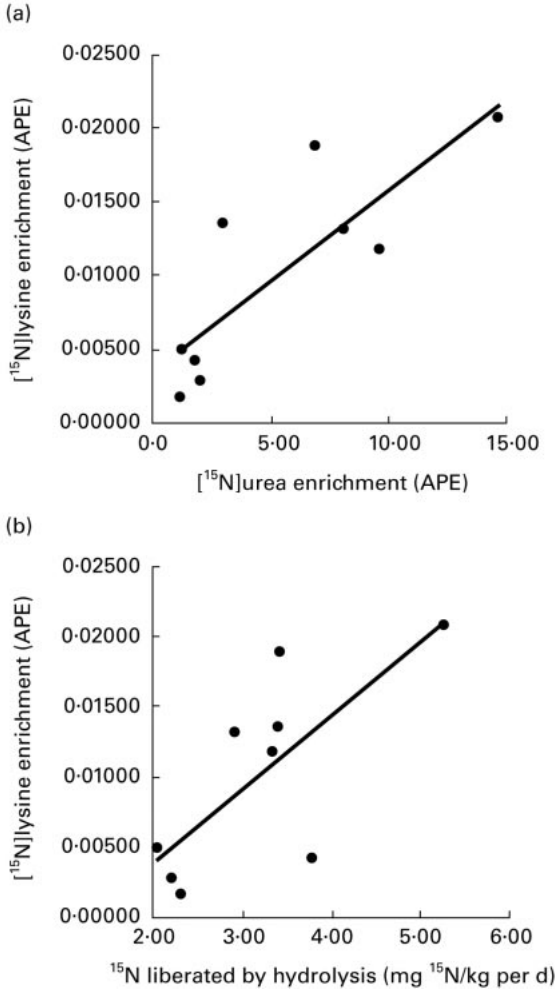

Fig. 2. Relationship between [^{15}N]lysine enrichment and (a) urea enrichment (R^2 0·6853) and (b) the extent of ^{15}N liberated by urea hydrolysis (R^2 0·54) in malnourished infants during nutritional rehabilitation. For details of procedures see p. 507.

Discussion

In the children studied here, urea synthesis was greater than urea excretion implying extensive urea hydrolysis (on average 50 % of synthesis) and recycling to the systemic circulation as observed in numerous previous studies in children and adults (Jackson, 1998). A more complete discussion of the urea hydrolysis results has recently been reported (Badaloo *et al*. 1999). Our concern here is with the fate of the N released during urea hydrolysis.

We assume that the small proportion which is recycled into urea synthesis (on average 13 %) reflects the fate of some or all of any absorbed as NH_3. However, in some but not all children there appears to be significant amounts of transfer of ^{15}N from ^{15}N-labelled urea to lysine and other amino acids. This indicates to us that urea hydrolysis can be of nutritional significance through the provision to the systemic circulation of essential amino acids synthesised *de novo* by intestinal microflora. While this is consistent with several previous studies, as will be reviewed, it is dependent on the validity of certain assumptions and potential errors in our measurements.

We assume oral urea to be absorbed intact from the upper gastrointestinal tract. Any significant hydrolysis before absorption, such as may occur with a *Helicobacter pylori* infection, or with significant bacterial overgrowth in the small intestine, would invalidate the method. All the children studied had been treated for presumptive small bowel overgrowth with a course of metronidazole. In any case any hydrolysis to $^{15}NH_3$ before absorption would have been identified as a much higher rate of recovery of [^{15}N^{14}N]urea in urine than the low level observed. Thus, urea hydrolysis can be assumed to be confined to the lower bowel, most likely the colon, although a contribution from bacteria in the terminal ileum can not be ruled out.

We assume that N released from urea hydrolysis in the colon is retained within the body with insignificant amounts of either ^{15}N or total N lost in the stool. Stool losses were not measured in the present studies but in previous infant studies after oral [^{15}N^{15}N]urea, enrichment of stool N was indistinguishable from background (Jackson *et al*. 1990). The digestibility of these diets in the children is known to be high, with stool N no more than 20–30 mg N/kg per d (Kennedy *et al.* 1990). Less than 10 % of any [^{15}N^{15}N]urea placed directly in the lumen of the right colon, either at colonoscopy or through a colostomy, is recovered in stools (Moran & Jackson, 1990*a*,*b*). Of the rest, about 5–10 % is recovered intact in urine as [^{15}N^{15}N]urea, up to 30 % is recovered as [^{15}N^{14}N]urea in urine, indicating some hydrolysis to $^{15}NH_3$ and reincorporation into urea, but with most label not accounted for and assumed to be retained within the system. Enrichment of stool N has been detected in the adult at 7 % of plasma urea (Wrong *et al*. 1985). This indicates a total colonic N flux diluting the urea of more than ten-fold that of urea hydrolysis (a flux of about 32 g N/d

for a urea production rate of 13 g N/d with 25 % hydrolysis (Jackson, 1998)). This is about 30-fold the normal daily stool N indicating the extent of absorption of N from the lower bowel.

It is unlikely that the colonic microflora represents an extensive 'sink' for label since after giving lactose–ureide to present labelled urea directly to the colon for fermentation (Bundy *et al*. 1996; Jackson *et al*. 1999), while 20 % of the tracer was recovered in stool, only 3 % was in bacterial N which in any case made only a minor contribution to the total stool N. This enrichment in stool and in bacterial isolates returned to background levels by 48–72 h. Furthermore, the fate of absorbed label (one-third lost in urine and two-thirds retained within the body) was very similar to the proportions and interpretations derived from the present studies.

As for the estimation of the values for urea synthesis and recycling from the relative magnitude of the urinary [^{15}N^{15}N]- and [^{15}N^{14}N]urea labelling, we have assumed a metabolic steady state from the plateau value of the enriched isotopic species. Other possible routes of loss of label, and likely associated errors in the interpretation of the data, have been discussed elsewhere (Jackson *et al*. 1984). Even allowing for generous errors in the assumptions we are left with a substantial retention of label, and urea N which it traces, which cannot be accounted for by other possible routes of loss.

Finally, we believe that our urea excretion values are subject to < 5 % error so that underestimation of urea excretion with overestimation of urea hydrolysis is unlikely. Our methods of urine collection and handling would have prevented significant bacterial degradation of urea post-collection. Although urinary tract infections are common in malnourished children, resulting in a poor appetite and failure to gain weight, this was not the case here since all our children were presumed to be infected and treated with broad spectrum antibiotics. Thus, bacterial hydrolysis of urea before its excretion with high levels of either $^{15}NH_3$ or [^{15}N^{14}N]urea can be reasonably excluded.

We can be confident, therefore, that our values for urea hydrolysis and the extent entering other pathways rather than recycling are not subject to serious error. As to the nature of these other pathways the systemic labelling of lysine with ^{15}N reported here points to amino acid synthesis as an important component. Since lysine does not transaminate in human subjects (Schoenheimer & Ratner, 1939) or in the rat (Torrallardona *et al*. 1996*a*), this implies *de novo* synthesis by the intestinal flora further implying the nutritional and physiological importance of urea hydrolysis with the potential to improve the quality of the protein supply by modifying the pattern of essential amino acids made available for protein synthesis. Previous indirect evidence suggesting that urea hydrolysis is of positive benefit includes studies by Snyderman (1967) showing that infant growth improved when a marginal intake of milk protein was supplemented with dietary urea, suggesting that nonessential N may limit the rate of growth. In addition, several studies of the fate of urea in human milk indicate that after oral [^{15}N]urea, stool losses are very small (Heine *et al*. 1984, 1986, 1987), with up to 40 % retained within the body (Heine *et al*. 1991), some in plasma protein. Other studies of incorporation of [^{15}N]urea into lysine and other amino acids include measurements in patients with chronic renal failure (Giordano *et al*. 1968; Furst, 1972), in children and adult Papua New Guinea highlanders and Japanese adults fed on LP diets (Tanaka *et al*. 1980; Rikimaru *et al*. 1984), and in rats fed on LP diets (Sheng *et al*. 1977). However, in the latter case the route of transfer may have involved coprophagy since no incorporation is observed when this is prevented (Torrallardona *et al*. 1996*b*). In young pigs caged to prevent coprophagy and fed on diets labelled with $^{15}NH_4Cl$ and [U-^{14}C]polyglucose, labelling with ^{14}C and ^{15}N was observed in all tissue amino acids including lysine (Torrallardona *et al*. 1994, 1996*c*). These authors interpret these data as indicating uptake from the gastrointestinal tract of amino acids deriving from bacterial amino acid biosynthesis.

The present studies extend these previous reports showing that in the infant the transfer of ^{15}N from urea to a non-transaminating essential amino acid can be demonstrated within 24 h of the administration of [^{15}N]urea. Whilst for histidine the labelling was similar to lysine, for glycine and alanine these were generally more enriched than lysine, the higher mean enrichment in alanine and glycine being consistent with their N originating in part via exchange reactions from $^{15}NH_3$. However, during the chromatographic separation of glycine and alanine, complete baseline collections of the peaks was not always achieved, so some isotope fractionation could have occurred accounting for the variability of enrichment with respect to lysine. Clearly, further work is needed to resolve whether the higher values for the glycine : lysine and alanine : lysine ratios (> 3) are more representative of the true values.

One weakness of these data is our failure to show labelling in all children. However, one simple explanation of this is that we terminated our studies too early to pick up labelling in all children. These studies are much shorter than most previous studies and it is clear from Fig. 1 that lysine enrichment was increasing at the end of the study period. A second weakness is the lack of well-described amino acid transporters in the colonic mucosa. However the non-ruminent large intestine does have a physiological capacity to absorb some amino acids, with absorption of substituted amino acids (asparagine, serine, threonine, tyrosine, arginine, hisidine, lysine) from sterilized ceca of adult pigs (Olszewski & Buraczewski, 1978). In addition, one amino acid transporter, at least for dipeptides is expressed in the colon (Dantzig *et al*. 1994).

Perhaps the most important issue relates to the magnitude and nutritional significance of the findings. If microbial amino acids in the colon are made available to the host in significant amounts, then the dietary amino acid pattern available for protein synthesis can be modified, improving dietary protein quality. Although the circulating lysine had not achieved equilibrium and the true enrichment of the intestinal pool of lysine is unknown, very approximate minimum values of *de novo* systemic lysine appearance can be calculated as ^{15}N-labelled lysine supply $= (Q_{lysine}) \times (\text{APE lysine}_{urine})/(\text{APE lysine}_{microbial})$, where Q_{lysine} is the appearance rate of lysine from whole body protein turnover and the diet and would have been between about 680 and 1000 mg/kg per d (whole-body protein turnover is about 8 g

protein/kg per d; i.e. 640 mg lysine/kg per d (Waterlow *et al*. 1978) with dietary lysine intake between 43 and 386 mg/kg per d); atom percent excess (APE) $\text{lysine}_{\text{urine}}$ as measured here is assumed to be at isotopic equilibrium. The assumed enrichment of microbial protein (APE $\text{lysine}_{\text{microbial}}$) is the maximum possible value, i.e. that of the urea pool at plateau (in these studies between 1 and 15 APE). The data reported here would indicate a lysine entry rate on average of 4·7 mg lysine/kg per d (range 1–20). Clearly, the assumptions made in these calculations make this an extremely conservative estimate and marked under-estimate of the true value for [^{15}N]lysine entry. Thus, true APE $\text{lysine}_{\text{urine}}$ at isotopic equilibrium would be higher (Fig. 1) and APE $\text{lysine}_{\text{microbial}}$ would be lower than the [^{15}N]urea APE given the other sources of N from the diet and endogenous secretions into the gut available for bacterial protein synthesis. With faecal NH_3 and/or bacterial N at only 8 % of the ^{15}N enrichment of plasma urea (Wrong *et al*. 1985) lysine appearance could be an order of magnitude higher, i.e. about 60 mg/kg per d. Indeed, support for this value comes from our other recent studies in adults given oral lactose–[^{15}N^{15}N]ureide to label colonic protein in which plasma lysine enrichment was 9 % of bacterial protein. If this ratio occurred in the children this would indicate an appearance rate of 58 mg/kg. Furthermore, these higher estimates are consistent with similar as yet unpublished data obtained in the pig referred to by Fuller & Reeds (1998).

In conclusion, these studies not only confirm the earlier studies showing that circulating amino acids can derive from urea N after hydrolysis and utilization of N by colonic micro-organisms but also show that when urea hydrolysis is extensive in these children, the process has the potential to provide nutritionally important quantities of lysine and presumably other essential amino acids. When this occurs, the quality of the dietary protein supply for these children will be less important than is usually assumed. Clearly more work is required to explore the mechanisms by which amino acids synthesized by gastrointestinal microflora can enter the systemic amino acid pool.

Acknowledgements

This work was generously supported by the Nestlé Foundation.

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